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Listing of chemicals in Annex III to the Rotterdam Convention: review of notifications of final regulatory actions to ban or severely restrict a chemical: azinphos-methyl

Azinphos-methyl

Note by the Secretariat

Addendum

Supporting documentation provided by Norway

The Secretariat has the honour to provide, in the annex to the present note, documentation received from Norway to support its notification of final regulatory action for azinphos-methyl as a pesticide. The documentation has been reproduced as received, without formal editing by the Secretariat.

* UNEP/FAO/RC/CRC.6/1.

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Annex

1. Focussed summary: Azinphos-methyl

2. Monograph, Azinphos-methyl, Rapporteur Member State: Germany. September 18, 1996.
   - 2.1 B-1 - B-4: Summary, Scientific Evaluation and Assessment
   - 2.2 B-5: toxicology and metabolism.
   - 2.3 B-7 + B-8: Environmental fate and behaviour.
   - 2.4 Addendum 3 B-8: Ecotoxicology.
   - 2.5 Addendum 7 B-9: Ecotoxicology.
   - 2.6 Addendum 8

3. Norwegian Agricultural Inspection Service – Pesticides Section: Information on Resolutions following recommendations from the Council for Pesticides (Council Meeting 20 September 2002)
   Available in Norwegian at: http://www.inchem.org/documents/jmpr/jmpmono/v91pr02.htm

4. Use and Findings of the azinphos-methyl insecticide in the JOVÅ programme

5. Joint FAO/WHO Meeting on Pesticide Residues Pesticide residues in food - 1991. Part 11 – Toxicology
   Available at: http://www.inchem.org/documents/jmpr/jmpmono/v91pr02.htm

6. Pesticide Manual (twelfth edition).

The following documents were provided in Norwegian. They are not attached to this document but hard copies will be made available at the meeting.

1. Letter of decision from the Norwegian Agricultural Inspection Service to Bayer Cropscience Agro. October 22, 2002

2. Holistic evaluation of Gusathion - azinphos-methyl. The Norwegian agricultural inspection Service. September 5, 2002

3. Recommendation from the Board of Pesticides (Rådet for plantevernmidler, møtebok). September 20, 2002

FOCUSED SUMMARY AZINPHOS-METHYL

I. INTRODUCTION

(a) The events that lead to the final regulatory action

During re-registration of the plant protection product Gusathion, The Norwegian Agricultural Inspection Service decided to phase out the active ingredient azinphos-methyl. This decision was a result of azinphos-methyl's very worrisome ecotoxicological properties and that the substance was found by monitoring despite the fact that the use had been restricted in the surveillance fields. The Agricultural Inspection Service was aware of azinphos-methyl's great agricultural importance, and therefore the plant protection product was authorized for one additional year. It was the hope that such an abbreviated registration period would expedite the process of getting alternative preparations on the market.

Gusathion was allowed to be imported until 31/12/2003 and allowed to be distributed until 31/12/2004. All use of Gusathion was strictly prohibited after 31/12/2005.

(b) Significance of final regulatory action, e.g. one use or many uses, level or degree of exposure

The decision aims at a complete reduction of risk of azinphos-methyl released to the environment from application of the substance. The decision encompassed all uses as azinphos-methyl was banned from 31/12/2005.

(c) An overview of the regulatory system in the notifying Party, if relevant

Pesticide registration in Norway is regulated by the Pesticide Act of 5 April 1963. In Norway, it is the Norwegian Agricultural Inspection Service (Norwegian Food Safety Authority after 2004) that is responsible for registering pesticides. There is also a Board of Pesticides (Rådet for plantevernmidler) that advises the Agricultural Inspection Service in questions regarding the approval of pesticides. The Board is comprised of 3 members appointed by the Ministry of Agriculture, one by the Norwegian Food Control Authority, one by the Local Government Ministry, two by the Ministry of Social Affairs and two by the Ministry of Environment. Registered plant protection products receive a registration period of (maximum) 5 years in Norway. According to the Pesticide Act, when an authorization is withdrawn, the product is allowed to be imported until the end of the calendar year, distributed until the end of the calendar year one year after the authorization expires, and the product is allowed to be used until the end of the calendar year two years after the authorization expires.

In the case of azinphos-methyl, the Board of Pesticides recommended at its meeting (20/09/2002) to phase out azinphos-methyl. However, because of the great demand for Gusathion, they recommended to extend the authorization for one additional year. The Norwegian Agricultural Inspection Service decided in its letter to the importer/applicant of Gusathion on 22/10/2002 (and also in the decision published on the internet III/2002) to ban all uses of azinphos-methyl after 31/12/2005.

(d) Scope of the regulatory action, precise description of the chemicals subject to the regulatory action

The scope of the regulatory action was both the plant protection product Gusathion and the active ingredient azinphos-methyl (CAS 86-50-0) as both are covered by the definition of a pesticide in the Pesticide Act.

II. RISK EVALUATION

(a) Key findings of the national evaluation

Norway's risk evaluation took into account toxicology, environmental fate and behaviour, ecotoxicology, residues and availability of alternatives. The review concluded that azinphos-methyl is extremely toxic to most aquatic organisms tested. Even a 30 meter buffer zone to surface water is not sufficient to protect the aquatic environment. By repeated use of azinphos-methyl it is possible that some populations of invertebrates are knocked out for a longer period.

(b) Key data reviews consulted together with a brief description

The ecotoxicological studies used in the Norwegian evaluation had also been reviewed by the Rapporteur Member State Germany as part of the EU Monograph (18/09/1996) with Addenda.

(c) Reference to national studies, e.g. toxicological and ecotoxicological studies.

Holistic evaluation of Gusathion - azinphos-methyl. The Norwegian Agricultural Inspection Service. 05.09.2002.

Recommendation from the Board of Pesticides (Rådet for plantevernmidler, møtebok).

Use and findings of the insecticide azinphos-methyl in the JOVÅ-programme. Memorandum from Gro Hege Ludvigsen and Olav Lunde, Jordforsk to Kristin Espeset, Norwegian Agricultural Inspection Service (Statens landbrukstilsyn), September 4, 2002.

(d) Summary of actual (or potential) human exposure and/or environmental fate

Through the agricultural and environmental monitoring programme of pesticides in Norway (JOVÅ), Norway's pesticide laboratory (Planteforsk Pesticidlaboratoriet) monitors catchments that represent agricultural areas in Norway. Azinphos-methyl has been a part of Planteforsk's analysis since 1996. Azinphos-methyl was detected on 5 occasions in rivers and streams and on one occasion in ditches. Azinphos-methyl has been detected at a maximum concentration of 0.64 µg/l (in 1998) and as recently as 2002 at a concentration of 0.55 µg/l.

In ecotoxicological studies, NOECs for fish (rainbow trout) range from 0.18-0.39 µg/l, NOEC for invertebrates (Daphnia magna) is established at 0.25 µg/l, and EC15 for

Chironomus riparius is established at 0.3 µg/l. A NOEC of 0.32 µg/l was established in an outdoor microcosm study.
Using the calculation method used at the time of the evaluation (Ganzelmeier, 1995), a maximum predicted environmental concentration (PEC) in surface water, taking into account a 30 meter buffer zone, of 1.53 µg/l is calculated. This is based on the application rate for apple fruit moths. This value is then compared to the NOEC of 0.32 µg/l established from a microcosm study. The ratio of these two figures is 5, indicating that the expected concentration in surface water is 5 times higher than an acceptable concentration for the protection of aquatic species. This conclusion was also supported by actual concentrations in Norway, in that concentrations detected in the monitoring program were twice as high as the acceptable concentration for the protection of aquatic species.

III. REDUCTION AND RELEVANCE TO OTHER STATES

(a) Estimates of the quantity of chemicals used, or imported/exported at the time of the regulatory action, and if possible information on ongoing trade

Azinphos-methyl had been on the market in Norway since before 1970. Annual sales (all of which are imported) from 1992 to 2001 range from 1410 kg/year (1992) to 2273 kg/year (1994). Average annual sales from the last five years prior to the regulatory action was 1861 kg/year.

(b) Relevance to other States, i.e. those with similar conditions of use.

Similar conditions of environmental exposure, such as contamination of surface water and exposure of aquatic organisms, are likely to occur also in other States and regions.

(c) Comments on the typical use of the chemical within the notifying country, with comments on possible misuse (if appropriate)

Azinphos-methyl had been used in pome fruit, stone fruit, blueberries, strawberries, cabbage and ornamentals. In fruit, application rates varied with different pests from 115-153 g a.i./ha to 574-765 g a.i./ha (against apple fruit moth). In blueberries, application rates were 689-765 g a.i./ha, in strawberries 510 g a.i./ha and in cabbage 1530 g a.i./ha

Monograph

18 September 1996

Azinphos-methyl

Volume 3

Annex B

Summary, Scientific Evaluation and Assessment

Rapporteur Member State: Germany

Azinphos-methyl - Annex B: Summary, scientific evaluation and assessment

Contents

Annex B

Azinphos-methyl

B-1: Identity

B.1 Identity

B.1.1 Identity of the active substance (Annex IIA 1)

See level 1.

B.1.2 Identity of the plant protection product (Annex IIA 3.1; Annex IIIA 1)

See level 1.

B.1.3 References relied on

Annex point(s) (91/414/EEC);	year. author(s). title. source. report number registration number.	GLP GEP Y/N	publ. Y/N	owner	data prot
EG:AIIA-1.11	1992. Imbeck Organic and Inorganic Samples Sodium by Flame Atomic Absorption Spectrometry FAAS. 2011-0267201-91E. CHE96-00097.	N	N	BAY	
EG:AIIA-1.11	1988. Krüger, K.W. Gusathion M, assey - HPLC method, external standard. 2201-0189301-88E. CHE96-00095.	N	N	BAY	
EG:AIIA-1.11	1992. Kulinna Gusathion M technical: methylchloride, ethylchloride, 1,2-dichloroethane, toluene - capillary gas chromatography. 2201-0246701-92E. CHE96-00093.	N	N	BAY	
EG:AIIA-1.11	1992. Kulinna Gusathion M technical: methylchloride, ethylchloride, 1,2-dichloroethane, toluene - capillary gas chromatography. 2201-0246101-92E. CHE96-00094.	N	N	BAY	
EG:AIIA-1.11	1987. Massil, S.E. and Fuss, L. HPLC analysis of Methyl-cotnion (Azinphos-methyl): calibration curve, precision and accuracy. CHE96-00124.	N	N	BAY	
EG:AIIA-1.11	1990. Ortner, G. Chemical Substances: Water-Volumetrie Method. 2011-0131301-90E. CHE96-00098.	N	N	BAY	
EG:AIIA-1.11	1991. Tengler, H.	N	N	BAY	

Annex point(s) (91/414/EEC);	year. author(s). title. source. report number registration number.	GLP GEP Y/N	publ. Y/N	owner	data prot
	Organic Solid Phases - Chloride, Phosphate, Nitrate and Sulfate - IC Method, external standard. 2201-0223901-91E. CHE96-00096.				
EG:AIIA-1.11	1992. Werner, T. Gusathion M: Purity and impurities - HPLC, external standard. 2201-0245501-92D. CHE96-00089.	N	N	BAY	
EG:AIIA-1.11	1992. Werner, T. Gusathion M: Impurities - High performance liquid chromatography. 2201-0245701-92D. CHE96-00090.	N	N	BAY	
EG:AIIA-1.11	1992. Werner, T. Gusathion M: Polar impurities - HPLC, external standard. 2201-0245601-92D. CHE96-00091.	N	N	BAY	
EG:AIIA-1.11	1992. Werner, T. Gusathion M: mercaptomethylbenzazimide - High performance liquid chromatography. 2201-0239901-92D. CHE96-00092.	N	N	BAY	
EG:AIIA-1.11	1992. Werner, T. Material accountability study. PC 507. CHE96-00075.	Y	N	BAY	
EG:AIIA-1.11	1992. Werner, T. Material accountability study. Revision of final report. PC 508. CHE96-00076.	Y	N	BAY	

Annex point(s) (91/414/EEC);	year. author(s). title. source. report number registration number.	GLP GEP Y/N	publ. Y/N	owner	data prot
EG:AIIA-1.11; EG:AIIIA-1.4	1995. Anonymous References (Makhteshim Agan ICC, Azinphos-methyl, Confidential). CHE96-00103.	N	N	BAY	

Annex B

Azinphos-methyl

B-2: Physical and chemical properties

B.2 Physical and chemical properties

B.2.1 Physical and chemical properties of the active substance Annex IIA 2)

B.2.1.1 Melting point and boiling point (Annex IIA 2.1)

B.2.1.1.1 Melting point:
Method: DSC (Differential Scanning Calorimetry):
T(0) (pure substance, extrapolated)
Test material: purified as 995 g/kg
Result: 73 °C
Comment: Acceptable
Ref.: Krohn, J., 1994

B.2.1.1.2 Boiling point:

Not measurable, decomposition at elevated temperature.
Comment: Acceptable
Ref.: Klusacek, H. and Krasemann, R., 1986

B.2.1.1.3 Temperature of decomposition or sublimation:

Method: OECD 113 - "Screening Test for Thermal Stability and Stability in Air": DSC (differential scanning calorimetry) in a closed ampoule and TGA (thermo-gravimetrical analysis) in an open crucible under air and nitrogen atmosphere.
Test material: purified as 991 g/kg
Results:
DSC-measurement: exothermic decomposition between 110 and 210 °C
TGA-measurement : weight loss due to decomposition between 140 and 200 °C. Azinphos-methyl is thermally stable at room temperature.
Comment: Acceptable
Ref.: Klusacek, H. and Krasemann, R., 1986

Thermal decomposition appeared to begin with the formation of mercaptans, dialkylsulfides and thiophosphates followed by conversion into polysulfides. With increasing temperature the compounds were further decomposed.
Ref.: Bertoni, G. et al., 1986

B.2.1.2 Relative density (Annex IIA 2.2)

Method: EEC A3
Test Material: Purified as 99.1 %
Result: 1.5184 at 21 °C
Comment: Acceptable
Ref.: Weber, D., 1987

B.2.1.3 Vapour pressure; volatility (Annex IIA 2.3)

B.2.1.3.1 Vapour pressure

Ref.: Seweko, 1974, LUF 95-50010
Method: OECD 104 Vapour pressure balance method (comparable with EC Test A4)
Material: OAL standard 1974

Results: values obtained from the vapour pressure curve:
70 °C $2.1 \cdot 10^{-4}$ hPa
100 °C $2.0 \cdot 10^{-3}$ hPa
values extrapolated from the vapour pressure curve:
20 °C $1.8 \cdot 10^{-6}$ hPa
25 °C $3.1 \cdot 10^{-6}$ hPa

Comment: The purity of test material is not specified.

Conclusion: Azinphos-methyl can be classified as semivolatile substance.

B.2.1.3.2 Volatility

Ref.: Krohn, 1994, WAS 95-50024
Method: Henry's law constant at 20 °C, calculated from vapour pressure and water solubility

Result: $2.0 \cdot 10^{-3}$ Pa · m^3/mole

Conclusion: The potential of volatility from aqueous surfaces should be low.

B.2.1.4 Appearance (Annex IIA 2.4)

Physical state, colour:
purified as: colourless crystals
technical as: yellow crystals

Odour:
purified as: none
technical as: mercaptane like

B.2.1.5 Spectra (Annex IIA 2.5)

Active substance
Test material: purified as 991 g/kg

The structure of azinphos-methyl is confirmed by various spectroscopic methods:

UV (methanol)
Absorption maxima at 220.6 and 284.0 nm (broad).

IR (KBr)

1H-NMR (250 MHz, CDCl3)
13C-NMR (62.89 MHz, CDCl3)
Mass spectra (70 eV electron impulse)

Comment: Acceptable
Ref.: Krohn, J., 1986

Impurities
None of the impurities present in the active substance as manufactured are of toxicological, ecotoxicological or environmental significance.

B.2.1.6 Solubility in water including effect of pH (Annex IIA 2.6)

Method: OECD 105 - flask method
Test material: Purified as 991 g/kg
Result: 27.9 ± 1.6 mg/l
Because the test substance did not show basic or acidic properties, solubility measurements under alkaline or acidic conditions were considered unnecessary.
Comment: Acceptable
Ref.: Krohn, J., 1987

B.2.1.7 Solubility in organic solvents (Annex IIA 2.7)

Method: OECD 105 - water solubility, adapted to organic solvents
Test material: purified a.i. 995 g/kg
Results: (all results given in g/l at 20 °C)

n-heptane	1.2
xylene	170
1,2-dichloroethane	> 250
2-propanol	8.5
1-octanol	7.8
polyethyleneglycol (lutrol)	180
acetone	> 250
acetonitrile	> 250
ethylacetate	> 250
dimethylsulfoxide	> 250

Comment: Acceptable
Ref.: Krohn, J., 1995

B.2.1.8 Partition coefficient n-octanol/water (Annex IIA 2.8)

Method: OECD 107 - flask shaking method; the method is comparable to the EC method A8.
Test material: Purified as 993 g/kg
Result: log P_{ow} lies within the range of 2.87 to 3.02 with a mean of 2.96.
Comment: Acceptable
Ref.: Bentz, F., 1983

B.2.1.9 Stability in water, hydrolysis rate, photochemical degradation, quantum yield and identity of breakdown product(s), dissociation constant including effect of pH (4 to 9)(Annex IIA 2.9)

B.2.1.9.1 Hydrolysis rate

(a) Ref.: Wilkes et al., 1979, WAS 95-50026
Material and Method:
Dissipation of [phenyl-UL-^{14}C]azinphos-methyl (27.8 mCi/mmole specific radioactivity; 96.8 % radiochemical purity) was studied in aqueous phosphate buffers at pH 4, 7 and 9 (measured pH 4.22, 6.95 and 9.25) under sterile conditions; fortification was at 1 mg/l and 10 mg/l. The samples were maintained in the dark at 30 °C and 40 °C. Samples from duplicate vials were analysed after 1, 2, 4, 7, 14, 21 and 30 d post-treatment before and after partitioning with ethyl acetate by LSC and two-dimensional TLC.

Results: Half-life (d) of ^{14}C-azinphos-methyl
(Rate constants (d^{-1}) are given in brackets):

	pH 4		pH 7		pH 9	
	1 mg/l	10 mg/l	1 mg/l	10 mg/l	1 mg/l	10 mg/l
30 °C	38.9 (0.0161)	42.2 (0.0145)	23.1 (0.0265)	25.5 (0.0256)	2.2 (0.284)	2.5 (0.266)
40 °C	17.8 (0.0355)	21.3 (0.0292)	11.0 (0.0550)	12.1 (0.0518)	1.1 (0.511)	1.3 (0.508)

Hydrolysis products:
1) trivial name: mercaptomethylbenzazimide
chemical name (CA): 3-(mercaptomethyl)-1,2,3-benzotriazin-4(3H)-one
CAS no.: 028527-10-8
2) trivial name: bis-(benzazimide-N-methyl)sulfide
chemical name (CA): 3,3'-[thiobis(methylene)]bis-[1,2,3-benzotriazin-4(3H)-one]
CAS no.: 025519-79-7
3) trivial name: (a) benzazimide and/or
(b) hydroxymethylbenzazimide
(could not be separated by two-dimensional TLC)
chemical name (CA): (a) 1,2,3-benzotriazin-4(3H)-one
(b) 3-(hydroxymethyl)-1,2,3-benzotriazin-4(3H)-one
CAS no.: (a) 000090-16-4
(b) 024310-40-5
4) trivial name: anthranilic acid
chemical name (CA): 2-aminobenzoic acid
CAS no.: 000118-92-3

Comment:
Acceptable, though the study report did not refer to any guideline. Half-lives at 22 °C were calculated by Wilmes (1984, WAS 95-50027).

based on these results (see below).

Conclusion:
Azinphos-methyl is most stable at low pH and at low temperature. It was slightly more stable at the higher concentration.

(b) Ref.: Wilmes, 1984, WAS 95-50027
Material and Method:
Calculation of half-lives at 22 °C from the experimental values (Wilkes et al., 1979, WAS 95-50026) obtained at the starting concentration of 1 mg/l.
Results: Hydrolytic half-lives of azinphos-methyl at 22 °C:

pH 4	87 d
pH 7	50 d
pH 9	4.1 d

B.2.1.9.2 Photochemical degradation

(a) Ref.: Wilkes et al., 1979, WAS 95-50028
Material and Method:
Photodegradation of [phenyl-UL-^{14}C]azinphos-methyl (27.8 mCi/mmole specific radioactivity; 97.2 % radiochemical purity) was studied in aqueous phosphate buffers at pH 4 (measured pH 4.35); fortification was at 10 mg/l. The samples were irradiated at approximately 30 °C in a Ace Photochemical Reaction Assembly Model 6515 equipped with a 200 W Hanovia Mercury Lamp No. 6515-32 in a borosilicate glass immersion well to exclude irradiation of 280 nm or less. Duplicate samples were collected until 48.25 h of continous exposure (19 sampling times). The samples were analysed before and after partitioning with ethyl acetate by LSC and two-dimensional TLC. Volatile photoproducts were collected in Chromosorb 102 traps; CO_2 was trapped in aqueous NaOH solution and were radioassayed, both.

Results:
- Radioactivity balance: 87.3 ... 113.3 % with an average of 102.4 %. The variation of the values from 100 % can be attributed to experimental errors. No significant volatilization losses occured.
- Half-life of as was 9.4 h at 30 °C and wavelengths > 280 nm
The photolytical reaction followed second-order kinetics. The second-order rate constant was calculated to be 0.001 $(h \cdot \%)^{-1}$
- Photolysis products representing > 10 % of initial radioactivity:

trivial name:	(a) benzazimide and/or
	(b) hydroxymethylbenzazimide
(could not be separated by two-dimensional TLC)	
chemical name (CA):	(a) 1,2,3-benzotriazin-4(3H)-one
	(b) 3-(hydroxymethyl)-1,2,3-benzotriazin-4(3H)-one
CAS no.:	(a) 000090-16-4
	(b) 024310-40-5

- Balance of radioactivity and distribution of degradation products after irradiation for 48 h:

azinphos-methyl	18.7 %
benzazimide and/or hydroxymethylbenzazimide	38.7 %
methylbenzazimide	1.5 %
anthranilic acid	9.8 %

unknown compound	1.5 %
^{14}C at TLC origin	5.2 %
^{14}C remaining in buffer solution	8.4 %

Comment:
The study report did not refer to any guideline. The results can be used only for orientation since wavelengths in the range of 280 - 290 nm were not excluded and because of irradiation with a mercury lamp and the high temperature. Morgan (1987, WAS95-50029) stated that the conditions were non-sterile.

(b) Ref.: Morgan, 1987, WAS 95-50029
Material and Method:
Photodegradation of [phenyl-UL-^{14}C]azinphos-methyl (46.9 mCi/mmole specific radioactivity; 98.9 % radiochemical purity) was studied according EPA guideline 161-2 in sterile 0.01 M acetate buffer at pH 4 (measured pH 4.34 ... 4.42), containing 1 % acetonitrile from stock solution; fortification was at 10 mg/l. The samples were exposed to natural sunlight of Kansas City, Missouri, USA, during the period January through March, 1987. Maxima of sunlight intensity were in the range of approximately 5000 - 80000 $\mu W/cm^2$ after 12, 37, 62, and 85 h. The average light energy per hour was 0.4033 $W \cdot min/(cm^2 \cdot h)$. The temperature of exposed fused quartz cells was 25 °C (17.3 - 29.0 °C). The sampling from both the exposed and dark cells was at approximately 0, 4, 5, 8, 32, 56, and 87 h post treatment. The samples were analysed by LSC, HPLC with radioactivity detection, GC-MS and TLC.

Results:
- Radioactivity balance:
  dark control: 97.5 - 103.3 % with an average of 100.0 %
  exposed cells: 99.2 - 105.0 % with an average of 102.6 %
- The photolytical reaction followed first-order kinetics.
The timed photochemical half-life (i.e. light energy half-life devided by average light energy per hour) at 25 °C was 76.7 h (3.2 d).
The dark cell showed no loss of as over the course of the study.
- Photolysis products representing > 10 % of initial radioactivity:

trivial name:	benzazimide
chemical name (CA):	1,2,3-benzotriazin-4(3H)-one
CAS no.:	000090-16-4

- Balance of radioactivity and distribution of degradation products after irradiation for 87 h:

azinphos-methyl	41.6 %
benzazimide	39.1 %
anthranilic acid	7.2 %
unknowns	12.1 %

(consisted of 5 distinct HPLC peaks none of which exceeded 2 % of initial radioactivity)

Comment:
The method does not meet the requirements specified in Annex II. Since the test was conducted under natural sunlight, reproducibility is not given and no comparability with standardized

experiments using artificial light is possible. Results can be used only for orientation and may be possibly utilized only for regions near to latitude 40 °N (Kansas City!).

B.2.1.9.3 Quantum yield of direct phototransformation

Ref.: Hellpointner, 1994, WAS 95-50030
Test Material: purified active substance 995 g/kg
Test guideline: Phototransformation of Chemicals in Water, Part A: Direct Phototransformation, Umweltbundesamt, Berlin, FRG (1990)

Method:
The quantum yield was determined according to ECETOC method in polychromatic light (> 295 nm). From the UV absorption data and the kinetic results of two photodegradation experiments with acetonitrile/water (1:9) at 4.9 mg as/l, 25 °C, in a merry-go-round irradiation apparatus (TQ 150 mercury lamp with Duran 50 filter) the quantum yield was calculated.
The irradiation intensity was about $6.2 \cdot 10^{16}$ photons/s/3 ml in the range of 295 - 490 nm.
The test system was not buffered; the pH was not reported.
Eleven samples were collected within 0 - 60 minutes of irradiation and were analysed by HPLC and UV/VIS spectroscopy.
The resulting quantum yield and UV absorption data were used to estimate the environmental half-life concerning direct phototransformation in water by two different simulation models.

Results:
- UV absorption properties:
Azinphos-methyl dissolved in water/acetonitrile (9/1) showed an absorption maximum at 225 nm (molar extinction coefficient = 29743 l/mole/cm) and a broad maximum at 284 nm (7388 l/mole/cm) which extends into the environmentally relevant range of wavelength at about 343 nm.
- The photodegradation followed a first order kinetic model with a rate constant k = 0.017 min^{-1} and a half-life of 40.5 min.
- The quantum yield was calculated to be 0.00204.
- Environmental photolysis half-life in (surface) water:

GC-Solar-program:

	Environmental half-life (days)			
Season	30th	40th	50th	60th degree of latitude
Spring	0.9	1.1	1.4	1.9
Summer	0.7	0.8	0.9	1.1
Fall	1.3	1.9	3.5	8.5
Winter	2.0	4.0	11	51

(pure water from close to the surface (0-5 cm), 10th degree of longitude, clear sky, typical ozone concentrations, half-lives integrated over the entire day)

Frank/Klöpffer-program:

	Environmental half-life (days)		
Month	Minimum	Mean	Maximum

April	1.3	2.3	9.2
May	1.0	1.6	6.6
June	0.9	1.4	5.5
July	1.0	1.5	5.1
August	1.1	1.6	5.5
September	1.8	3.1	12

(pure water from close to the surface (0-1 cm), stagnant water, geographic and climatic conditions of Germany (50th degree of latitude), no contribution of another mono- and bimolecular elimination process, half-lives integrated over the entire day)

Comment: Accepted regarding quantum yield and environmental half-life. Not accepted regarding aqueous photolysis because of irradiation with a mercury lamp.

Conclusion:
The results indicate that direct photodegradation in water essentially contributes to the overall elimination of azinphos-methyl in the environment. The half-life concerning direct photolysis is expected in the range between 1 and 4 d, for the months of main use.

B.2.1.9.4 Dissociation constant

Ref.: Stupp, 1991, WAS 95-50023
Material: Azinphos-methyl, content: 995 g/kg
Method: Titration method on the basis of OECD Guideline No. 112. On account of the solubility, the titration was carried out contrary to the OECD Guidelines in the present of 2-propanol as co-solvent. Additional titration was carried out in acetic acid as solvent and perchloric acid as titration agent.

Result:
The resulting titration curves do not show a neutralisation reaction of the test substance. It is not possible to specify a pK value in aqueous systems.

Conclusion:
Azinphos-methyl does not have acid or alkaline properties.

B.2.1.10 Stability in air, photochemical degradation, identity of breakdown product(s)

Ref.: Hellpointner, 1994, LUF 95-50011
Method:
The estimation of photochemical oxidative degradation of azinphos-methyl in the troposphere was conducted by using the "Atmospheric Oxidative Program" (Meylan and Howard, 1993, LUF 96-50001) which is based on a calculation procedure by means of quantitative structure reactivity relations (QSAR) developed by Atkinson (1985, LUF 95-00019; 1988, LUF 95-00018). Based on the molecular structure of as and a comprehensive set of experimental data on the reaction of organic compounds with photochemically

produced OH radicals and with ozone, a conservative estimation of rate constant, half-life and chemical lifetime in the troposphere was made.

Result:
On the basis of the molecular structure of azinphos-methyl it is apparent that reactions with photochemically produced OH-radicals and to a lesser extent ozone or direct photolysis contribute to degradation in air.
Main reactions are hydrogen abstraction at the methylen group and reaction at the P=S-group.
Based on a mean OH-radical concentration of $1.5 \cdot 10^6$ OH radicals/ cm^3 (global 12 h day time concentration, excluding the night) and an calculated overall OH reaction rate of $145.22 \cdot 10^{-12}$ cm^3/molecule/s, a half-life of 0.9 h corresponding to a chemical lifetime of 1.3 h was assessed, with respect to the OH radical reaction only.
A more conservative assessment using an accuracy factor of 2 resulted in a maximum half-life of < 2 h and a maximum chemical lifetime of < 3 h.

Conclusion:
On account of the short chemical lifetime of azinphos-methyl in the air, it is not to be expected that the as can be transported in gaseous phase over large distances or can accumulate in the air.

B.2.1.11 Flammability including auto-flammability (Annex IIA 2.11)

B.2.1.11.1 Flammability

Method: EEC A10
Test material: Active substance as manufactured - 900 g/kg
Results: Not easily flammable, the substance melts by approach of the ignition flame.
Comment: Acceptable
Ref.: Mix, K. H., 1995

B.2.1.11.2 Auto-flammability

Method: EEC A16
Test material: Active substance as manufactured - 900 g/kg
Results: Not auto-flammable, no exothermic effects occur till reaching the melting point.
Comment: Acceptable
Ref.: Mix, K. H., 1995

B.2.1.12 Flash point (Annex IIA 2.12)

Not applicable. The active substance is a solid; its melting point is > 40 °C.

B.2.1.13 Explosive properties (Annex IIA 2.13)

Method: EEC A14
Test material: Active substance as manufactured - 900 g/kg
Results: Mechanical sensitivity - negative; thermal sensitivity - negative.
Comment: Applicable
Ref.: Mix, K. H., 1995

B.2.1.14 Surface tension (Annex IIA 2.14)

Method: OECD 115 - ring method; the method is comparable to the EEC method A5.
Test material: purified as 995 g/kg
Results: 66 mN/m (saturated aqueous solution), non-surface active
Comment: Acceptable
Ref.: Krohn, J., 1995

B.2.1.15 Oxidising properties (Annex IIA 2.15)

The examination of the chemical structure of azinphos-methyl establishes that the active substance is incapable of reacting exothermically with a combustible material.
Comment: Acceptable

B.2.1.16 Summary of physical and chemical properties

Azinphos-methyl has a melting point of 73 °C. Its water solubility is 28 mg/l. The log of the partition coefficient n-octanol/water lies within the range of 2.87 to 3.02 with a mean of 2.96.
The vapour pressure of azinphos-methyl amounts to $1.8 \cdot 10^{-4}$ Pa at 20 °C. Because of Henry's law constant of $2.0 \cdot 10^{-3}$ Pa - m^3/mole the potential of volatility from aqueous surfaces should be low.
The substance does not have acid or alkaline properties.
The hydrolytic half-lives of azinphos-methyl at 22 °C amount to 87, 50, and 4.1 d at pH 4, 7, and 9, respectively.
The major hydrolysis products are bis-(benzazimide-N-methyl)sulfide (3,3'-[thiobis(methylene)]bis-[1,2,3-benzotriazin-4(3H)-one]), benzazimide and/or hydroxymethylbenzazimide (1,2,3-benzotriazin-4(3H)-one and/or 3-(hydroxymethyl)-1,2,3-benzotriazin-4(3H)-one), and anthranilic acid (2-aminobenzoic acid).
Photolytic half-lives were determined to be in the range of 9.4 - 76.7 h. However, the results on photolysis can be used only for orientation because of various deviations from the requirements (e.g. the range of 280 - 290 nm were not excluded, non-sterile conditions, natural sunlight, higher temperature).
The major photolysis products are benzazimide and/or hydroxymethyl-benzazimide.
The environmental half-lives concerning direct photolysis are expected in the range between 1 and 4 d, for the months of main use.
Concerning indirect photolysis and based on the reactivity against OH radicals in the troposphere, a short half-life of < 2 h corresponding to a chemical lifetime of < 3 h was assessed conservatively.
Its flammability, explosive or oxidizing properties are not critical.

B.2.2 Physical, chemical and technical properties of the plant protection products (Annex IIIA 2)

Gusathion M EC 19.5

B.2.2.1 Appearance (Annex IIIA 2.1)

Physical state: liquid
Colour: yellow to brown
Odour: intense bad smell
Ref.: Schröder, P., 1995 (a)

B.2.2.2 Explosivity and oxidizing properties (Annex IIIA 2.2)

B.2.2.2.1 Explosivity

Currently not available, study in progress

B.2.2.2.2 Oxidising properties

As neither the active substance nor the co-formulants are oxidising, the product is not expected to be oxidising

B.2.2.3 Flash point and other indications of flammability or spontaneous ignition (Annex IIIA 2.3)

Flash point:

Method: EEC A9
Result: 28 °C
Comment: Acceptable
Ref.: Schröder, P., 1995 (b)

Ignition temperature:

Currently not available, study in progress

B.2.2.4 Acidity/alkalinity and if necessary pH value (Annex IIIA 2.4)

Method: CIPAC MT 75.2
Result: pH = 4.7 (1 % solution)
Acidity max. 0.1 % H_2SO_4
Comment: Acceptable
Ref.: Schröder, P., 1995 (c)
Schröder, P., 1995 (d)

B.2.2.5 Viscosity and surface tension (Annex IIIA 2.5)

B.2.2.5.1 Viscosity for newtonian liquids

Method: OECD 114 (rotation viscometer)
Result: 3.4 mPa·s
Comment: Acceptable
Ref.: Schröder, P., 1995 (f)

B.2.2.5.2 Surface tension

Method: EEC A5 (ring method)
Result: 34.8 mN/m
Comment: Acceptable
Ref.: Schröder, P., 1995 (e)

B.2.2.6 Relative density and bulk density (Annex IIIA 2.6)

B.2.2.6.1 Relative density of liquid preparations

Method: EEC A3 (Oscillating Densitimeter)
Result: 1.14 at 20 °C
Comment: Acceptable
Ref.: Schröder, P., 1995 (g)

B.2.2.7 Storage — stability and shelf-life. Effects of light, temperature and humidity on technical characteristics of the plant protection product (Annex IIIA 2.7)

B.2.2.7.1 Effect of high temperatures

Method: CIPAC MT 46.1
Result: The formulation is chemically stable to storage at 54 °C for 14 days.
Comment: Acceptable
Ref.: Schröder, P., 1995 (h)

B.2.2.7.2 Effect of low temperatures

Method: CIPAC MT 39.2
Result: 5.0 ml sediment (crystals)
Comment: Acceptable
Ref.: Schröder, P., 1995 (i)

B.2.2.7.3 Shelf life at ambient temperatures

Result: Stable for 2 years under ambient temperature conditions.
Comment: Acceptable
Ref.: Schröder, P., 1995 (j)

B.2.2.8 Technical characteristics of the preparation (Annex IIIA 2.8)

B.2.2.8.1 Wettability

Not applicable

B.2.2.8.2 Persistent foaming

Method: CIPAC MT 47
Results: 2.0 ml after 1 minute
2.0 ml after 15 minutes
Comment: Acceptable

Ref.: Schröder, P., 1995 (k)

B.2.2.8.3 Suspensibility and suspension stability

Not applicable

B.2.2.8.4 Dilution stability

Not applicable

B.2.2.8.5 Dry sieve test and wet sieve test

Not applicable

B.2.2.8.6 Particle size distribution

Not applicable

B.2.2.8.7 Emulsifiability, re-emulsifiability, emulsion stability

Result:
Emulsion stability:
Separation: max. 1.0 ml cream (bottom), 2.0 ml foam after 2 hours
Re-emulsifiability:
Completely re-emulsifiable after 24 h

Comment: Acceptable
Ref.: Schröder, P., 1995 (l)

B.2.2.8.8 Flowability, pourability (rinsability) and dustability

Not applicable

B.2.2.9 Physical and chemical compatibility with other products (Annex IIIA 2.9)

No comment of the applicant to this point at this stage of approval.

B.2.2.10 Adherence and distribution to seeds (Annex IIIA 2.10)

Not applicable

B.2.2.11 Summary of physical and chemical properties (plant protection product) (Annex IIIA 2.11)

Gusathion M EC 19.5 is not oxidising, its pH is within the range that naturally occurs e.g. in soil. Its stability allows storage under practical and commercial conditions. At low temperatures the risk of crystallization exists. Its technical properties indicate that no particular problems are to be expected when it is used as recommended. The formulation is classified as flammable.

B.2.2 Physical, chemical and technical properties of the plant protection products (Annex IIIA 2)

Gusathion M WP 25

B.2.2.1 Appearance (Annex IIIA 2.1)

Physical state: powder
Colour: beige
Odour: strong, characteristic
Ref.: Albanese, P., 1994

B.2.2.2 Explosivity and oxidizing properties (Annex IIIA 2.2)

B.2.2.2.1 Explosivity

Method: EEC A14
Result: Not explosive
Comment: Acceptable
Ref.: Albanese, P., 1994

B.2.2.2.2 Oxidizing properties

As neither the active substance nor the formulants are oxidising, the product is not expected to be oxidising.

B.2.2.3 Flash point and other indications of flammability or spontaneous ignition (Annex IIIA 2.3)

Flammability: No flame propagation (EEC A10)
Auto-flammability: Not auto-flammable (EEC A16)
Comment: Acceptable

B.2.2.4 Acidity/alkalinity and if necessary pH value (Annex IIIA 2.4)

Method: CIPAC MT 75.2
Result: pH = 4.98 (1% in water)
(Acidity not determined since pH > 4.0)
Comment: Acceptable
Ref.: Albanese, P., 1994

B.2.2.5 Viscosity and surface tension (Annex IIIA 2.5)

Not applicable

B.2.2.6 Relative density and bulk density (Annex IIIA 2.6)

Method: CIPAC MT 33
Result: Bulk density = 0.58 g/ml
Comment: Acceptable
Ref.: Albanese, P., 1994

B.2.2.7 Storage — stability and shelf-life. Effects of light, temperature and humidity on technical characteristics of the plant protection product (Annex IIIA 2.7)

B.2.2.7.1 Effect of high temperatures

Method: CIPAC MT 46.1
Result: The formulation is chemically stable to storage at 40 °C for 8 weeks (as degradation of 4.1 %).
Comment: Acceptable
Ref.: Wolf, 1994

B.2.2.7.2 Shelf life at ambient temperatures

No document provided for a storage test at ambient temperature. However, the manufacturer reports that the active substance degrades about 5.3 % after one year at ambient temperature.
Comment: Acceptable but there has to be a statement on the label that the product when stored in its unopened original container, away from direct sunlight, at a temperature not above 30 °C will be fit for use for at least 12 months.

B.2.2.8 Technical characteristics of the preparation (Annex IIIA 2.8)

B.2.2.8.1 Wettability

Method: CIPAC MT 53.3
Results: 25 s without swirling
12 s with swirling
Comment: Acceptable
Ref.: Albanese, P., 1994

B.2.2.8.2 Persistent foaming

Method: CIPAC MT 47
Results: 3 ml at 0 minutes
0 ml after 5 minutes
Comment: Acceptable
Ref.: Albanese, P. 1994

B.2.2.8.3 Suspensibility and suspension stability

Method: CIPAC MT 15.1
Result: > 80 %
Comment: Acceptable
Ref.: Albanese, P., 1994

B.2.2.8.4 Dilution stability

Not applicable (not soluble in water)

B.2.2.8.5 Dry sieve test and wet sieve test

Wet sieving

Method: CIPAC MT 59.3
Result: No visible residue (> 0.04 mm screen)
Comment: Applicable

Ref.: Albanese, P., 1994

B.2.2.8.6 Particle size distribution

No data provided

B.2.2.9 Physical and chemical compatibility with other products (Annex IIIA 2.9)

Method: Static miscibility (in-house method): Homogeneous aqueous dilutions of the test material and some other products are prepared. After specified periods of time (0.5 - 24 h) possibly formed sediment or cream is determined and compared with the behaviour of aqueous dilutions of the singular formulations.

Physical compatible with: Folicur WG 25 (tebuconazol)
Captan 80 % (captan)
Pomarsol Z WG (ziram)
Pomarsol WG 50 (thiram)
Mancozeb WP 80 (mancozeb)
Antracol WP 70 (propineb)
Baycor - Captan WP 43,75
(bitertanol/captan)

Chemical compatibility: Based on practical experience Gusathion M WP 25 is considered to be chemically compatible with the a. m. products.

B.2.2.10 Adherence and distribution to seeds (Annex IIIA 2.10)

Not relevant.

B.2.2.11 Summary of physical and chemical properties (plant protection product) (Annex IIIA 2.11)

Gusathion M WP 25 is not explosive, not oxidising, its pH is within the range that naturally occurs e.g. in soil. Because of its limited stability at elevated and ambient temperatures an expiring date on the label is necessary for storage under practical and commercial conditions. Its technical properties indicate that no particular problems are to be expected when it is used as recommended.

B.2.2 Physical, chemical and technical properties of the plant protection products (Annex IIIA 2)

Gusathion M WP 35

Several studies on residues, toxicity etc. were performed with the Gusathion M WP 35 formulation instead of the WP 25 formulation. All the physical and chemical properties are comparable.
Ref.: Albanese, P., 1994 (a)
Wolf, 1994 (a)

B.2.2 Physical, chemical and technical properties of the plant

protection products (Annex IIIA 2)

Cotnion Methyl 20 SC

No data are available.

B.2.3 References relied on

Annex point(s) (91/414/EEC);	year. author(s). title. source. report number registration number.	GLP GEP Y/N	publ. Y/N	owner	data prot
EG:AIIA-2.1	1986. Bertoni, G., Liberti, A., Agostinone, C.B., D'Antonio, M., Pettinari, L. and Leoni, V. Identification by gaschromatography mass-spectrometry of the products obtained from thermal decomposition of azinphosmethyl (Guthion). Annali di Chimica 76, 1986, 19-28. CHE96-00079.	N	Y		
EG:AIIA-2.1	1986. Klusacek, H. and Krasemann, R. Thermal stability of the agrochemical active ingredient azinphos methyl. PC 153. CHE96-00078.	N	N	BAY	
EG:AIIA-2.1	1994. Krohn, J. Melting point of azinphos-methyl. PC 156. CHE96-00077.	N	N	BAY	
EG:AIIA-2.2	1987. Weber, D. Determination of density according to GLP: Azinphos-methyl. PC 142. CHE96-00080.	N	N	BAY	
EG:AIIA-2.3	1994. Krohn, J. Calculation of the Henry law constant of azinphosmethyl. BAYER FILE NO.: PC 157. WAS95-50024.	N	N	BAY	
EG:AIIA-2.3	1974. Sewekow, B. Determination of vapour pressure of azinphos-methyl (Methylgusathion). Generated by: Bayer AG, submitted by: Bayer AG, 1974. 7.2.2.1 /01 ! Bayer PC143. LUF95-50010.	N	N	BAY	

Annex point(s) (91/414/EEC);	year. author(s). title. source. report number registration number.	GLP GEP Y/N	publ. Y/N	owner	data prot
EG:AIIA-2.3; EG:AIIA-2.9	1984. Wilmes, R. Properties of pesticides in water. Active ingredient: Azinphos- methyl (Guthion) (revision of 10.07.1984). Generated by: Bayer AG, submitted by: Bayer AG. 7.2.1.1 /04 ! Bayer M1039. WAS95-50027.	N	N	BAY	
EG:AIIA-2.5	1986. Krohn, J. Azinphos-methyl - spectra of the active ingredient. PC 141. CHE96-00081.	N	N	BAY	
EG:AIIA-2.6	1987. Krohn, J. Water solubility of azinphos-methyl (Guthion M, Gusathion M) at 20° C. PC 146. CHE96-00082.	N	N	BAY	
EG:AIIA-2.7	1995. Krohn, J. Solubility of azinphos-methyl in representative organic solvents. PC 710. CHE96-00083.	Y	N	BAY	
EG:AIIA-2.8	1983. Bentz, F. GUTHION/partition coefficient in n-octanol/water. PC 151. CHE96-00084.	N	N	BAY	
EG:AIIA-2.9	1994. Hellpointner, E. Determination of the quantum yield and assessment of the environmental half-life of the direct photodegradation of azinphos-methyl in water. Generated by: Bayer AG, submitted by: Bayer AG. 7.2.1.2 /03 ! Bayer PF3990. WAS95-50030.	Y	N	BAY	

Annex point(s) (91/414/EEC);	year. author(s). title. source. report number registration number.	GLP GEP Y/N	publ. Y/N	owner	data prot
EG:AIIA-2.9	1974. Heuer, B., Yaron, B. and Birk, Y. Guthion half-life in aqueous solutions and on glass surfaces. Bulletin of Environmental Contamination and Toxicology, 11, 6, 1974, 532-537. 7.2.1.1 /02 ! Bayer IM145. WAS95-50025.	N	Y		
EG:AIIA-2.9	1987. Morgan, J.G. The aqueous photolysis of Guthion-phenyl-UL-14C. Generated by: Mobay Corporation, submitted by: Bayer AG. 7.2.1.2 /02 ! Bayer MR94709. WAS95-50029.	N	N	BAY	
EG:AIIA-2.9	1991. Stupp, H.-P. Dissociation constant of E 1582. BAYER FILE NO.: PC 144. WAS95-50023.	N	N	BAY	
EG:AIIA-2.9	1979. Wilkes, L.C., Wargo, J.P. and Gronberg, R.R. Dissipation of Guthion in buffered aqueous solution. Generated by: Mobay Corporation, submitted by: Bayer AG. 7.2.1.1 /03 ! Bayer MR67983. WAS95-50026.	N	N	BAY	
EG:AIIA-2.9	1981. Wilkes, L.C., Wargo, J.P. and Gronberg, R.R. Photodegradation of Guthion in aqueous solution (revision of 13.08.1981). Generated by: Mobay Corporation, submitted by: Bayer AG. 7.2.1.2 /01 ! Bayer MR67980. WAS95-50028.	N	N	BAY	
EG:AIIA-2.10	1994. Hellpointner, E. Calculation of the chemical lifetime of	N	Y		

Annex point(s) (91/414/EEC);	year. author(s). title. source. report number registration number.	GLP GEP Y/N	publ. Y/N	owner	data prot
	azinphos-methyl in the troposphere. Generated by: Bayer AG, submitted by: Bayer AG. 7.2.2.3 /01 ! Bayer PF3989. LUP95-50011.				
EG:AIIA-2.11; EG:AIIA-2.13	1995. Mix, K.H. Determination of safety-relevant parameters of E 1582 (Gusathion M). PC 667. CHE96-00085.	Y	N	BAY	
EG:AIIA-2.14	1995. Krohn, J. Surface tension of azinphos-methyl. PC 706. CHE96-00086.	Y	N	BAY	
EG:AIIIA-2	1994. Albanese, P. PHYSICAL, CHEMICAL AND TECHNICAL CHARACTERIZATION OF GUSATHION M WP 25. 021/94. CHE96-00105.	N	N	BAY	
EG:AIIIA-2	1994. Albanese, P. PHYSICAL, CHEMICAL AND TECHNICAL CHARACTERIZATION OF GUSATHION M WP 35. 017/94. CHE96-00106.	N	N	BAY	
EG:AIIIA-2	1995. Schröder, P. Storage Stability Report (Gusathion M EC 19.5). CHE96-00120.	N	N	BAY	
EG:AIIIA-2	1994. Wolf, H. Storage Stability Report (Azinphos-Methyl WP 25). CHE96-00104.	N	N	BAY	
EG:AIIIA-2	1994. Wolf, H. Sorage Stability Report	N	N	BAY	

Annex point(s) (91/414/EEC);	year. author(s). title. source. report number registration number.	GLP GEP Y/N	publ. Y/N	owner	data prot
	(Azinphos-Methyl WP 35). CHE96-00107.				
EG:AIIIA-2.1	1995. Schröder, P. Appearance (Gusathion M EC 19.5). CHE96-00108.	N	N	BAY	
EG:AIIIA-2.3	1995. Schröder, P. Flash point (Gusathion M EC 19.5). CHE96-00109.	N	N	BAY	
EG:AIIIA-2.4	1995. Schröder, P. Acidity/Alkalinity (Gusathion M EC 19.5). CHE96-00110.	N	N	BAY	
EG:AIIIA-2.4	1995. Schröder, P. pH (Gusathion M EC 19.5). CHE96-00111.	N	N	BAY	
EG:AIIIA-2.5	1995. Schröder, P. Surface tension (Gusathion M EC 19.5). CHE96-00112.	N	N	BAY	
EG:AIIIA-2.5	1995. Schröder, P. Kinematic Viscosity (Gusathion M EC 19.5). CHE96-00113.	N	N	BAY	
EG:AIIIA-2.6	1995. Schröder, P. Relative density of liquid preparations (Gusathion M EC 19.5). CHE96-00114.	N	N	BAY	
EG:AIIIA-2.7	1995. Schröder, P. Effect of high temperatures (Gusathion M EC 19.5). CHE96-00115.	N	N	BAY	
EG:AIIIA-2.7	1995.	N	N	BAY	

Annex point(s) (91/414/EEC);	year. author(s). title. source. report number registration number.	GLP GEP Y/N	publ. Y/N	owner	data prot
	Schröder, P. Effect of low temperatures (Gusathion M EC 19.5). CHE96-00116.				
EG:AIIIA-2.7	1995. Schröder, P. Shelf life at ambient temperatures (Gusathion M EC 19.5). CHE96-00119.	N	N	BAY	
EG:AIIIA-2.8	1995. Schröder, P. Persistent foaming (Gusathion M EC 19.5). CHE96-00117.	N	N	BAY	
EG:AIIIA-2.8.7	1995. Schröder, P. Emulsion stability/Re-emulsifiability (Gusathion M EC 19.5). CHE96-00118.	N	N	BAY	

Annex B

Azinphos-methyl

B-3: Further information and efficacy

B.3 Data on application and further information

B.3.1 Data on application relevant to the active substance (Annex IIA 3.1 to 3.6)

B.3.1.1 Function (Annex IIA 3.1)

Insecticide, Acaricide

B.3.1.2 Effects on harmfull organisms (Annex IIA 3.2)

Azinphos-methyl is an organophosphorus insecticide. The mode of action of this group of insecticides is well known (Kerkut & Gilbert, 1985).
In brief, nerve impulses transmitted to the next nerve fibre (or to a muscle) by acetylcholine is then immediately broken down by the enzyme acetylcholinesterase. The rapid destruction accounts for the brevity and unity of each normal propagated impulse.
Organophosphorus insecticides interfere with this destruction, as they inhibit the enzyme acetylcholinesterase. Thus, the nervous system is blocked by nerves being permanently stimulated.

Azinphos-methyl is acting with neurotoxic effects by way of contact or feeding ("contact and stomach poison"). There is a rapid knock down effect and long lasting efficacy.

B.3.1.3 Field of use (Annex IIA 3.3)

Plant protection products containing azinphos-methyl are authorized for use in agriculture, horticulture (field and protected crops), citrus and viniculture.
The active substance is mostly used in Southern Europe, in a wide range of crops but mostly in horticulture. Horticulture means growing of fruits, ornamentals and vegetables. Application is made by spraying, e.g. with a field crop sprayer, portable sprayer or air-assisted fruit tree sprayer.
The active substance has been in commercial use since 1956.

B.3.1.4 Summary of intended uses (Annex IIA 3.4)

The intended uses are listed in table B.3.1.4-1.

B.3.1.5 Mode of action (Annex IIA 3.5)

Azinphos-methyl is an organophosphorus insecticide. In principle, organophosphorus insecticides work by interfering with the transmission of nervous impulses, thereby disrupting the pest's nervous system, resulting in death.

B.3.1.6 Information on the occurrence or possible occurrence of the development of resistance and appropriate management strategies (Annex IIA 3.6; Annex IIIA 6.3)

Resistance to organophosphorus insecticides is well known. It exists in varying degrees depending on the pest species, the location and the

individual organophosphorus compound (GIFAP IRAC Newsletter, 1987). The "resistance factor" indicates the concentration of the active substance which is necessary to reach the same level of effectiveness on a resistant strain in comparison to a sensitive strain. For example laboratory tests with azinphos-methyl have shown a "resistance factor" of 25 for Plutella xylostella.

It is clear that resistance to azinphos-methyl can develop. However, azinphos-methyl remains in use, delivering efficacy is acceptable in practice. But no wide spread resistance was observed, except perhaps in some regionally occuring populations of pest species which are well known or easily developing resistance against many chemical based pests.
There are other pest species where no problems due to resistance have been confirmed in spite of commercial use since 1956.

Where resistance may be a problem, it is useful to keep azinphos-methyl available as a weapon for use with other active substances in strategies to combat the problem. For example a combination with imidacloprid, an insecticide with a different mode of action, is under development.

B.3.2 Data on application relevant to the plant protection product (Annex IIIA 3)

B.3.2.1 Field of use envisaged (Annex IIIA 3.1)

All approved uses without berries, vegetables and sugar-beets and without all uses registered in "cold climate zones" (Denmark, Sweden, Finland).

B.3.2.2 Effects on harmful organisms, systemic or not in plants (Annex IIIA 3.2)

Azinphos-methyl is taken up by target organisms by way of contact or feeding ("contact and stomach poison"). The substance is not systemic in plants.

B.3.2.3 Details of intended uses (Annex IIIA 3.3; 3.4; 3.5; 3.6; 3.7; 3.9)

The intended uses are listed in table 3.1.4-1.
This table is including data on the intended application rates, the concentrations of active substances in diluted sprays as well as the methods of application (spraying; diluant water), the number and timing of applications.
For control of most of the insect pests it is necessary to do several applications per season, but in order to fulfil the conditions of modern resistance management strategies changes with plant protection products with other mode of action as far as available for the special uses have to be preferred.

B.3.2.4 Necessary waiting periods or other precautions to avoid phytotoxic effects on succeeding crops (Annex IIIA 3.8)

There are no phytotoxic effects neither on treated nor on succeeding crops.

B.3.2.5 Preliminary range finding tests and field experimentation (Annex IIIA 6.1; 6.2)

Precise and detailed data concerning this topic were not submitted and are not regarded to be necessary by the notifier for the inclusion of the active substance into Annex I, because many plant protection products with azinphos-methyl have been approved and profitably used for many years in a wide range of crops in the Member States of the EU and around the world.
The data submitted by the notifier and that concerning the dosage of plant protection products (referring to the amount of active substance) in various crops reflect the different registration situations in the Member States of the EU.

B.3.2.6 Effects on the quality and where appropriate on the yield of treated plants or effects on the quality of treated plant products (Annex IIIA 6.4) and other effects (Annex IIIA 6.5; 6.6)

There is no detailed information concerning this topic. But there is enough experience from commercial use of azinphos-methyl in many countries with a wide range of pests and in a wide range of crops to conclude that there are no adverse effects, neither on the quality nor on the yield.

This statement applies likewise to
- the effects on the quality and yield of treated plants (Annex IIIA 6.4.1; Annex IIIA 6.4.3),
- the effects on transmormation processes (Annex IIIA 6.4.2),
- phytotoxicity (target plants; Annex IIIA 6.5),
- the impact on succeeding crops (Annex IIIA 6.6.1),
- the impact on other plants, including adjacent crops (Annex IIIA 6.6.2),
- the impact on plants or plant products used for propagation (Annex IIIA 6.6.3).

B.3.3 Summary of data on application

Because it is not regarded as necessary by the notifier to advocate the inclusion of an "existing" active substance into Annex I with detailed results and information concering efficacy and possible phytotoxicity of the formulated products already authorized and in fact well known in the EU the submitted information is rather condensed any may be summarized as follows:
Azinphos-methyl is the active substance in many plant protection products with different formulations. To be authorized, all the products had to undergo careful efficacy and phytotoxicity testing in many Member States of the EU.
Authorizations have been granted for use in a wide range of crops with many different pests.
Apart from several pests which have developed resistance to azinphos-methyl it is proven by experience from field testing and

commercial use that the plant protection products with azinphos-methyl
- are sufficiently effective and therefore may contribute to avoid severe crop losses,
- do not cause phytotoxicity (target plants),
- do not exert adverse effects on succeeding crops,
- do not exert adverse effects on other plants including adjacent crops and
- do not exert adverse effects on the quality of plants or plant products used for propagation and on transformation processes.

B.3.4 Further information on the active substance (Annex IIA 3.7 to 3.9)

B.3.4.1 Recommended methods and precautions concerning handling, storage, transport or fire (Annex IIA 3.7)

Handling and storage

To preserve quality, store in a dry place. Keep container tightly closed and in a well-ventilated place. Store sealed, or so that only qualified persons or their staff have access. Only handle under local ventilation hood. Keep away from food, drink and animal feeding stuffs.

Transport

GGVSee/IMDG Code: 6.1	UN No.: 2783 MFAG: 505 EmS: 6.1 04
PG: II	MPO: PP
GGVE/GGVS: Class 6.1	No. 71B RID/ADR: Class 6.1 No. 71B
Warning sign: Hazard no. 060	Substance no. 2783
ADNR: Class 6.1 No. 81A	Cat -- ICAO/IATA-DGR: 6.1 2783 II
Declaration for land shipment:	AZINPHOSMETHYL (FEST)
Declaration for sea shipment:	Organophosphorus pesticides, solid, toxic, n.o.s. AZINPHOS-METHYL (SOLID)

Declaration for shipment by air: --
Other information:
Toxic. Evil smelling. Marine pollutants. Keep separated from foodstuffs. Keep away from cargo susceptible to odour.

Fire

Extinguishing media: Sprayed water jet, foam, extinguishing powder, CO2, sand.
Fight fire in early stages if safe to do so.
Wear respiratory protection.
Well ventilated areas: Full mask with combination filter, e.g. ABEK-P2 (offers no protection from carbon monoxide!).
Enclosed premises: respirator with independent air supply.
Contain firefighting water.

B.3.4.2 Procedures for destruction or decontamination (Annex IIA 3.8)

B.3.4.2.1 Controlled incineration

Stability and reactivity
Thermal decomposition: At 90 °C or higher, highly exothermic reaction from 110 °C.
Hazardous reactions: Specific consideration of halogens is not necessary.
Combustion gases: In the event of fire, the formation of hydrogen cyanide, carbon monoxide, phosphorus pentoxide, sulfur dioxide and nitrogen oxides must be anticipated.

B.3.4.2.2 Others

Disposal considerations (as listed in safety data sheet):
Package product wastes. Close and label the waste receptacles and, likewise, any uncleaned empty containers. Dispose of them at a suitable waste incineration plant in accordance with the official regulations. Where large quantities are concerned, consult the supplier.
Adsorption by activated charcoal has been suggested for purifying liquid waste which has been contaminated with azinphos-methyl.

B.3.4.3 Emergency measures in the case of an accident (Annex IIA 3.9)

Fire fighting water is to be contained and decontaminated in a suitable sewage plant or incinerated.
Adsorption by activated charcoal has been suggested for purifying liquid waste which has been contaminated with azinphos-methyl.

Safety data sheet
Ref.: Anonymous, 1994

B.3.5 Further information on the plant protection product (Annex IIIA 4)

Gusathion M EC 19.5

B.3.5.1 Packaging (type, materials, size, etc.), compatibility on the preparation with proposed packaging materials (Annex IIIA 4.1)

PACKAGING (type, materials, size etc)

1 litre bottle: material: HDPE-COEX with barrier of E/VAL or PA
alternativ: Al bottle
shape/size: round / 88.5 x 234
opening: 42 mm diameter
closure: screw cap with additional tamper evident, e.g. sealing disk

Test results: satisfactory (ADR), UN registration No.

1888810 (10 x 1 l) 1888839 (20 x 1 l)

5 litre container: material: HDPE/PA-COEX with barrier of PA
shape/size: square / 194 x 112 x 362, handle isolated from the content
opening: 51 mm diameter
closure: screw cap with additional tamper evident, e.g. sealing disk

Test results: satisfactory (ADR), UN registration No. 188868 (4 x 5 l)

B.3.5.2 Procedures for cleaning application equipment (Annex IIIA 4.2)

Rinsing with water and detergent

B.3.5.4 Recommended methods and precautions concerning handling, storage, transport or fire (Annex IIIA 4.4)

HANDLING AND STORAGE
To protect the quality of the product, avoid temperatures below 10 °C and above 40 °C. Keep container tightly closed and in a well-ventilated place. Store sealed, or so that only qualified persons or their staff have access. Only handle under local ventilation hood. Keep away from food, drink and animal feeding stuffs.
Take precautions to prevent formation of explosive mixtures:
Keep away from sources of ignition - No smoking.
Follow the explosion protection guidelines of the "Berufsgenossenschaft der Chemischen Industrie" (Employers' Liability Insurance Association for the German Chemical Industry).
Keep container tightly closed and in a well-ventilated place.
Take precautionary measures against static discharges.

TRANSPORT
No data submitted.

FIRE
Extinguishing media: sprayed water jet, foam, extinguishing powder, CO2, sand.
Fight fire in early stages if safe to do so.
Wear respiratory protection.
Well ventilated areas: full face mask with combination filter, e.g. ABEK-P2 (offers no protection from carbon monoxide!)
Enclosed premises: respirator with independent air supply.
Contain firefighting water.
In the event of fire, the formation of hydrogen cyanide, carbon monoxide, phosphorus pentoxide, sulphur dioxide and nitrogen oxides must be anticipated.

B.3.5.5 Emergency measures in case of an accident (Annex IIIA 4.5)

Use the following personal protective equipment:

If product is handled while not enclosed, and if skin contact may occur:
Respiratory protection: full mask with filter ABEK-P3
Follow the recommendations of the respiratory protection leaflet ZH1/134 of the "Berufsgenossenschaft der Chemischen Industrie" (Employers' Liability Insurance Association for the German Chemical Industry).
Hand protection: protective gloves for chemicals
Other protective equipment: In special cases additional measures for personal protection may be necessary, e.g. wearing protective hood, chemical-resistant and possibly antistatic protective gloves or boots, and chemical-resistant suit with or without independent air supply.
Keep the place of work clean. Avoid contact with product. Keep working clothes separate. Remove soiled or soaked clothing immediately. Clean it separately, taking suitable precautions, or destroy if necessary. Wash the hands before breaks. Take a shower or a bath at end of work.
Prevent entry into drains, waters or soil. Avoid sources of ignition. Thake up spilled product with absorbent material (e.g. sawdust, peat, chemical binder). Fill materials taken up into closable container. To clean the floor and all objects contaminated by this material, use damp cloth. Also place used cleaning materials into closable receptacles.

B.3.5.6 Procedures for destruction or decontamination of the plant protection product and its packaging (Annex IIIA 4.6)

Package product wastes. Close and label the waste receptacles and, likewise, any uncleaned empty containers. Dispose of them at a suitable waste incineration plant in accordance with the official regulations. Where large quantities ar concerned, consult the supplier.

Safety data sheet
Ref.: Anonymous, 1994

B.3.5 Further information on the plant protection product (Annex IIIA 4)

Gusathion M WP 25

B.3.5.1 Packaging (type, materials, size, etc.), compatibility on the preparation with proposed packaging materials (Annex IIIA 4.1)

PACKAGING (type, materials, size etc)

The following packaging types are currently used for Gusathion M WP 25:

1 kg prelined carton: carton board, composite film: paper/LDPE/alu./LDPE, sealed, dimensions: 190 x 76 x 280 mm

10 kg LDPE sack in tin plate drum, dimensions, sack: 1100 x 550 mm, dimensions, drum: hight 393 mm, diameter 344 mm, opening 328 mm

B.3.5.2 Procedures for cleaning application equipment (Annex IIIA 4.2)

Rinsing with water and detergent.

B.3.5.4 Recommended methods and precautions concerning handling, storage, transport or fire (Annex IIIA 4.4)

HANDLING AND STORAGE
Information on safe handling:
Suitable container materials: LDPE (low density polyethylene).
Only handle under local ventilation hood. Keep container tightly closed. Make provision for product and fire-fighting water to be retained.
Information on fire and explosion prevention:
Take precautions to prevent formation of explosive mixtures:
Keep away from sources of ignition - No smoking.
Follow the explosion protection guidelines of the "Berufsgenossenschaft der Chemischen Industrie" (Employers' Liability Insurance Association for the German Chemical Industry).
Keep container tightly closed.
Prevent formation of dust deposits and whirling-up of dust.
Information on storage:
See Chapter 15 for storage regulations.
Observe the rules contained in the VCI concept for separate/common storage. For reasons of quality assurance, keep dry at temperatures under 40 °C. Store sealed, or so that only qualified persons or their staff have access. Keep away from food, drink and animal feeding stuffs.

TRANSPORT
Since no data for this topic are submitted by the applicant, underneath there are the data for Gusathion M WP 35 (comparable with WP 25):

GGVSee/IMDG	Code: 6.1	UN No.: 2783	MFAG: 505	EmS: 6.1 04	
PG:	II	MPO: PP			
GGVE/GGVS:	Class 6.1	No. 71B	RID/ADR: Class 6.1	No. 71B	
Warning sign:	Hazard No. 060	Substance no. 2783			
ADNR:	Class 6.1	No. 81A	Cat -- ICAO/IATA-DGR: 6.1 2783 II		

Declaration for land shipment : 35 % Azinphos-methyl (fest)
Declaration for sea shipment : Organophosphorus pesticides, solid, toxic, n.o.s. 35 % Azinphos-methyl (solid)
Declaration for shipment by air: Organophosphorus pesticides, solid, toxic, n.o.s. 35 % Azinphos-methyl (solid)

Other information:
Toxic. Slightly smelling. Marine pollutants. Keep dry. Avoid heat above +50 °C. Keep separated from foodstuffs.

FIRE
Extinguishing media: sprayed water jet, foam, extinguishing powder, CO2, sand.
Fight fire in early stages if safe to do so.
Wear respiratory protection.
Well ventilated areas: full face mask with combination filter, e.g. ABEK-P2 (offers no protection from carbon monoxide!)
Enclosed premises; respirator with independent air supply.
Contain firefighting water.
In the event of fire, the formation of hydrogen cyanide, carbon monoxide, phosphorus pentoxide, sulphur dioxide and nitrogen oxides must be anticipated.

B.3.5.5 Emergency measures in case of an accident (Annex IIIA 4.5)

Use the following personal equipment:
If product is handled while not enclosed, and if skin contact may occur.
Respiratory protection: full mask with filter ABEK-P3
Follow the recommendations of the respiratory protection leaflet ZH1/134 of the "Berufsgenossenschaft der Chemischen Industrie" (Employers' Liability Insurance Association for the German Chemical Industry).
Hand protection: protective gloves for chemicals
Other protective equipment: In special cases additional measures for personal protection may be necessary, e.g. wearing protective hood, chemical-resistant and possibly antistatic protective gloves or boots, and chemical-resistant suit with or without independent air supply.
Do not empty into drains or waters. Take up spilled product with dust-binding material or suitable vaccum cleaner. Avoid formation of dust. Fill materials taken up into closable container. To clean the floor and all objects contaminated by this material, use damp cloth. Also place used cleaning materials into closable receptacles.

B.3.5.6 Procedures for destruction or decontamination of the plant protection product and its packaging (Annex IIIA 4.6)

Package product wastes. Close and label the waste receptacles and, likewise, any uncleaned empty containers. Dispose of them at a suitable waste incineration plant in accordance with the official regulations. Where large quantities are concerned, consult the supplier.
Wast code number:
53103 Old stock and remainders of crop protection and pest control products.
53104 Production waste from crop protection and pest control products.

Safety data sheets (Gusathion M WP 25 and WP 35)
Ref.: Anonymous, 1994

B.3.5 Further information on the plant protection product (Annex IIIA 4)

Cotnion Methyl 20 SC

No data submitted.

B.3.6 References relied on

Annex point(s) (91/414/EEC);	year. author(s). title. source. report number registration number.	GLP GEP Y/N	publ. Y/N	owner	data prot
EG:AIIA-3.5	1996. Kerkut, G.A. and Gilbert, L.I. Comprehensive insect physiology, biochemistry and pharmacology Vol. 12 Insect control. Pergamon Press, 12, 115-129. BIO96-00100.	N	Y		
EG:AIIA-3.6	1996. BIFAP Insecticide / Acaricide Resistance: Survey and Recommendations by Industry. IRAC Newsletter, 1, 1987. BIO96-00101.	N	Y		
EG:AIIA-3.7	1994. Anonymous Safety Data Sheet (Gusathion M techn.). 060848/10. CHE96-00102.	N	N	BAY	
EG:AIIA-3.8.1	1986. Bertoni, G., Liberti, A., Agostinone, C.B., D'Antonio, M., Pettinari, L. and Leoni, V. Identification by gaschromatography mass-spectrometry of the products obtained from thermal decomposition of azinphosmethyl (Guthion). Annali di Chimica 76, 1986, 19-28. CHE96-00079.	N	Y		
EG:AIIA-3.8.2	1990. Farran, A. and de Pablo, J. Study on the adsorption of three organophosphorus pesticides: Diazinon, Azinphos-methyl and Fenthion by activated carbon. Revista de agroquimica y technologia de alimentos, Vol. 30 (1), 1990, 76-82. CHE96-00087.	N	Y		
EG:AIIA-3.9	1990. Farran, A. and de Pablo, J. Study on the adsorption of three organophosphorus pesticides: Diazinon, Azinphos-methyl and Fenthion by	N	Y		

Annex point(s) (91/414/EEC);	year. author(s). title. source. report number registration number.	GLP GEP Y/N	publ. Y/N	owner	data prot
	activated carbon. Revista de agroquimica y technologia de alimentos, Vol. 30 (1), 1990, 76-82. CHE96-00088.				
EG:AIIIA-4.4; EG:AIIIA-4.5; EG:AIIIA-4.6	1994. Anonymous Safety Data Sheet (Gusathion M EC 19,5). 347551/02. CHE96-00099.	N	N	BAY	
EG:AIIIA-4.4; EG:AIIIA-4.5; EG:AIIIA-4.6	1994. Anonymous Safety Data Sheet (Gusathion M WP 25). 806378/01. CHE96-00100.	N	N	BAY	
EG:AIIIA-4.4; EG:AIIIA-4.5; EG:AIIIA-4.6	1994. Anonymous Safety Data Sheet (Gusathion M WP 35). 806386/01. CHE96-00101.	N	N	BAY	

Table B.3.1.4-1 : Details of " intended uses " in the EU

Crop	Pest controlled	Rate: kg. a.s./ha (maximum per application)	Rate: kg a.s./ha (maximum per season)	No. of applications (maximum per season)	Pre-harvest interval in days (range)
apple	bit./suc. Ins. Mites	1,5	7,5	5	14-35
apricot	bit./suc. Ins.	1,05	1,5	2	20
stonefruit	bit./suc. Ins.	1,3	3,9	3	20-21
citrus	bit. Ins., Mites	3,0	6,0	3	14-28
cotton	bit./suc. Ins.	1,0	1,0	1	14-28
grape	bit./suc. Ins.	1,0	3,0	3	14-28
mustard	bit. Ins.	0,196	0,588	3	21
olive	bit. Ins.	1,8	5,4	3	20-60
ornamentals	bit./suc. Ins.	0,4	1,6	4	-
peach	bit./suc. Ins.	1,1183	2,2366	3	15-35
pear	bit./suc. Ins.	1,3419	3,6	5	14-35
plum	bit./suc. Ins.	1,05	2,25	3	20-30
potato	bit./suc. Ins.	0,7	1,0	2	14-28
rape	bit. Ins.	0,294	0,588	3	21
soybean	bit./suc. Ins.	0,4	0,8	2	20
tabacco	bit./suc. Ins.	0,38	1,14	3	21

Annex B

Azinphos-methyl

B-4: Methods of analysis

B.4 Methods of analysis

B.4.1 Analytical methods for formulation analysis (Annex IIA 4.1; Annex IIIA 5.1)

B.4.1.1 Analytical methods for analysis of the active substance as manufactured

For the determination of the active substance as manufactured by Bayer AG, Germany, General Quimica S.A., Spain, and Makhteshim Chemical Works, Israel, analytical methods based on HPLC with reversed or normal phase and UV-detection with external standardization are available.
The method (Werner, T., 1992a) with reversed phase chromatography on a Lichrospher 100 RP-18 column, UV-detection at 210 nm, and acetonitrile/water as mobile phase is applicable for the assay of the active substance and the determination of most impurities in azinphos-methyl technical grade produced by Bayer AG and General Quimica.
The normal phase method (Krüger, K.W., 1988) with a Lichrosorb Si-60 column, UV-detection at 254 nm, and n-heptane/tetrahydrofuran as mobile phase is also applicable for the assay of the active substance as manufactured by Bayer AG and General Quimica.
For the determination of the active substance in technical grade azinphos-methyl as produced by Makhteshim Chemical Works, Israel, a reversed phase method (Massil, S. E. and Fuss, L., 1987) with a Phase Separation ODS-1 column, UV-detection at 254 nm, and methanol/water as mobile phase is applicable
Ref.: Werner, T., 1992 (a)
Krüger, K. W., 1988
Massil, S. E. and Fuss, L., 1987

B.4.1.2 Analytical methods for analysis of the formulations

For the determination of the active substance in EC- and WP-preparations the HPLC-method (Krüger, K. W., 1988) is applicable.

The principle is normal phase chromatography on a Lichrosorb Si-60 column with UV-detection at 254 nm, and n-heptane/tetrahydrofuran as mobile phase and evaluation by external standard.
For the SC-preparation from Makhteshim no method was submitted.
Ref.: Krüger, K. W., 1988

B.4.2 Analytical methods (residue) for food and feed (II 4.2.1; III 5.2.1)

B.4.2.1 Analytical methods (residue) for plants and plant products

Residues of azinphos-methyl in plant material were determined according the multi-residue method DFG S 19 (Specht und Thier, 1987) and supplements to this method (Siebers, 1995).
Azinphos-methyl is extracted from the sample material with acetone/water. Water is added in such an amount that in consideration of the

sample's natural water content the acetone/water ratio is 2/1. Sodium chloride and dichloromethane are added to the extract leading to a separation of the organic and the aqueous phase. The organic phase is evaporated and a cleanup of the residue by gel chromatography on Bio-Beads S-X3 follows. The elution is done with a cyclohexane/ethyl acetate mixture. The fraction containing the residues of azinphos-methyl is cleaned on a silica gel column. Azinphos-methyl is eluted with toluene/acetone 95/5. The active substance is determined by gas chromatography with an electron capture or thermionic detector.

For validation data see table B.4.2 and B.4.3.

Residues of azinphos-methyl in plant material can also be determined according to the multi-residue DFG-method S 8 (Becker, 1985).

The plant sample is extracted with acetone and water is added to the filtered extract before partitioning with dichloromethane. The organic layer is dried over sodium sulfate and concentrated on a rotary evaporator; the remaining solvent is dried and the residue is dissolved in dichloromethane. This solution is cleaned-up on a column packed with silica gel and activated carbon; the residue is eluted with a mixture of dichloromethane, toluene and acetone. The eluate is concentrated and the residue is dissolved in n-hexane. Azinphos-methyl is determined by gas chromatography with an alkali flame ionisation detector.
Recovery experiments are performed with apples, pears and witloof chicory at 0.5 mg/kg. The recoveries were > 70 %. Informations about mean, range, coefficient of variation are not given. The routine limit of determination is 0.2 mg/kg.

B.4.2.2 Analytical methods (residue) for food of animal origin

For determination of azinphos-methyl and azinphos-methyl oxygen analogue residues in bovine tissues and milk Mobay-method 66439 was developed (Wargo et al., 1978). Both compounds are removed from the sample matrix by organic solvent extraction. Milk and tissues except fat are first extracted with acetone and then with dichloromethane. The extracts are combined, partitioned and the dichloromethane layer is evaporated to dryness. The residue then is partitioned in acetonitrile/hexane. Fat samples are first extracted with hexane and then with acetonitrile; the extracts are combined and partitioned. The acetonitrile fraction from each extraction procedure is taken for further clean-up. The organic solvent is evaporated and the residue is partitioned in dichloromethane/water. The organic layer is evaporated and the dry residue is dissolved in benzene; azinphos-methyl and azinphos-methyl oxygen analogue are separated via chromatography on a silica gel column. Azinphos-methyl is determined by gas chromatography with a flame photometric detector. Azinphos-methyl oxygen analogue is determined by HPLC with UV-detection.

For validation data see table B.4.2 and B.4.3.

B.4.3 Analytical methods (residue) for soil, water and air (II 4.2.2-II4.2.4; III 5.2.2-III 5.2.4)

B.4.3.1 Soil (II 4.2.2; III 5.2.2)

According to the method 67084 (Wargo and Gronberg, 1979) azinphos-methyl and azinphos-methyl oxygen analogue are extracted from soil with a methanol/dichloromethane mixture under reflux. After evaporation to dryness the extract is partitioned in hexane/acetonitrile; the acetonitrile phase is evaporated to dryness and the residue is partitioned in aqueous-methanol/dichloromethane. The dichloromethane layer is evaporated to dryness, again dissolved in dichloromethane and azinphos-methyl and azinphos-methyl oxygen analogue are separated on a silica gel column. The fraction containing azinphos-methyl is then partitioned in hexane/acetonitrile and azinphos-methyl is determined by gas chromatography with a flame photometric detector. The fraction containing the azinphos-methyl oxygen analogue is cleaned-up on a Florisil column and determined by reversed- phase HPLC.

In connection with a freezer storage stability study, method 67084 was slightly modified: the clean-up procedure after the silica gel column is no longer performed and both compounds are determined by capillary gas chromatography with a flame photometric detector (Grace, 1990; Wiedmann 1990). The recoveries determined in this study can be regarded as a validation of method 67084.

For validation data see table B.4.2 and B.4.3.

B.4.3.2 Water (including drinking water) (II 4.2.3; III 5.2.3)

Residues of azinphos-methyl in ground and drinking water are analysed according to the method H231 (Burger, 1988). One liter of water is concentrated by solid-phase extraction on RP- 18 material; the residue is eluted with methanol/acetonitrile and then with dichloromethane. The eluate is passed through a silica gel column which is eluted with methanol. This eluate is evaporated to dryness and the residue is dissolved in a mixture of methanol, acetonitrile and n-heptane. Azinphos-methyl is determined by TLC on silica gel plates using an elution gradient; evaluation of the chromatograms is done by reflection densitometry at 190-350 nm.

For validation data see table B.4.2 and B.4.3.

B.4.3.3 Air (II 4.2.4; III 5.2.4)

Bayer-method 00391 (Riegner, 1995) describes the determination of azinphos-methyl in air. According to this method air is sucked through a Tenax-adsorption-tube. The active ingredient is extracted with acetonitril and determined by HPLC with an UV detector (220 nm).
The method is validated according to Nachrichtenbl. Deut. Pflanzenschutzd. 46, 60-61 (1994).

For validation data see table B.4.2 and B.4.3.

B.4.4 Analytical methods (residue) for human and animal body fluids and tissues (II 4.2.5; III 5.2.5)

For determination of azinphos-methyl residues in animal tissues the HPLC-method (Wargo et al., 1978) was submitted (see 3.4.2.2).
For validation data see table B.4.2 and B.4.3.

Additionally a method for estimating the blood cholinesterase activity was submitted (Fleisher et al., 1956). This method is not validated for azinphos-methyl.

B.4.5 Evaluation and assessment

B.4.5.1 Formulation analysis

HPLC-methods with normal phase (Si-60) or reversed phase (RP-18) chromatography with UV-detection at 210 nm or 254 nm and evaluation by the method of external standardization are applicable for the assay of the active substance as manufactured.
For EC- and WP-preparations a normal phase HPLC-method with a Si-60 column, UV-detection at 254 nm, and external standardization is applicable.
No method was submitted for the SC-preparation.
For all methods submitted by Bayer AG no data on specifity, accuracy, interference, linearity, and precision are provided.

B.4.5.2 Residue analysis

For assessment of the analytical methodology of azinphos-methyl residues the following criteria (Blacha-Puller und Siebers, 1993) are used:

- Limits of determination adequate for checking the relevant residue. For azinphos the relevant residue limits are as follows:

  plants and plant products: 0.05 mg/kg or >0.05 mg/kg (MRL)
  food of animal origin:
    meat and eggs : 0.05 mg/kg (proposed MRL)
    milk : 0.02 mg/kg (proposedl MRL)
  soil: 0.05 mg/kg (general upper limit)
  water: 0.1 µg/l (EU drinking water limit)
  air: 250 µg/m³ (Heimann, 1995; NOEL-based calculation)

- Mean recovery rates 70 to 110 %
- Relative standard deviation of the recovery rates <20 %
- No interfering blanks
- Acceptable experimental and apparatus expenditure

According to these criteria for assessment an adequate analytical methodology for azinphos-methyl is available for crops, soil, water,

air, meat and milk. According to the residue definition (see B.6.3 and B.7.9) analytical methods for metabolites are not necessary.

The recoveries of muscle tissues show a coefficient of variation > 20 %.
For eggs no methods were submitted.

Analytical methods for determination of azinphos-methyl in human body fluids and human tissues are considered as not relevant.

B.4.6 References relied on

Annex point(s) (91/414/EEC):	year. author(s). title. source. report number registration number.	GLP GEP Y/N	publ. Y/N	owner	data prot.
EG:AIIA-4.1	1988. Krüger, K.W. Gusathion M, assey - HPLC method, external standard. 2201-0189301-88E. CHE96-00095.	N	N	BAY	
EG:AIIA-4.1	1987. Massil, S.E. and Fuss, L. HPLC analysis of Methyl-cotnion (Azinphos-methyl): calibration curve, precision and accuracy. CHE96-00124.	N	N	BAY	
EG:AIIA-4.1	1992. Werner, T. Gusathion M: Purity and impurities - HPLC, external standard. 2201-0245501-92D. CHE96-00089.	N	N	BAY	
EG:AIIA-4.2.1	1987. Becker, G. Organohalogen, Organophsphorus and Triazine Compounds - S8. Manual of Pesticide Residue Analysis, 1, 1987, 283-295. MET95-00024.	N	Y		
EG:AIIA-4.2.1	1995. Siebers, J. Untersuchungen zur Anwendbarkeit verschiedener Modifikationen der DFG-Multimethode S19 am Beispiel des Wirkstoffs Azinphos-methyl. MET96-00001.	N	N	BBA	
EG:AIIA-4.2.1	1987. Specht, W. and Thier, H.P. Organochlorine, organophosphorus nitrogen-containing and other pesticides - S 19. Manual of Pesticide Residue Analysis, 1, 1987, 383-399. MET95-00029.	N	Y		
EG:AIIA-4.2.1	1978.	N	N	BAY	

Annex point(s) (91/414/EEC);	year. author(s). title. source. report number registration number.	GLP GEP Y/N	publ. Y/N	owner	data prot
	Wargo, J.P.; Pollock, R.J.; Gronberg, R.R. A method for the determination of Guthion and Guthion oxygen analog in bovine tissues and milk utilizing gas chromatography and high pressure chromatography. Method 66439. 1978. MET95-00031.				
EG:AIIA-4.2.2	1990. Grace, T.J. Freezer storage stability of Azinphos-methyl and azinphos-methyl oxygen analogue in soil; MR100165. 1990. MET95-00027.	-	N	BAY	
EG:AIIA-4.2.2	1979. Wargo, J.P.; Gronberg, R.R. An analytical residue method for the determination of Guthion, Guthion oxygen analog, and total Guthion and metabolite residues in soil Methode 67084. 1979. MET95-00030.	N	N	BAY	
EG:AIIA-4.2.3	1988. Burger, K. Multiple method for ultrarace determination: Pesticide active ingrdients in ground and drinking water analyzed by TLC/AMD (automated multiple development). Pflanzenschutz-Nachrichten Bayer, 41, 1988, 175-228. MET95-00025.	N	Y		
EG:AIIA-4.2.4	1995. Heimann, K.G. Azinphos-methyl Begründung der Bestimmungsgrenze in der Luft. 1995. MET95-00032.	N	N	BAY	
EG:AIIA-4.2.4	1995. Riegner, K. Methode zur Bestimmung von	Y	N	BAY	

Annex point(s) (91/414/EEC);	year. author(s). title. source. report number registration number.	GLP GEP Y/N	publ. Y/N	owner	data prot
	Azinphos-methyl in Luft Methode 00391. 1995. MET95-00028.				
EG:AIIA-4.2.5	1956. Fleisher, J., Woodson, G.S., Simet, L. A Visual Method for Estimating Blood Cholinesterase Activity. AMA Arch. Industr. Health, 1, 1956, 510-520. MET95-00026.	N	Y		

Table B.4.2 and B.4.3: Validation data of the residue analytical methods for azinphos-methyl

REFERENCE	TEST MATERIAL	FORTIFICATION LEVEL	RECOVERY (%) MEAN AND/OR (RANGE)	CV OF RECOVERIES (%)	LIMIT OF DETERMINATION
Specht und Thier, 1987 amendment: Siebers, 1995 (GC-ECD or NPD)	apples, tomatoes	0.1 0.05-1 mg/kg	< 90 * 108 (97-117)	no data 6.1 (n=6)	no data 0.05 mg/kg
Wargo et al.,1979 (GC-FPD)	milk, bovine muscle tissue fat kidney liver	0.005-0.02 mg/kg 0.05-0.1 mg/kg 0.05-0.1 mg/kg 0.05-0.1 mg/kg 0.05-0.1 mg/kg	82 (74-95) 82 (53-104) 94 (87-98) 107 (98-112) 87 (81-94)	8.6 (n=6) 27 (n=4) 5.4 (n=4) 6.1 (n=4) 6.6 (n=4)	0.001 mg/kg 0.01 mg/kg 0.01 mg/kg 0.01 mg/kg 0.01 mg/kg
Wargo and Gronberg, 1979 (GC-FPD)	soil (sandy loam)	0.05 mg/kg 0.1 mg/kg	73 91	no data no data	no data
Grace, 1990 (GC-FPD)	soil (Fresno) (Chualar)	0.01-1.0 mg/kg 0.01-0.5 mg/kg	92 (70-120) 97 (90-104)	18 6.8	0.01 mg/kg 0.01 mg/kg
Wiedmann, 1990 (GC-FPD)	soil	0.01-1.0 mg/kg 0.05 mg/kg	86-120 77 (62-92)	no data 14 (n=8)	0.01 mg/kg
Burger, 1988 (HPTLC-UV)	water	0.05-0.4 µg/l	98 (70-112)	14 (n=7)	0.05 µg/l
Riegner, 1995 (HPLC-UV)	air	0.026 0.3 mg/m³	98 (97-101) 99 (97-102)	1.6 1.3	0.023 mg/m³

* data corrected by the Raporteur Member State

Annex B

Azinphos-methyl

B-5: Toxicology and metabolism

B.5 TOXICOLOGY AND METABOLISM

B.5.1 ABSORPTION, DISTRIBUTION, EXCRETION AND METABOLISM (TOXICOKINETICS)

The studies on rats showed a high degree of absorption of the radioactivity (three hours after oral dosing the concentration of radioactivity in the blood had reached its highest value) followed by fast elimination from the body. Thus, >94 % of the orally administered dose had been eliminated after two days. The bile-fistulated animals eliminated 27 % of the applied amount with the bile within one day, of which more than 60 % was eliminated within eight hours. A part of the radioactivity eliminated with the bile was subject to enterohepatic circulation.
After distribution of the radioactivity the highest levels were found in highly perfused organs such as liver and kidney. Sixteen days post-administration extremely low levels were found in all organs.
Benzazimide, a metabolite of azinphos-methyl, had a very similar biokinetic behaviour to the parent molecule. The radioactivity of benzazimide was quickly and completely absorbed in the GIT, followed by fast elimination from the body. About 75 % of the total dose was identified in metabolism studies. None of the individual compounds constituted more than 5 % of the total dose. The major compounds found in urine and feces 72 hours post-administration are methylsulfinylmethylbenzazimide (M10), methylsulfonylmethylbenzazimide (M11), cysteinylmethylbenzazimide sulfoxide (M20) and cysteinylmethylbenzazimide sulfone (M21). The first step in the process of degradation is cleavage of the organophosphorus ester with the benzazimide moiety followed by methylation and oxidation reactions. Reactions catalysed by transferases produce the cysteine conjugates.
The kinetic studies on farm animals are summarized in chapter B.6.2. However, as for the rat the proposed metabolic pathways for the laying hen and the lactating goat are shown in this chapter (Figure B.5.1.-1).

B.5.1 ABSORPTION, DISTRIBUTION, EXCRETION AND METABOLISM (TOXICOKINETICS IN THE RAT)

The following studies were performed in the rat in order to investigate the toxicokinetics and metabolism of azinphos-methyl and benzazimide, a metabolite of azinphos-methyl:

Patzschke, Wagner and Weber, 1976: ^{14}C-Azinphos-methyl (^{14}C-Gusathion M): Biokinetic investigations on rats. Report no.: PF1054 of 11 October 1976; Bayer AG, Institute for Pharmacokinetics, Elberfeld, Germany. Dates of experimental work: not specified.
Guidelines and GLP:
The method employed was the scientific standard at the time the study was performed. However, in general the study is in compliance with the demands of OECD-Guideline 417 (adopted 4 April 1984). When the study was performed, GLP was not compulsory. The study is acceptable.
Material and methods:
Test substance: Azinphos-methyl (ISO common name), batch no., chemical purity and radiochemical purity not specified, specific radioactivity 8.75 mCi/mmol, position of labelling [carbonyl-^{14}C]azinphos-methyl.
Test species: Male Sprague-Dawley rats (source Mus Rattus AG, München, FRG, average weight 170 g), usually 5 rats/group.
Administration: Single doses of 0.1 or 2.0 mg/kg bw (intravenous), 0.1, 2.0 or 6.0 mg/kg bw (oral) in physiological salt solution with 5 % Cremophor EL vehicle. All doses were given in a total volume of 1.7 ml.
Biliary cannulation: Performed on the day prior to administration.

Sampling: Animals were killed and samples were taken between 6 hours and 16 days after administration. Blood and organs were taken for determination of radioactivity.
Radioassay: Liquids by liquid scintillation counting (LSC) and solids by combustion + LSC, and autoradiography.

Rao, 1988: Disposition and metabolism of azinphos-methyl in rats. Report no.: MR98327 of 30 September 1988; Mobay Corp., Research and Development Dept., Stilwell, Kansas, USA. Dates of experimental work: November 1987 to August 1988.
Guidelines and GLP:
The test method employed was EPA Ref: 85-1. In general the study is in compliance with the demands of OECD-Guideline 417 (adopted 4 April 1984). The tests followed the OECD principles of GLP (declaration of test facility). The study is acceptable.
Material and methods:
Test substance: Azinphos-methyl, batch no. and chemical purity of parent compound not specified; radiochemical substance: batch no. # C-108, radiochemical purity >99 %, specific radioactivity 22.32 mCi/mmol, position of labelling [phenyl-UL-^{14}C]azinphos-methyl.
Test species: Male and female Sprague-Dawley rats (source: SASCO Inc. Omaha, NE, body weight about 200 g for both sexes), 5 animals/sex/group (single dose experiments) and 7 animals/sex/group (multiple dose experiments).
Administration: 0.125 and 2.5 mg/kg bw (single oral dose) and 0.125 mg/kg bw (14 days unlabeled and at day 15 labeled substance, termed as "chronic dosing") in Cremophor EL diluted with water, volume administered approx. 0.5 ml. An additional *in vitro* metabolism-experiment was conducted with 32 µg/ml reaction mixture (hepatic subcellular fraction, buffer and required cofactors).
Sampling: Urine and feces were collected 8, 24, 48 and 72 hours after treatment. The animals were killed at 72 hours and samples of blood, bone, brain, fat, gonad, heart, kidney, liver, lung, muscle, spleen, gastrointestinal tract (GIT) and the remaining carcass were taken for analysis.
Radioassay: Liquids by liquid scintillation counting (LSC), radioactivity monitor coupled to HPLC, solids by combustion and LSC, and thin layer chromatography (TLC)-plates by radiochromatogram scanner.

Weber, Patschke and Wegner, 1980: [Phenyl-UL-^{14}C] Benzazimide: Biokinetic study on rats. Report no.: PH9005 of 10 March 1980; Bayer AG, Institute for Pharmacokinetics, Elberfeld, Germany. Dates of experimental work: not specified.
Guidelines and GLP:
The method employed was the scientific standard at the time the study was performed. However, in general the study is in compliance with the demands of OECD-Guideline 417 (adopted 4 April 1984). When the study was performed, GLP was not compulsory. The study is acceptable.
Material and methods:
Test substance: Benzazimide (ISO common name), batch no. and chemical purity of parent compound not specified; radiochemical substance: batch no. C-235, purity approx. 99 %, specific radioactivity 20 mCi/mmol, position of labelling [phenyl-UL-^{14}C]benzazimide.
Test species: Male and female Sprague-Dawley rats (source Mus Rattus AG, München, FRG, average weight 170-210 g), usually 5 males per examination time and dose, for the 1 mg/kg bw oral group also 5 females, 1 or 2 males for autoradiography.
Administration: Single doses of 0.05, 1.0 and 5.0 mg/kg bw (oral), 1 mg/kg bw (intravenous) and 1.0 mg/kg bw (intraduodenal) in physiological saline/Cremophor EL vehicle at dose volume of 10 ml/kg (oral and iv) or 1 ml/kg (intraduodenal).
Biliary cannulation: Performed on the day prior to administration.
Sampling: Animals were killed and samples of blood and organs taken between 5 minutes and 10 days after dosing to establish kinetic data.

Radioassay: Liquids by liquid scintillation counting (LSC), windowless proportional counting tubes, solids by oxidation and LSC, end window proportional counting tubes, autoradiography.

B.5.1.1 ABSORPTION, DISTRIBUTION AND EXCRETION

Absorption
Following oral administration the radioactivity was absorbed almost completely (90-100 %) within three hours by male rats **(Patzschke et al., 1976)**. The amounts of radioactivity determined in the whole animal excluding GIT (gastrointestinal tract) were independent of the route of administration and of the applied dose. Six hours post-application about 60 % of the applied radioactivity was distributed within the animal. At this time already 25 % had been renally eliminated, the remainder was contained in the GIT. The results of the study on rats with bile fistulae confirmed the assumption of almost complete absorption in the GIT. Within 24 hours 27 % of the radioactivity of the intravenous dose was recovered in the bile and only 6 % in feces. It was assumed that in non-fistulated animals the bile was partly re-absorbed in the GIT and eliminated with the urine (enterohepatic circulation).

Excretion
The radioactivity was excreted from the body at a high rate. Two days after oral administration <3 to 5 % of the recovered radioactivity was still present in the animals excluding the GIT. During the first 24 hours <0.1 % was exhaled in the breath, whereas half of the renally eliminated radioactivity was excreted within 8 to 10 hours. After 16 days the radioactivity recovered within the animal was <1 %. There was no dependence of the excretion of radioactivity from the route or dose of administration **(Patzschke et al., 1976)**. Also between sexes and the dosing regime (single or chronic) no differences in the excretion rates were observed. Within 24 hours >60 % of the administered radioactivity was excreted with urine and feces whereas only approximately 0.2 and 0.1 % was expired as $^{14}CO_2$ by male and female rats **(Kao, 1988)**. A summary of the excretion of total radioactivity and radioactive residues is shown in Table B.5.1.1-1.

Distribution
The distribution of radioactivity into the tissues was very similar after oral and intravenous administration (Table B.5.1.1-2). After oral administration of 6.0 mg/kg bw the highest concentration of radioactivity in the blood was found three hours post-application. Six hours post-application of 2 mg/kg bw the highest concentrations of radioactivity were found in the highly perfused excretion organs, i.e. kidneys and liver, as well as in the adrenal gland (corresponding to concentrations of 2-3 µg azinphos-methyl equivalents per gram tissue). The concentrations in the other organs were in the range of 1.2-1.4 µg/g. Only the concentration in the serum was slightly higher (1.7 µg/g). The concentrations declined relatively quickly until day 2 post-application and then the radioactivity was more slowly eliminated from the organs (half-life initially 10 hours, later about 10 days). On day 16 post-application, the radioactivity in the whole animal (without GIT) amounted to about 0.05 µg/g. The highest concentration was found in the blood (erythrocytes) whereas in skeletal muscles and fat, values were below the detection limit. The results of distribution of radioactivity within the body were also confirmed by whole body autoradiography **(Patzschke et al., 1976)**. **Kao (1988)** determined the distribution of radioactivity in the rat three days post-application. The concentration levels were higher in highly perfused tissues or organs (Table B.5.1.1-3).

B.5.1.2 METABOLISM

The metabolism of [phenyl-UL-^{14}C]azinphos-methyl in the rat was investigated in the study of **Kao (1988)**. Table B.5.1.2-1 gives the distribution of metabolites in the urine and feces (composite samples of the urine collected at 8, 24, 48 and 72 hours and of the feces collected at 8, 24 and 48 hours post-application). Twelve metabolites were found in the urine by HPLC analysis; among them, eight compounds were identified. None of the unknown metabolites constituted more than 5 % of the total dose. Sulfate or glucuronyl conjugates were not detected. Desmethyl isoazinphos-methyl (M04) was the only compound still having intact the organophosphorus ester with the benzazimide moiety (0-6 % of the administered dose). All other identified compounds were metabolites produced after hydrolysis of this ester linkage. Methylsulfinylmethylbenzazimide (M10), methylsulfonylmethylbenzazimide (M11), cysteinylmethylbenzazimide sulfoxide (M20) and cysteinylmethylbenzazimide sulfone (M21) were the major compounds. Benzazimide (M08) as well as cysteinyl- and glutathionyl methylbenzazimide (M17, M22) were also identified.
The extractability of the radioactivity from feces ranged from 16 to 25 % of the total dose. Thereof the major part was organosoluble and corresponded to 12 to 18 % of the radioactivity administered. The water soluble portion amounted to 1 to 2 % of the total radioactivity. In the feces of the high dose experiment desmethyl isoazinphos-methyl (M04) and azinphos-methyl oxygen analogue (M01) as well as methylsulfonylmethylbenzazimide (M11), cysteinylmethylbenzazimide sulfoxide (M20) and methylthiomethylbenzazimide (M09) were identified. None of the unknown metabolites exceeded 5 % of the applied radioactivity. No unchanged azinphos-methyl was detected in urine or feces.
In *in vitro* experiments with hepatic subcellular fractions it was demonstrated that glutathion transferases metabolised azinphos-methyl to desmethyl isoazinphos-methyl (M04) and glutathionyl methylbenzazimide (M22). Mixed function oxidases catalysed the oxidation to azinphos-methyl oxygen analogue (M01), its hydrolysis and further metabolisation to benzazimide (M08).
A proposed metabolic pathway in rats is shown in Figure B.5.1-1. The first step in the process of degradation of azinphos-methyl (P) is the cleavage of the organophosphorus ester bond probably yielding mercaptomethylbenzazimide (M05). This compound is rather unstable and undergoes methylation to methylthiomethyl-benzazimide (M09). Oxidation of the sulfur atom yields methylsulfinylmethylbenzazimide (M10) and methylsulfonylmethylbenzazimide (M11). In a transferase reaction the parent compound is transformed to glutathionyl methylbenzazimide (M22). Via a transferase, glutathion is changed with cysteine yielding cysteinylmethylbenzazimide (M17) that is oxidised to cysteinylmethylbenzazimide sulfoxide (M20) and cysteinylmethyl-benzazimide sulfone (M21). Two minor pathways transform azinphos-methyl leaving the organophosphorus ester with the benzazimide moiety intact. In an oxidase reaction azinphos-methyl oxygen analogue (M01) and in a transferase reaction desmethyl isoazinphos-methyl (M04) are produced. However, the major compounds found 72 hours post-application are methylsulfinylmethylbenzazimide (M10), methylsulfonylmethylbenzazimide (M11), cysteinylmethylbenzazimide sulfoxide (M20) and cysteinylmethylbenzazimide sulfone (M21).

B.5.1.3 BIOKINETIC BEHAVIOUR OF BENZAZIMIDE, A METABOLITE OF AZINPHOS-METHYL

In an additional study, the biokinetic behaviour of a metabolite of azinphos-methyl, [phenyl-UL-^{14}C]benzazimide, was investigated in rats **(Weber et al., 1980)**.

Absorption
Following oral administration of [phenyl-UL-^{14}C]benzazimide the radioactivity was absorbed almost completely (>95 %). Two hours after administration, the

maximum absorption is obtained and in the plasma the highest concentration of radioactivity was determined. Three hours post-application about 56 % of the applied radioactivity was distributed within the animal (excluding the GIT). At this time, 18 % had been renally eliminated, the remainder was contained in the GIT. The results of the study on rats with bile fistulae confirmed the assumption of almost complete absorption in the GIT. Within 24 hours after intravenous administration 42 % of the applied radioactivity was recovered in the bile and only 1.6 % in feces.

Excretion

The radioactivity was excreted from the body at a high rate. Two days after oral administration only 0.67 to 0.84 % of the administered radioactivity was still present in the animals excluding the GIT. During the first 24 hours <0.04 % was expired as $^{14}CO_2$, whereas half of the renally eliminated radioactivity was excreted within four hours and 90 % within 12 hours. After 10 days, the radioactivity still present in the animal amounted to approximately 0.17 %. There was no dependence of the excretion of radioactivity from the route or dose of administration. Also between sexes no differences in the excretion rates were observed (Table B.5.1.3-1). Within 48 hours, 54-66 % was excreted in the urine and 33-45 % in the feces; >99 % of the administered radioactivity was eliminated from the body within 48 hours.

Distribution

After oral administration of 1 mg/kg bw, the highest concentration of radioactivity in the blood was found two hours post-application. Three hours post-application, when the first rats were sacrificed, the highest concentrations of radioactivity were found in tissues and organs (Table B.5.1.3-2). Significantly higher concentrations were determined in the highly perfused excretion organs kidneys and liver. The concentrations declined relatively quickly until day 1 post-application and then the radioactivity was more slowly eliminated from the organs. The half-life initially was 2-3 hours (determined in the plasma during the first 24 hours), between days 2 and 10 the half-life was about four days. On day 10 post-application, the equivalent concentration of radioactivity (based on the unchanged parent compound) in the whole animal (without GIT) amounted to about 0.0019 µg/g fresh tissue. The highest concentration was found in the blood (i.e. erythrocytes). The results of distribution of radioactivity within the body were also confirmed by whole body autoradiography.

Table B. 5.1.1-1: Excretion of total radioactivity and radioactive residues in the rat after application of [carbonyl-14C]azinphos-methyl (Patzschke et al., 1976) or [phenyl-UL-14C]azinphos-methyl (Kao, 1988) (values are given in percent of administered radioactivity)

Report	Administration	Dose	Sex	Time (h)	CO_2	Bile	Urine	Feces	Total excreted	Body without GIT	GIT	Recovery
Patzschke	oral	0.1	male	48	-	-	62	34	96	< 3	< 0.10	
et al.,	oral	2.0	male	48	<0.1 2)	-	68	26	94	5	1.0	97
1976	intravenous	0.1	male	48	-	-	63	29	92	n.d. 1)	n.d. 1)	to
	intravenous	2.0	male	48	<0.1 2)	-	68	26	94	4	0.8	100
	intravenous	2.0	male	24	-	27	54	6	87	11	2.0	
Kao, 1988	oral	0.125	male	72	~0.2 2)	-	70.5	25.4	97.0 *		3.0	106.3
	oral	0.125	female	72	~0.1 2)	-	73.2	23.3	96.7 *		3.3	107.7
	oral	2.5	male	72	-	-	69.8	24.7	95.7 *		4.3	103.9
	oral	2.5	female	72	-	-	71.7	23.1	96.1 *		3.9	100.2
	(chronic) oral	0.125	male	72	-	-	67.8	26.0	94.7 *		5.3	92.2
	(chronic) oral	0.125	female	72	-	-	72.9	21.3	95.0 *		5.0	95.6

GIT gastrointestinal tract
* includes radioactivity recovered in the cage wash
1) not determined 2) value after 24 hours

Table B.5.1.1-2: Relative concentration of radioactivity (P) in individual parts of the body of rats after application of [carbonyl-^{14}C]azinphos-methyl (2.0 mg/kg bw) (all values are multiplied by a factor of 100) P = (measured radioactivity / g tissue or plasma):(administered radioactivity / g body weight)

Report	Administration	Time (h)	Body without GIT	Blood	Erythrocytes	Serum	Liver	Kidney	Adrenal Gland	Brain	Skeletal Muscle	Skin	Fat
Patzschke et al., 1976	Oral	6	62	66	66	86	97	110	110	63	61	65	63
		24	23	29	31	32	32	40	31	23	19	22	21
		48	6	15	17	12	10	14	11	7	4	7	5
		96	2	10	16	4	5	5	5	3	2	3	< 5
		192	< 2	8	< 20	2	2	3	3	3	< 2	< 2	< 5
		384 [1]	1	5	11	< 1	1	2	2	2	< 1	< 1	< 2
	Intravenous	6	73	71	66	85	100	130	130	63	63	62	61
		24	16	29	27	27	27	35	29	19	16	19	19
		48	5	13	17	10	9	12	8	6	3	6	5
		96	2	10	14	4	5	5	5	3	1	3	< 5
		192	< 2	7	< 20	2	2	3	2	3	<2	< 2	< 5

GIT gastrointestinal tract
1) values from experiment with 6 mg/kg bw administered

Table B.5.1.1-3: Concentration of radioactivity in individual parts of the body of rats 72 hours after oral application of [phenyl-UL-^{14}C]azinphos-methyl (values are given as µg azinphos-methyl equivalents per gram tissue)

Report	Dose (mg/kg bw)	Sex	Blood	Bone	Brain	Fat	Gonad	Heart	Kidney	Liver	Lung	Muscle	Spleen	GIT
Kao LRM, 1988	0.125	male	0.013	<0.004	0.004	<0.004	<0.004	0.004	0.008	0.005	0.008	<0.004	<0.004	<0.004
	0.125	female	0.013	<0.004	0.005	<0.004	<0.004	0.006	0.013	0.007	0.010	<0.004	0.005	<0.004
	chronic 0.125	male	0.016	<0.004	0.006	<0.004	0.004	0.006	0.013	0.006	0.011	<0.004	0.004	<0.004
	chronic 0.125	female	0.020	<0.004	0.007	<0.004	0.004	0.006	0.018	0.007	0.012	<0.004	0.007	<0.004
	2.5	male	0.266	0.042	0.126	0.050	0.090	0.101	0.206	0.121	0.159	0.070	0.084	0.060
	2.5	female	0.319	0.038	0.118	0.026	0.043	0.088	0.257	0.117	0.172	0.061	0.088	0.047

Table B.5.1.2-1: Distribution of metabolites in the excreta of rats 48 hours after oral administration of [phenyl-UL-^{14}C]azinphos-methyl (values are given in percent of the applied radioactivity).
For structures of the metabolites see figure B.5.1-1

Report	Dose (mg/kg bw)	Sex	Excretion	M01	M04	M08	M09	M10	M11	M17	M20	M21	M22	Unknown
Kao,	0.125	male	urine	-	2	2	-	3	20	2	0	30	0	7
1988		female	urine	-	0	2	-	8	14	2	1	20	14	8
	chronic	male	urine	-	5	0	-	2	19	0	1	30	7	2
	0.125	female	urine	-	6	4	-	4	20	1	9	20	4	3
	2.5	male	urine	-	3	3	-	9	18	2	6	16	3	9
			feces	3	4	-	1	-	2	-	2	-	-	7
			Σ	**3**	**7**	**3**	**1**	**9**	**20**	**2**	**8**	**16**	**3**	**16**
		female	urine	-	6	4	-	13	15	<1	12	13	4	3
			feces	2	<1	-	3	-	3	-	<1	-	-	5
			Σ	**2**	**6**	**4**	**3**	**13**	**18**	**<1**	**12**	**13**	**4**	**8**

\- not found
M01 azinphos-methyl oxygen analogue
M04 desmethyl isoazinphos-methyl
M08 benzazimide
M09 methylthiomethylbenzazimide
M10 methylsulfinylmethylbenzazimide
M11 methylsulfonylmethylbenzazimide
M17 cysteinylmethylbenzazimide
M20 cysteinylmethylbenzazimide sulfoxide
M21 cysteinylmethylbenzazimide sulfone
M22 glutathionyl methylbenzazimide

Table B.5.1.3-1: Excretion of total radioactivity and radioactive residues in the rat after application of [phenyl-UL-^{14}C]benzazimide within 48 hours (values are given in percent of administered radioactivity).

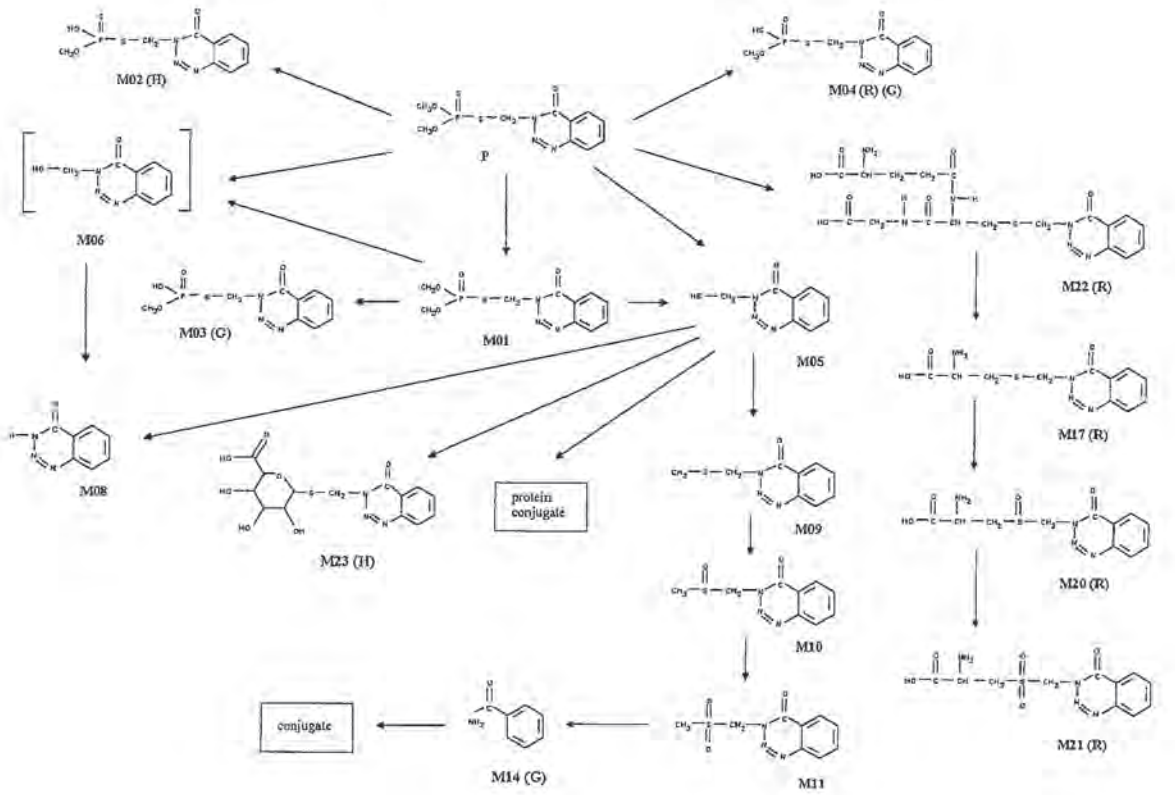
Report	Administration	Dose (mg/kg bw)	Sex	CO_2	Bile	Urine	Feces	Total Elimination	Body without GIT	GIT	Recovery (% of applied)
Weber et al.,	oral	0.05	male	-	-	54.3	45.0	99.3	0.67	0.084	105
1980	oral	1.0	male	<0.04 1)	-	57.0	42.3	99.3	0.67	0.084	106
	oral	1.0	female	-	-	66.4	32.7	99.1	0.84	0.100	100
	oral	5.0	male	-	-	58.4	40.8	99.2	0.68	0.068	109
	intravenous	1.0	male	-	-	58.4	40.9	99.3	0.61	0.095	106
	intravenous	1.0	male	-	42.3	55.0	1.6	98.9	0.98	0.16	112
	intraduodenal	1.0	male	-	38.2	57.8	2.8	98.8	1.00	0.14	101

GIT gastrointestinal tract
1) value after 24 hours

Table B.5.1.3-2: Relative concentration of radioactivity (P) in individual parts of the body of rats after single oral application of [phenyl-UL-^{14}C]benzazimide (1 mg/kg bw) (all values are multiplied by a factor of 100) P = (measured radioactivity / g tissue or plasma): (administered radioactivity / g body weight)

Report	Time (hours)	Body without GIT	Blood	Erythro -cytes	Plasma	Liver	Kidney	Adrenal Gland	Brain	Muscle	Skin	Testes	Suet (peri-renal fat)
Weber et al.,	3	63	99	69	128	110	170	72	50	50	59	49	49
1980	6	35	45	40	49	71	110	40	27	27	34	30	27
	24	1.4	5.9	11	0.88	12	6.3	1.1	0.44	0.26	0.51	0.32	<1.6
	48	0.73	6.2	12	0.30	8.4	4.1	0.74	0.25	0.17	0.23	0.13	<1.6
	72	0.62	5.6	11	0.19	6.8	3.0	0.77	0.23	0.15	0.16	0.10	<1.6
	144	0.45	5.0	9.9	0.14	5.0	1.9	0.70	0.23	0.16	0.15	0.080	<1.6
	240	0.19	3.1	6.2	0.037	1.5	0.73	<0.6	0.11	0.099	0.078	0.039	<1.6

Figure B.5.1-1 Proposed metabolic pathways for azinphos-methyl in the laying hen (H), lactating goat (G) and rat (R)



Proposed metabolic pathways for azinphos-methyl (continuation from figure B.5.1.-1)
The following metabolites were identified (the letter indicates where a metabolite was found only in hen (H), goat (G) or rat (R):

P	azinphos-methyl
M01	azinphos-methyl oxygen analogue
M02	desmethyl azinphos-methyl
M03	desmethyl azinphos-methyl oxygen analogue
M04	desmethyl isoazinphos-methyl
M05	mercaptomethylbenzazimide
M06	hydroxymethylbenzazimide
M08	benzazimide
M09	methylthiomethylbenzazimide
M10	methylsulfinylmethylbenzazimide
M11	methylsulfonylmethylbenzazimide
M14	benzamide
M17	cysteinylmethylbenzazimide
M20	cysteinylmethylbenzazimide sulfoxide
M21	cysteinylmethylbenzazimide sulfone
M22	glutathionylmethylbenzazimide
M23	conjugate of mercaptomethylbenzazimide with glucuronic acid

B.5.2 ACUTE TOXICITY, INCLUDING IRRITANCY AND SKIN SENSITIZATION

Azinphos-methyl is very toxic after single oral administration in both aqueous and non-aqueous vehicles (oral LD50 in rats: 4.4-26 mg/kg bw) and after intraperitoneal injection (ip LD50 in rats: 5.7-11.6 mg/kg bw). The greatest inhibition of plasma and erythrocyte ChE activity following a single oral dose was measured 5 to 24 hours after treatment while the peak brain ChE inhibition was already determined two hours after the treatment. Whereas the mouse (oral LD50: 11-20 mg/kg bw) is as susceptible as the rat, the guinea pig (oral LD50: 80 mg/kg bw) is less susceptible to the acute oral toxicity of azinphos-methyl.
Azinphos-methyl applied dermally is moderately toxic in both aqueous and non-aqueous vehicles (dermal LD_{50} in the rat 72.5-250 mg/kg bw) although in one study using a 25 % Cremophor emulsion as vehicle, the LD50 was determined as 2500-5000 mg/kg bw. It is very toxic by inhalation (4h LC50 in the rat: 0.132-0.155 mg/l air) when administered as an aerosol diluted with PEG400/ethanol.
Clinical symptoms of acute azinphos-methyl intoxication in laboratory rodents are qualitatively similar for the oral, dermal, inhalation and intraperitoneal routes of administration. Generally, they are typical of organophosphate poisoning, predominantly comprising palmospasm, ataxia, clonic cramps, prostration, salivation and dyspnea, followed by apathy and piloerection. Clinical symptoms are generally evident shortly after administration (within 5 to 20 minutes of treatment in the lethal dose range), except in the case of dermal exposure where symptoms appear on the day after dosing. Some symptoms of intoxication persist for up to 11 days after exposure.
The substance is not an irritant to either intact or abraded skin of the rabbit, but it elicits mild conjunctival irritation which is reversible within 48 hours. It is not, however, classified as a primary eye irritant.
Using the guinea pig maximization test of Magnusson and Kligman, evidence was obtained of skin-sensitizing properties of technical azinphos-methyl. Two additional Bühler patch tests on guinea pigs using induction concentrations of 25 or 12.5 % and challenge concentrations of 25 and 6.0 % (respectively) elicited sensitization in both studies. A subsequent challenge at a concentration of 0.6 % in the second study did not elicit a sensitization response.
With regard to the high acute oral/dermal toxicity in both aqueous and non-aqueous media, the high (4 hour) toxicity by inhalation and sensitizing potential the following classification and labelling of azinphos-methyl, according to Directive 67/548/EEC, 18th adaptation, Annex IV (General Classification and Labelling Requirements for Dangerous Substances and Preparations), is proposed:

- T+ (very toxic)
- R26 (very toxic by inhalation)
- R28 (very toxic if swallowed)
- R24 (toxic in contact with skin)
- X_i (irritant)
- R43 (may cause sensitization by skin contact)

Table B.5.2-1: Summary of the acute toxicity studies

Study	Vehicle	Species/sex	Result	Reference
Oral			LD_{50} (mg/kg bw)	
*	Ethanol/PG	Rat/f	16.4	DuBois et al., 1957
*	Panasol AN-2	Rat/f	12.2-15	Nelson, 1968
*	Peanut oil	Rat/m, f	13, 11	Gaines, 1969
(Non-fasting)	PG	Rat/m+f	12	Crawford and Doull, 1970
(Fasting)	DMSO	Rat/m, f	5.6, 6.4	Crawford and Anderson, 1974
(Fasting)	CMC	Rat/m, f	19, 16	Lamb and Anderson, 1974
(Non-fasting)	CMC	Rat/m, f	19, 10	Lamb and Anderson, 1974
*	MC/Arabi- gum	Rat/m, f	26, 24	Pasquet et al., 1976
*	Cremophor/water	Rat/m	16.75	Thyssen, 1976
(Fasting)	Cremophor/water	Rat/m	9.7	Thyssen, 1977a; 1977b
(Fasting)	Cremophor/water	Rat/m	5.3	Thyssen, 1977c
(Fasting)	Cremophor/water	Rat/m, f	4.6, 4.4	Mihail, 1978
*	Cremophor/water	Rat/m	25.4	Flucke, 1979
(Fasting)	Cremophor/water	Rat/m	9.1	Heimann, 1981
(Non-fasting)	Cremophor/water	Rat/m	17.25	Heimann, 1981
(Fasting)	Cremophor/water	Rat/m	6.7	Heimann, 1982
(Non-fasting)	Cremophor/water	Rat/m	12.8	Heimann, 1982
(Fasting)	Cremophor/water	Rat/m	7.1	Heimann, 1987b
(Non-fasting)	PG	Mouse/m+f	20	Crawford and Doull, 1970
*	PG	Mouse/f	11	Sterri et al., 1979
*	Ethanol/PG	Guinea pig/m	80	DuBois et al., 1957
(Fasting)	Cremophor/water	Dog/m	>10	Mihail, 1978
Dermal			LD_{50} (mg/kg bw)	
	Panasol AN-2	Rat/f	72.5	Nelson, 1968
	Xylene	Rat/m, f	220, 220	Gaines, 1969
	Acetone/EtOH/oil	Rat/f	90	Pasquet et al., 1976
	Cremophor/water	Rat/m, f	2500-5000	Mihail, 1978
	Cremophor/water	Rat/m, f	200-250, 155	Heimann, 1982
Inhalation			LC_{50} (mg/l air)	
1 hour		Rat/m	0.385	Kimmerle and Lorke, 1968
4 hour		Rat/m	0.152	Kimmerle and Lorke, 1968
4 hour	Ethanol/PEG 400	Rat/m, f	0.155, 0.132	Shiotsuka, 1987a
Intraperitoneal			LD_{50} (mg/kg bw)	
	Ethanol/PG	Rat/m, f	11.6, 5.7	DuBois et al., 1957
	Panasol AN-2	Rat/f	8.5-8.9	Nelson, 1968
	Cremophor/saline	Rat/m, f	6.9, 9-10	Krötlinger, 1993
	Ethanol/PG	Mouse/m, f	5.4, 3.4	DuBois et al., 1957
		Mouse/m	4.2	Kamienski and Murphy, 1971
		Mouse	3-4.5	Benke et al., 1973
	Ethanol/PG	Guinea pig/m	40.0	DuBois et al., 1957
Skin irritation				
(24 h exposure)		Rabbit/m, f	No irritation	Thyssen, 1981
(4 h exposure)	Water	Rabbit/m	No irritation	Zorbas, 1994a
Eye irritation				
(5 min exposure)		Rabbit	Minimal irritation	Thyssen, 1981
(24 h exposure)		Rabbit	Mild irritation	Thyssen, 1981
		Rabbit	Mild irritation	Zorbas, 1994b
Sensitization				
(Maximisation)	Cremophor/saline	Guinea pig	Skin sensitization	Flucke, 1986
(Buehler test)	Ethanol/water	Guinea pig	Skin sensitization	Porter et al., 1987
(Buehler test)	Cremophor/saline	Guinea pig	Skin sensitization	Heimann, 1987a

* Feeding status not specified

B.5.2.1 ORAL STUDIES

B.5.2.1.1 RAT

DuBois, Thursh and Murphy, 1957: Studies on the toxicity and pharmacologic actions of the dimethoxy ester of benzotriazine dithiophosphoric acid (DBD,

Guthion). Department of Pharmacology, University of Chicago, Chicago, Illinois. Dates of experimental work: not specified. Published in: J Pharmacol Exp Ther 119:208-218, 1957.

Guidelines and GLP:
The method employed was the scientific standard at the time the study was performed. When the study was performed, GLP was not compulsory.
Main deviations from current OECD guidelines: Only female rats were used. Body weights, individual data for clinical observations, and necrospy findings were not reported.
The study is considered supplementary.

Material and methods:
Azinphos-methyl (source: Chemagro Corp., New York, purity: >97 %) was emulsified in 20 % ethanol and 80 % propylene glycol and administered once by gavage (volume <0,3 % of bw) to female Sprague-Dawley rats (source: not specified; body weight range: 175-250 g). A total of 40 animals was used, but the number of animals per group and the dose levels were not specified. The mortality data were based on a 10-day observation period and the approximate LD_{50} values were calculated by the log probability method.

Findings:
The clinical symptoms observed were increased respiration, unsteadiness, lack of coordination, scattered muscular twitches; lacrimation, urination, defecation, salivation; tonic and clonic convulsions, prostration, and respiratory failure. The time of onset of clinical symptoms or deaths was not specified. The LD_{50} value was calculated to be 16.4 mg/kg bw.

Conclusions:
Azinphos-methyl is of very high toxicity by the oral route. The LD50 value was 16.4 mg/kg bw for female rats.

B.5.2.1.2 RAT

Nelson, 1968: The acute mammalian toxicity of two samples of Guthion technical to adult female rats. Report no. 22579 of 26 April 1968; Chemagro Corporation, Research and Development Department. Dates of experimental work: not specified.

Guidelines and GLP:
The method employed was the internal standard at the time the study was performed. When the study was performed, GLP was not compulsory.
Main deviations from current OECD guidelines: Only female rats and only 4 animals per dose group were used. Body weights, clinical observations, and necrospy findings were not reported.
The 3-page report contains also an acute intraperitoneal and an acute dermal toxicity study in rats. The study is considered supplementary.

Material and methods:
Guthion technical (samples nos. PF1966 and 31064, source and purity: not specified) as a solution in Panasol AN-2 was administered once by gavage to groups of 4 adult female Sprague-Dawley rats (source and body weight range: not specified) at dose levels of 7.5, 10, 15 and 20 mg/kg bw. Mortality was recorded for 14 days after dosing and the LD_{50} value estimated by an unspecified method.

Findings:
Onset and nature of clinical symptoms and time of death were not reported. Mortality occurred at dose levels of 10 mg/kg bw and above. The LD50 values were calculated to be 12.2 and 15 mg/kg bw for the samples PF1966 and 31064, respectively.

Conclusions:
Azinphos-methyl is of very high toxicity by the oral route. The LD50 values were 12.2 and 15 mg/kg bw for female rats.

B.5.2.1.3 RAT

Gaines, 1969: Acute toxicity of pesticides. Toxicology Laboratory, Pesticides Program, National Communicable Disease Center, U.S. Department of Health, Education, and Welfare, Atlanta, Georgia 30333. Dates of experimental work: not specified. Published in: Toxicol Appl Pharmacol 14:515-534, 1969.

Guidelines and GLP:
The method employed was the internal standard at the time the study was performed. When the study was performed, GLP was not compulsory.
Main deviations from current OECD guidelines: Body weights, clinical observations, and necrospy findings were not reported.
The study is considered supplementary.

Material and methods:
Azinphos-methyl (technical grade, source and purity: not specified) as a solution in peanut oil was administered once by gavage to groups of adult male and female Sherman rats. The strain, age, body weight of the rats, and the dosing procedures were described in an earlier publication. The LD50 values were calculated by the method of Litchfield and Wilcoxon, 1949.

Findings:
Onset and nature of clinical symptoms and time of death were not reported.
The lowest lethal doses were 12 or 7.5 mg/kg bw for male or female rats, respectively. The LD50 values were calculated to be 13 mg/kg bw for males and 11 mg/kg bw for females.

Conclusions:
Azinphos-methyl is of very high toxicity by the oral route. The LD50 values were 13 mg/kg bw for male rats and 11 mg/kg bw for female rats.

B.5.2.1.4 RAT

Crawford and Doull, 1970: Antagonism of the lethal effects of Dipterex and Guthion with atropine and related drugs. University of Kansas Medical Center, Kansas City, Kansas 66103, USA. Dates of experimental work: not specified. Abstract, published in: Fed. Proc. 29:349, 1970.

Guidelines and GLP:
The method employed was not specified. When the study was performed, GLP was not compulsory.
Main deviations from current OECD guidelines: Test conditions, body weights, individual data for clinical observations, and necrospy findings were not reported.
The study is considered supplementary.

Material and methods:
Guthion (batch no. and purity not specified) in polypropylene glycol was administered once by gavage at unspecified dose levels to groups (number of groups and number/group not specified) of non-fasting adult male and female rats (source: Charles River, body weight range not specified). Symptoms and mortality were recorded for 10 days after dosing and the LD_{50} value calculated (method not specified).

Findings:
Time of death and nature and onset of clinical symptoms were not specified.
The LD_{50} value was calculated to be 12 ± 1.3 mg/kg bw for non-fasting rats.

Conclusion:
Azinphos-methyl is of very high toxicity by the oral route. The LD50 value was 12 mg/kg bw for rats.

B.5.2.1.5 RAT

Crawford and Anderson, 1974: The acute oral toxicity of Guthion technical, benzazimide and methyl benzazimide to rats. Report no. 41190 of 23 July 1974; Chemagro Division of Baychem Corporation, Research and Development, USA. Dates of experimental work: not specified.

Guidelines and GLP:
The method employed was the scientific standard at the time the study was performed. When the study was performed, GLP was not compulsory.
Main deviations from current OECD guidelines: Only 4 animals/sex per test group were used. Body weights, individual data for clinical observations, and necrospy findings were not reported.
The 3-page report contains also the acute oral toxicity study of benzazimide and methyl benzazimide in rats.
The study is considered supplementary.

Material and methods:
Guthion technical (batch no. not specified, 99.0 % a.i.) in DMSO was administered once by gavage to groups of 4 male and 4 female Sprague-Dawley rats (source: not specified; body weight range: 245-305 g for males and 190-236 g for females) fasting for 24 h prior to treatment at dose levels of 2, 4, 8, and 16 mg/kg bw. Symptoms and mortality were recorded for 14 days after dosing and the LD_{50} values calculated by the method of Weil, 1952.

Findings:
Clinical signs: The clinical symptoms were typical of ChE inhibition (not specified). Symptoms occurred between 5 and 20 minutes after dosing and persisted only during the day of dosing. Mortality occurred 10 to 80 minutes after application at dose levels of 4 mg/kg bw and above.
Gross pathology: not reported.

Table B.5.2.1.5-1: LD_{50} values in rats by sex

Sex	Formulation agent	NOEL (mg/kg bw)*	LD_{50} mg/kg bw (95 % confidence limits)
Male	DMSO	<2	5.6 (3.5-9.2)
Female	DMSO	<2	6.4 (3.1-12.9)

* = maximum dose level without clinical symptoms

Conclusion:
Azinphos-methyl is of very high toxicity by the oral route. The LD50 values were 5.6 mg/kg bw for male rats and 6.4 mg/kg bw for female rats.

B.5.2.1.6 RAT

Lamb and Anderson, 1974: The acute oral toxicity of Guthion, benzazimide and methyl benzazimide to fasted and nonfasted rats using CMC as the excipient. Report no. 41621 of 04 September 1974; Chemagro Corporation, Research and Development, USA. Dates of experimental work: not specified.

Guidelines and GLP:
The method employed was the scientific standard at the time the study was performed. When the study was performed, GLP was not compulsory.
Main deviations from current OECD guidelines: Only 4 animals/sex per test group were used. Body weights, individual data for clinical observations, and necrospy findings were not reported.
The 5-page report contains also the acute oral toxicity study of benzazimide and methyl benzazimide in rats.
The study is considered supplementary.

Material and methods:
Guthion technical (batch no. not specified, 99.0 % a.i.) in 2 % carboxymethylcellulose (CMC) was administered once by gavage to groups of 4 male and 4 female Sprague-Dawley rats (source: not specified; body weight range: 223-355 g for males and 188-260 g for females) fasting for 22-24 h prior treatment or non-fasting. The dose levels were 8, 16, 32 and 64 mg/kg bw for males and 4, 8, 16 and 32 mg/kg bw for females. Symptoms and mortality were recorded for 14 days after dosing and the LD_{50} values calculated by the method of Weil, 1952.
Findings:
Clinical signs: The clinical symptoms were typical of ChE inhibition (not specified). Symptoms occurred between 12 and 60 minutes after dosing and persisted only during the day of dosing. Mortality occurred at dose levels of 16 mg/kg bw and above.
Gross pathology: not reported.

Table B.5.2.1.6-1: LD_{50} values in rats by sex and feed status

Sex/feed status	Formulation agent	NOEL (mg/kg bw)*	LD_{50} mg/kg bw (95 % confidence limits)
Male non-fasting	2 % CMC	<8	19 (13-27)
Male fasting	2 % CMC	<8	19 (13-27)
Female non-fasting	2 % CMC	<4	10 (impossible to calculate)
Female fasting	2 % CMC	4	16 (11-24)

* = maximum dose level without clinical symptoms

Conclusion:
Azinphos-methyl is of very high toxicity by the oral route. The LD50 values were 19 mg/kg bw for male rats and 10 or 16 mg/kg bw for female rats, the feeding status did not markedly influence the acute oral toxicity.

B.5.2.1.7 RAT

Pasquet, Mazuret, Fournel and Koenig, 1976: Acute oral and percutaneous toxicity of phosalone in the rat, in comparison with azinphosmethyl and parathion. Rhône-Poulenc Recherches, Centre Nicolas Grillet, 94400 Vitry-Sur-Seine, France. Dates of experimental work: not specified. Published in: Toxicol Appl Pharmacol 37:85-92, 1976.
Guidelines and GLP:
The method employed was the scientific standard at the time the study was performed. When the study was performed, GLP was not compulsory.
Main deviations from current OECD guidelines: Body weights, individual data for clinical observations, and necropsy findings were not reported.
The study is considered supplementary.
Material and methods:
Azinphos-methyl (source and batch no.: not specified, purity: >95 %) was emulsified in methylene chlorid/arabic gum/Tween 80 and administered once by gavage in a volume of 5 ml/kg bw to groups of 10 male and 10 female rats (strain: CD (COBS), source: Charles River, France; body weight range: 120-220 g) at 5 dose levels (not specified). Mortality was recorded for 10 days after dosing and the LD_{50} value calculated by the method of Litchfield and Wilcoxon, 1949. Plasma, erythrocyte and brain ChE activities were determined 2, 5 and 24 h after the oral administration of single doses of 0, 2, 6 and 18 mg/kg bw to groups of 6 female rats.
Findings:
Clinical symptoms (not specified) occurred 5 minutes after application. Time of death and necropsy findings were not reported. Mortality occurred at dose levels of 20 mg/kg bw (males) or 13 mg/kg bw (females) and above. The LD50 values (and

confidence limits) were calculated to be 26 (21-32) mg/kg bw for male rats and 24 (20-29) mg/kg bw for female rats.

Table B.5.2.1.7-1: ChE activities in female rats 2, 5 and 24 h post administration (in % of control)

Dose level (mg/kg bw)	Plasma (2/5/24 h)	Erythrocytes (2/5/24 h)	Brain (2/5/24 h)
2	94/87/87	77/76/77	79/94/95
6	69/62/80	54/47/56	47/71/92
18	56/53/45	33/24/18	25/35/32

Conclusions:
Azinphos-methyl is of very high toxicity by the oral route. The LD50 values were 26 mg/kg bw for male rats and 24 mg/kg bw for female rats.

B.5.2.1.8 RAT

Thyssen, 1976: Studies to determine the toxic effects of the simultaneous application of azinphos-methyl or azinphos-ethyl and methamidophos. Report no.: 6354 of 24 September 1976; Bayer AG, Institute of Toxicology, Wuppertal-Elberfeld, Germany. Dates of experimental work: not specified.
Guidelines and GLP:
The method employed was the scientific standard at the time the study was performed. When the study was performed, GLP was not compulsory.
Main deviations from current OECD guidelines: Only male rats were used. Body weights, individual data for clinical observations, and necropsy findings were not reported.
The 7-page report contains also a study for combination toxicity.
The study is considered supplementary.
Material and methods:
Azinphos-methyl (batch not specified, technical purity) was emulsified in distilled water and Cremophor EL and administered once by gavage in a volume of 1.0 ml/100 g bw to groups of 10 male Wistar II albino rats (source: Winkelmann, Borchen, Germany; body weight range 160-180 g) at dose levels of 5, 10, 15, 20 and 25 mg/kg bw. The feeding status of the animals was not specified. Symptoms and mortality were recorded for 14 days after dosing and the LD_{50} value calculated by the method of Litchfield and Wilcoxon, 1949.
Findings:
Clinical signs: The clinical symptoms were typical of ChE inhibition (not specified) and occurred at all dose levels. Mortality occurred within 1 h after dosing at dose levels of 10 mg/kg bw and above.
The LD_{50} value (and 95 % confidence limits) for male rats were calculated to be 16.75 (13.1-21.42) mg/kg bw.
Gross pathology: not reported.
Conclusions:
Azinphos-methyl is of very high toxicity by the oral route. The LD50 value for male rats was 16.75 mg/kg bw.

B.5.2.1.9 RAT

Thyssen, 1977: Study for combination toxicity of azinphos-methyl and propoxur. Report no.: 7174 of 14 December 1977; Bayer AG, Institute of Toxicology, Wuppertal-Elberfeld, Germany. Dates of experimental work: not specified.
Thyssen J: Study for combination toxicity of azinphos methyl and azinphos ethyl. Report no.: 7178 of 14 December 1977; Bayer AG, Institute of Toxicology, Wuppertal-Elberfeld, Germany. Dates of experimental work: not specified.

Guidelines and GLP:
The method employed was the scientific standard at the time the study was performed (Kimmerle & Lorke, 1968). When the study was performed, GLP was not compulsory.
Main deviations from current OECD guidelines: Only male rats were used. Body weights, individual data for clinical observations, and necrospy findings were not reported.
The 7-page and 6-page reports (no. 7174 and 7178) contain also studies for combination toxicity.
The studies are considered supplementary.
Material and methods:
Azinphos-methyl (batch 6/05010, purity 93.0 %) was emulsified in distilled water and Cremophor EL and administered once by gavage in a volume of 1.0 ml/100 g bw to groups of 15 male Wistar II albino rats (source: Winkelmann, Borchen, Germany; body weight range 160-180 g) fasting for 16 h prior to and 3-4 h after treatment. The dose levels were 5, 7.5, 8.5, 10 and 12.5 mg/kg bw. Symptoms and mortality were recorded for 14 days after dosing and the LD_{50} value calculated by the method of Litchfield and Wilcoxon, 1949.
Findings:
Clinical signs: The clinical symptoms were typical of ChE inhibition (not specified) and occurred at all dose levels. First deaths appeared 12-31 minutes after application at dose levels of 8.5 mg/kg bw and above.
The LD_{50} value (and 95 % confidence limits) for fasting male rats were calculated to be 9.7 (9.0-10.6) mg/kg bw.
Gross pathology: not reported.
Conclusions:
Azinphos-methyl is of very high toxicity by the oral route. The LD50 value for male rats was 9.7 mg/kg bw.

B.5.2.1.10 **RAT**

Thyssen, 1977: Study for combination toxicity of Chlorpyrifos, Cytrolane, Cyolane, Tamaron, Gusathion-ethyl, and Gusathion-methyl active ingredient. Report no.: 7179 of 14 December 1977; Bayer AG, Institute of Toxicology, Wuppertal-Elberfeld, Germany. Dates of experimental work: not specified.
Guidelines and GLP:
The method employed was the scientific standard at the time the study was performed (Kimmerle & Lorke, 1968). When the study was performed, GLP was not compulsory.
Main deviations from current OECD guidelines: Only male rats were used. Body weights, individual data for clinical observations, and necrospy findings were not reported.
The 15-page report contains also studies for combination toxicity.
The study is considered supplementary.
Material and methods:
Azinphos-methyl (batch 6/05010, purity 93.0 %) was emulsified in distilled water and Cremophor EL and administered once by gavage in a volume of 1.0 ml/100 g bw to groups of 15 male Wistar II albino rats (source: Winkelmann, Borchen, Germany; body weight range 160-180 g) fasting prior to treatment. The dose levels were 3.5, 5, 7.5, and 10 mg/kg bw. Symptoms and mortality were recorded for 7 days after dosing and the LD_{50} value calculated by the method of Litchfield and Wilcoxon, 1949.
Findings:
Clinical signs: The clinical symptoms were typical of ChE inhibition (not specified) and occurred at all dose levels. First deaths appeared 9-90 minutes after application at all dose levels.
The LD_{50} value (and 95 % confidence limits) were calculated to be 5.3 (4.4-6.4) mg/kg bw for fasting male rats.
Gross pathology: not reported.

Conclusions:
Azinphos-methyl is of very high toxicity by the oral route. The LD50 value for male rats was 5.3 mg/kg bw.

B.5.2.1.11 **RAT**

Mihail, 1978: R1582 (Gusathion M active ingredient) Acute toxicity studies. Bayer report no.: 7618 of 15 June 1978; Bayer AG, Institute for Toxicology, Wuppertal-Elberfeld, Germany. Dates of experimental work: November 1977 to February 1978.
Guidelines and GLP:
The method employed was the scientific standard at the time the study was performed. When the study was performed, GLP was not compulsory.
Main deviations from current OECD guidelines: Body weights and individual data for clinical observations necrospy findings were not reported.
The 8-page report contains also an acute dermal study in rats and an acute oral study in dogs.
The study is considered acceptable.
Material and methods:
R1582 (batch no. not specified, rcvd. 15.3.1977, purity 91.6 %) was emulsified in distilled water and Cremophor EL and administered once by gavage in a volume of 1.0 ml/100 g bw to groups of 15 male and 15 female Wistar albino rats (source: Winkelmann, Borchen, Germany; body weight range 170-210 g) fasting for 16 h prior to treatment. The dose levels were 1.0, 2.5, 3.5, 4.0, 5.0, 6.0, 7.5 and 10.0 mg/kg bw for males and 1.0, 2.5, 3.5, 5.0, 5.5, 6.0 and 7.5 mg/kg bw for females. Symptoms and mortality were recorded for 14 days after dosing, necropsy was performed on the animals that died during the study and the LD_{50} value calculated by the method of Litchfield and Wilcoxon, 1949.
Findings:
Clinical signs: The clinical symptoms were typical of organophosphate poisoning, impairment of general health condition, muscle twitching, clonic cramps, salivation and breathing disorders. The appearance of symptoms occurred between 5 and 20 minutes after dosing and persisted for a maximum of 24 hours in survivors. Mortality occurred between 7 and 50 minutes after appplication at dose levels of 3.5 mg/kg bw and above.
Gross pathology: No abnormal findings.

Table B.5.2.1.11-1: LD_{50} values in rats by sex

Sex	Formulation agent	NOEL (mg/kg bw)*	LD_{50} mg/kg bw (95 % confidence limits)
Male	Cremophor EL/water	1.0	4.6 (4.1-5.3)
Female	Cremophor EL/water	1.0	4.4 (3.9-4.9)

* = maximum dose level without clinical symptoms

Conclusions:
Azinphos-methyl is of very high toxicity by the oral route. The LD50 values were 4.6 mg/kg bw for male rats and 4.4 mg/kg bw for female rats.

B.5.2.1.12 **RAT**

Flucke, 1979: Gusathion M (R1582): Determination of acute toxicity (LD_{50}). Report of 24 January 1979; Bayer AG, Institute for Toxicology, Wuppertal-Elberfeld, Germany. Dates of experimental work: not reported.
Guidelines and GLP:
The method employed was the internal laboratory standard at the time the study was performed. When the study was performed, GLP was not compulsory.

Main deviations from current OECD guidelines: The 1-page report contains no information about the test substance (batch no., purity), the test animals (strain, age, source), housing conditions, and diet. Only male rats were used. Body weights, data for clinical observations, and necrospy findings were not reported.
The study is considered supplementary.
Material and methods:
Gusathion M (batch no. and purity not specified) was emulsified in distilled water and Cremophor EL and administered once by gavage to groups of 10 male rats (strain, age, source, feeding status: not specified) at dose levels of 20, 25, 30, 35, and 50 mg/kg bw. Mortality was recorded for 14 days after dosing and the LD_{50} value calculated (method of calculation not specified).
Findings:
Clinical symptoms and mortality occurred at all dose levels. Time of death, onset of clinical symptoms and necropsy findings were not reported.
The LD_{50} value (and confidence limits) for male rats were calculated to be 25.4 mg/kg bw (22.0-29.3 mg/kg bw).
Conclusion:
Azinphos-methyl is of very high toxicity by the oral route. The LD50 value was 25.4 mg/kg bw for male rats.

B.5.2.1.13 RAT

Heimann, 1981: R1582 (Azinphos-methyl, active ingredient of Gusathion M). Determination of acute toxicity (LD_{50}). Report of 15 July 1981; Bayer AG, Institute for Toxicology, Wuppertal-Elberfeld, Germany. Dates of experimental work: not specified.
Guidelines and GLP:
The method employed was the laboratory standard for quality control purposes at the time the study was performed. When the study was performed, GLP was not compulsory.
Main deviations from current OECD guidelines: The 1-page report contains no information about the test animals (strain, age, source), housing conditions, and diet. Only male rats were used. Body weights, clinical observations, and necrospy findings were not reported.
The study is considered supplementary.
Material and methods:
R1582 (batch no. 230105019, purity 92.4 %) was emulsified in distilled water and Cremophor EL and administered once by gavage in a volume of 10 ml/kg bw to groups of 10 male rats (strain, age, source: not specified) at dose levels of 5, 7.5, 8.5, 9, and 10 mg/kg bw (fasting) or 10, 15 and 25 mg/kg bw (non-fasting). Mortality was recorded for 7 days after dosing and the LD_{50} value calculated (method of calculation not specified).
Findings:
Mortality occurred at dose levels of 7.5 mg/kg bw and above. Time of death, onset of clinical symptoms, and necopsy findings were not reported.

Table B.5.2.1.13-1: LD_{50} values in male rats by feed status

Feed status	Formulation agent	NOEL (mg/kg bw)*	LD_{50} mg/kg bw (confidence limits)
Fasting	Cremophor EL/water	<5	9.10 (8.43-9.83)
Non-fasting	Cremophor EL/water	<10	17.25(13.30-22.37)

* = maximum dose level without clinical symptoms

Conclusion:
Azinphos-methyl is of very high toxicity by the oral route. The LD50 values were 9.10 mg/kg bw for fasting and 17.25 mg/kg bw for non-fasting male rats.

B.5.2.1.14 RAT

Heimann, 1982: R1582 (Azinphos-methyl, the active ingredient of ®Guthion). Study of the acute oral and dermal toxicity to rats. Report of 30 June 1982; Bayer AG, Institute for Toxicology, Wuppertal-Elberfeld, Germany. Dates of experimental work: March 1982 to May 1982.
Guidelines and GLP:
The method employed was the internal laboratory standard at the time the study was performed. When the study was performed, GLP was not compulsory. Main deviations from current OECD guidelines: Only male rats were used. Body weights, individual data for clinical observations, and necrospy findings were not reported.
The 6-page report contains also an acute dermal study in rats.
The study is considered supplementary.
Material and methods:
R1582 (batch no. 79-R-225-42, purity 88.9 %) was emulsified in distilled water and Cremophor EL and administered once by gavage in a volume of 1 ml/100 g bw to groups of 10 (or 20) male Wistar albino rats (WISP strain, SPF-CPB; source: Winkelmann, Borchen, Germany; body weight range 160-200 g) fasting for 16 h prior to treatment or non-fasting. The dose levels were 5.0, 6.3, 6.7 and 8.0 mg/kg bw (fasting) or 10.0, 12.3, 16.0 and 20.0 mg/kg bw (non-fasting). Symptoms and mortality were recorded for 14 days after dosing and the LD_{50} values were calculated by the method of Litchfield and Wilcoxon (1949).
Findings:
Clinical signs: tremors; spastic, staggering gait; sternal recumbency; salivation; difficult breathing; lethargy; piloerection. Symptoms were observed about 10 minutes after dosing, mortality occurred within 30 minutes (fasting) or within 1 hour (non-fasting) after dosing at all dose levels.
Gross pathology: not reported.

Table B.5.2.1.14-1: LD_{50} values in male rats by feed status

Feed status	Formulation agent	NOEL (mg/kg bw)*	LD_{50} mg/kg bw (confidence limits)
Fasting	Cremophor EL/water	<5	6.7 (5.8-7.7)
Non-fasting	Cremophor EL/water	<10	12.8 (11-14.7)

* = maximum dose level without clinical symptoms

Conclusion:
Azinphos-methyl is of very high toxicity by the oral route. The LD50 values were 6.7 mg/kg bw for fasting male rats and 12.8 mg/kg bw for non-fasting male rats.

B.5.2.1.15 RAT

Heimann, 1987: E1582 techn.: Determination of acute toxicity (LD_{50}). Report of 23 November 1987; Bayer AG, Institute for Toxicology, Wuppertal-Elberfeld, Germany. Dates of experimental work: not specified.
Guidelines and GLP:
The method employed was the laboratory standard for quality control purposes at the time the study was performed. When the study was performed, GLP was not compulsory.
Main deviations from current OECD guidelines: The 1-page report contains no information about the test animals (strain, age, source), housing conditions, and diet. Only male rats were used. Body weights, clinical observations, and necrospy findings were not reported.
The study is considered supplementary.

Material and methods:
E1582 (batch no. 233 796 036, purity 92.0 %) was emulsified in distilled water and Cremophor EL and administered once by gavage in a volume of 10 ml/kg bw to groups of 5 fasting male rats (strain, age, source: not specified) at dose levels of 5.0, 6.3, 8.0, 8.5 and 9.0 mg/kg bw. Mortality was recorded for 14 days after dosing and the LD_{50} value calculated (method of calculation not specified).
Findings:
Symptoms were observed at all dose levels, mortality occurred at dose levels of 6.3 mg/kg bw and above. Time of death, onset of clinical symptoms, and necropsy findings were not reported.
The LD_{50} value (and confidence limits) were calculated to be 7.1 mg/kg bw (6.4-8.1 mg/kg bw) for fasting male rats.
Conclusion:
Azinphos-methyl is of very high toxicity by the oral route. The LD50 value was 7.1 mg/kg bw for fasting male rats.

B.5.2.1.16 MOUSE

Crawford and Doull, 1970: Antagonism of the lethal effects of Dipterex and Guthion with atropine and related drugs. University of Kansas Medical Center, Kansas City, Kansas 66103, USA. Dates of experimental work: not specified. Abstract, published in: Fed. Proc. 29:349, 1970.
Guidelines and GLP:
The method employed was not specified. When the study was performed, GLP was not compulsory.
Main deviations from current OECD guidelines: Test conditions, body weights, individual data for clinical observations, and necrospy findings were not reported.
The study is considered supplementary.
Material and methods:
Guthion (batch no. and purity not specified) in polypropylene glycol was administered once by gavage at unspecified dose levels to groups (number of groups and number/group not specified) of non-fasting adult male and female CF1 mice (source: Carworth Farms, body weight range not specified). Symptoms and mortality were recorded for 10 days after dosing and the LD_{50} value calculated (method not specified).
Findings:
Time of death and nature and onset of clinical symptoms were not specified. The LD_{50} value was calculated to be 20 ± 0.9 mg/kg bw for non-fasting mice.
Conclusion:
Azinphos-methyl is of very high toxicity by the oral route. The LD50 value was 20 mg/kg bw for mice.

B.5.2.1.17 MOUSE

Sterri, Rognerud, Fiskum and Lyngaas, 1979: Effect of toxogonin and P2S on the toxicity of carbamates and organophosphorus compounds. Norwegian Defence Research Establishment, Division of Toxicology, N-2007 Kjeller, Norway. Dates of experimental work: not specified. Published in: Acta Pharmacol et Toxicol 45:9-15, 1979.
Guidelines and GLP:
The method employed was not specified. When the study was performed, GLP was not compulsory.
Main deviations from current OECD guidelines: Only female mice were used. Test conditions, body weights, individual data for clinical observations, and necrospy findings were not reported.

The study is considered supplementary.
Material and methods:
Azinphos-methyl (source: Bayer AG, batch no. not specified, purity 93.3-94.5 %) in propylene glycol was administered once by gavage at dose levels of 10, 20 and 40 mg/kg bw to 3 groups (30, 38 and 10 animals respectively) of non-fasting female NMRI mice (source: in house or National Institute of Public Health, Norway; body weight range: 18-26 g). Mortality was recorded 20 hours after dosing, at which time survivors were killed and used for the analysis of acetylcholinesterase (AChE) activity (by the radiochemical method of Sterri & Fonnum, 1978) in RBC and cerebrum. AChE activities were expressed as the percentage of control (unintoxicated animal) values ± standard error of the mean. The LD_{50} value was calculated by an unspecified method.
Findings:
Clinical signs, time of onset and duration, maximum dose level without clinical symptoms, time of death and necropsy findings were not specified.
The LD_{50} value was calculated to be 11 mg/kg bw.

Table: B.5.2.1.17-1: Mortality and average AChE activity in survivors after 20 hours

Dose level (mg/kg bw)	Mortality	Average AChE activity (% of control) ± S.E.M.	
		Erythrocytes	Cerebrum
10	12/30	33 ± 4	56 ± 4
20	37/38	17	37
40	10/10	-	-

Conclusion:
Azinphos-methyl is of very high toxicity by the oral route. The LD50 value was 11 mg/kg bw for female mice.

B.5.2.1.18 GUINEA PIG

DuBois, Thursh and Murphy, 1957: Studies on the toxicity and pharmacologic actions of the dimethoxy ester of benzotriazine dithiophosphoric acid (DBD, Guthion). Department of Pharmacology, University of Chicago, Chicago, Illinois. Dates of experimental work: not specified. Published in: J Pharmacol Exp Ther 119:208-218, 1957.
Guidelines and GLP:
The method employed was the scientific standard at the time the study was performed. When the study was performed, GLP was not compulsory.
Main deviations from current OECD guidelines: Only male guinea pigs were used. Body weights, individual data for clinical observations, and necrospy findings were not reported.
The study is considered supplementary.
Material and methods:
Azinphos-methyl (source: Chemagro Corp., New York, purity: >97 %) was emulsified in 20 % ethanol and 80 % propylene glycol and administered once by gavage (volume <0,3 % of bw) to male guinea pigs (source: not specified; body weight range: 300-400 g). A total of 34 animals was used, but the number of animals per group and the dose levels were not specified. The mortality data were based on a 10-day observation period and the approximate LD_{50} values were calculated by the log probability method.
Findings:
The clinical symptoms observed were increased respiration, unsteadiness, lack of coordination, scattered muscular twitches; lacrimation, urination, defecation, salivation; tonic and clonic convulsions, prostration, and respiratory failure.

The time of onset of clinical symptoms or deaths was not specified. The LD_{50} value was calculated to be 80 mg/kg bw.
Conclusions:
Azinphos-methyl is of high toxicity by the oral route. The LD50 value was 80 mg/kg bw for male guinea pigs.

B.5.2.1.19 DOG

Mihail, 1978: R1582 (Gusathion M active ingredient) Acute toxicity studies, Bayer report no.: 7618 of 15 June 1978; Bayer AG, Institute for Toxicology, Wuppertal-Elberfeld, Germany. Dates of experimental work: November 1977 to February 1978.
Guidelines and GLP:
The method employed was the scientific standard at the time the study was performed. When the study was performed, GLP was not compulsory.
Main deviations from current OECD guidelines: Only male animals and only one or two animals per dose level were used. Body weights and necrospy findings were not reported.
The 8-page report contains also an acute oral and an acute dermal study in rats.
The study is considered supplementary.
Material and methods:
R1582 (batch no. not specified, rcvd. 15.3.1977, purity 91.6 %) was emulsified in distilled water and Cremophor EL and administered once by gavage in a volume of 2 ml/kg bw to single fasting male Beagle dogs (source: Gräfl. Degenfeld-Schomburgsches Rentamt, Geislingen/Steige, Germany; body weight range: 12.7-16.2 kg) at dose levels of 1.0, 2.5 and 5.0 mg/kg or to two dogs at a dose of 10.0 mg/kg bw. Symptoms and mortality were recorded for 14 days after dosing.
Findings:
No deaths occurred and all dogs but one tolerated these dose levels without showing any symptoms. One dog at 10 mg/kg bw vomited the test compound about 2 h after administration.
Conclusions:
Dogs tolerated azinphos-methyl at dose levels up to 5 mg/kg bw without clinical symptoms. The LD_{50} was greater than 10 mg/kg bw.
According to the study author and the notifier, the maximum dose level without clinical symptoms was 10 mg/kg bw.

B.5.2.2 PERCUTANEOUS STUDIES

B.5.2.2.1 RAT

Nelson, 1968: The acute mammalian toxicity of two samples of Guthion technical to adult female rats. Report no. 22579 of 26 April 1968; Chemagro Corporation, Research and Development Department. Dates of experimental work: not specified.
Guidelines and GLP:
The method employed was the internal standard at the time the study was performed. When the study was performed, GLP was not compulsory.
Main deviations from current OECD guidelines: Only female rats and only 4 animals per dose group were used. Test conditions, body weights, clinical observations, and necrospy findings were not reported.
The 3-page report contains also an acute oral and an acute intraperitoneal toxicity study in rats.
The study is considered supplementary.
Material and methods:
Guthion technical (sample no. PF1966, source and purity: not specified) as a solution in Panasol AN-2 was applied once, dermally, at dose levels of 50, 62.5, 70, 75, 100, 125 and 150 mg/kg bw to groups of 4 adult female Sprague-Dawley rats (source and body weight range: not specified). Duration of contact, abraded

or intact skin, occlusive dressing or open application were not specified. Mortality was recorded for 14 days after dosing and the LD50 value estimated by an unspecified method.
Findings:
Onset and nature of clinical symptoms and time of death were not reported. Mortality occurred at dose levels of 75 mg/kg bw and above. The LD50 value was calculated to be 72.5 mg/kg bw.
Conclusion:
Azinphos-methyl is of high toxicity by the dermal route. The LD50 value was 72.5 mg/kg bw for female rats.

B.5.2.2.2 RAT

Gaines, 1969: Acute toxicity of pesticides. Toxicology Laboratory, Pesticides Program, National Communicable Disease Center, U.S. Department of Health, Education, and Welfare, Atlanta, Georgia 30333. Dates of experimental work: not specified. Published in: Toxicol Appl Pharmacol 14:515-534, 1969.
Guidelines and GLP:
The method employed was the internal standard at the time the study was performed. When the study was performed, GLP was not compulsory.
Main deviations from current OECD guidelines: Body weights, clinical observations, and necrospy findings were not reported.
The study is considered supplementary.
Material and methods:
Azinphos-methyl (technical grade, source and purity: not specified) as a solution in xylene was administered once, dermally, to groups of adult male and female Sherman rats. The strain, age, body weight of the rats, and the test conditions were described in an earlier publication. The LD50 values were calculated by the method of Litchfield and Wilcoxon, 1949.
Findings:
Onset and nature of clinical symptoms and time of death were not reported. The lowest lethal doses were 160 mg/kg bw for male and female rats each. The LD50 values were calculated to be 220 mg/kg bw for males and for females.
Conclusion:
Azinphos-methyl is of high toxicity by the dermal route. The LD50 values were 220 mg/kg bw for male and for female rats.

B.5.2.2.3 RAT

Pasquet, Mazuret, Fournel and Koenig, 1976: Acute oral and percutaneous toxicity of phosalone in the rat, in comparison with azinphosmethyl and parathion. Rhône-Poulenc Recherches, Centre Nicolas Grillet, 94400 Vitry-Sur-Seine, France. Dates of experimental work: not specified. Published in: Toxicol Appl Pharmacol 37:85-92, 1976.
Guidelines and GLP:
The method employed was the scientific standard at the time the study was performed. When the study was performed, GLP was not compulsory.
Main deviations from current OECD guidelines: Only female animals were used. Body weights, individual data for clinical observations, and necrospy findings were not reported.
The study is considered supplementary.
Material and methods:
Azinphos-methyl (source and batch no.: not specified, purity: >95 %) was dissolved in a mixture of acetone/ethanol/peanut oil (1:1:2) and administered once, dermally, in a volume of 2.5 ml/kg bw to groups of 10 female rats (strain: CD (COBS), source: Charles River, France; body weight range: 120-220 g) at 3 or 5 dose levels (not specified). The test substance was removed after 24 h.

Mortality was recorded for 10 days after dosing and the LD50 value was calculated by the method of Litchfield and Wilcoxon, 1949.
Findings:
Clinical symptoms, time of death and necropsy findings were not reported.
Mortality occurred at dose levels of 40 mg/kg bw and above. The LD50 value (and confidence limits) were calculated to be 90 (60-130) mg/kg bw.
Conclusion:
Azinphos-methyl is of high toxicity by the dermal route. The LD50 value was 90 mg/kg bw for female rats.

B.5.2.2.4 RAT

Mihail, 1978: R1582 (Gusathion M active ingredient) Acute toxicity studies. Bayer report no.: 7618 of 15 June 1978; Bayer AG, Institute for Toxicology, Wuppertal-Elberfeld, Germany. Dates of experimental work: November 1977 to February 1978.
Guidelines and GLP:
The method employed was an in house method according to the method of Noakes & Sanderson (1969), conforming in principle to the EPA proposed guidelines for registering pesticides in the US, Fed. Reg., vol 43, No. 163, August 22, 1978. When the study was performed, GLP was not compulsory.
Main deviations from current OECD guidelines: Body weights and individual data for clinical observations and necrospy findings were not reported.
The 8-page report contains also an acute oral study in rats and an acute oral study in dogs.
The study is considered acceptable.
Material and methods:
R1582 (batch no. not specified, rcvd. 15.3.77, purity 91.6 %) as an emulsion in distilled water/Cremophor EL (100 mg/kg bw dose) or as a paste in Cremophor EL (all dose levels of 500 mg/kg bw or more) was applied once, dermally for 24 hours to shaved, intact dorsal skin, at dose levels of 100, 500, 1000, 1500, 2500 and 5000 mg/kg bw to groups of 5 or 10 Wistar albino rats of each sex (source: Winkelmann, Borchen, Germany; body weight range: 170-210 g). The application sites were occluded for 24 hours and then washed with soap and water to remove the test compound. Symptoms and mortality were recorded for 14 days after dosing, necropsy was performed on the animals that died during the study and the LD_{50} value was calculated by the method of Litchfield and Wilcoxon, 1949.
Findings:
Clinical signs: Deaths occurred between 2 and 10 days after dosing. The clinical symptoms were impairment of general health condition, clonic cramps, salivation and breathing disorders. The symptoms occurred on the day after treatment and persisted for 2 to 11 days.
Gross pathology: Animals dying during the study revealed occasional pulmonary emphysemas and apparently enlarged adrenals.

Table B.5.2.2.4-1: Dermal LD_{50} values in rats by sex

Sex	Formulation agent	NOEL (mg/kg bw)*	LD_{50} mg/kg bw
Male	Cremophor EL/water	<100	2500-5000
Female	Cremophor EL/water	<100	2500-5000

* = maximum dose level without clinical symptoms

Conclusion:
Azinphos-methyl was of low toxicity by the dermal route. The LD50 values were 2500-5000 mg/kg bw for male and female rats.
This result, however, is inconsistent with the rest of the studies.

B.5.2.2.5 RAT

Heimann, 1982: R1582 (Azinphos-methyl, the active ingredient of ®Guthion). Study of the acute oral and dermal toxicity to rats. Report of 30 June 1982; Bayer AG, Institute for Toxicology, Wuppertal-Elberfeld, Germany. Dates of experimental work: March 1982 to May 1982.
Guidelines and GLP:
The method employed was the internal laboratory standard at the time the study was performed, based on the wrapping method of Noakes & Sanderson (1969). When the study was performed, GLP was not compulsory.
Main deviations from current OECD guidelines: Body weights and individual data for clinical observations and necrospy findings were not reported.
The 6-page report contains also an acute oral study in rats.
The study is considered acceptable.
Material and methods:
R1582 (batch no. 79-R-225-42, purity 88.9 %) as an emulsion in distilled water/Cremophor EL was applied once, dermally for 24 hours to the clipped intact dorsal skin of groups of 5 or 10 male and female Wistar rats (WISP strain, SPF-CPB, source: Winkelmann, Borchen, Germany, body weight range: 160-200 g). The dose levels were 100, 160, 200, 250, 315 and 400 mg/kg bw for males and 63, 100, 160 and 250 mg/kg bw for females. The application sites were occluded for 24 hours and then washed with soap and water. The animals were observed for 14 days after treatment. Animals dying during the study and those surviving to the end of the study were necropsied. The LD_{50} values were calculated by the method of Litchfield and Wilcoxon (1949).
Findings:
Clinical signs: The symptoms were similar to those observed following oral administration (tremors; spastic, staggering gait; sternal recumbency; salivation; difficult breathing; lethargy; piloerection). Symptoms occurred at all dose levels within one hour to one day after application and persisted for up to 7 days. In the case of surviving animals, lethargy persisting for up to 9 days was observed. The application site skin areas were without clinical signs. Mortality occurred at dose levels of 100 or 200 mg/kg bw and above (females or males) between one and 7 days after application.
Gross pathology: Examination of animals dying during the study revealed dark liver with lobular pattern, pale spleen, reddened renal medulla and ulcer-like foci on the lining of the gastrointestinal tract. Animals killed at the end of the experiment showed no treatment-related damage.

Table B.5.2.2.5-1: Dermal LD_{50} values in rats by sex

Sex	Formulation agent	NOEL (mg/kg bw)*	LD_{50} mg/kg bw (confidence limits)
Male	Cremophor EL/water	<100	approx. 200-250
Female	Cremophor EL/water	<63	155 (115-209)

* = maximum dose level without clinical symptoms

Conclusion:
Azinphos-methyl is of high toxicity by the dermal route. The LD50 values were approximately 200-250 mg/kg bw for male rats and 155 mg/kg bw for female rats.

B.5.2.3 INHALATION STUDIES

B.5.2.3.1 RAT

Kimmerle and Lorke, 1968: Toxicology of insecticidal organophosphates. Bayer AG, Institute of Toxicology, Wuppertal-Elberfeld, Germany. Dates of experimental work: not specified. Published in: Pflanzenschutz-Nachrichten Bayer 21:111-142, 1968.

Guidelines and GLP:
The method employed was the internal standard at the time the study was performed. When the study was performed, GLP was not compulsory.
Main deviations from current OECD guidelines: Only male rats were used. Test conditions, clinical observations and necrospy findings were not reported.
The study is considered supplementary.
Material and methods:
Groups of 20 male rats (strain, source, body weight: not specified) were exposed for 1 h or 4 h to Gusathion technical (batch no., source and purity: not specified) in the dynamic inhalation apparatus described by Niessen et al. (Arch Toxicol 20:44, 1963). The exposure concentrations were not reported. After a 14-day post-exposure observation period, the LC50 values were calculated by an unspecified method.
Findings:
Onset and nature of clinical symptoms and time of death were not reported. The LC50 values were calculated to be 0.385 and 0.152 mg/l for the 1-hour and the 4-hour exposure, respectively.
Conclusion:
Azinphos-methyl is of very high toxicity by the inhalative route. The LC50 values for male rats were 0.385 and 0.152 mg/l for the 1-hour and the 4-hour exposure, respectively.

B.5.2.3.2 RAT

Shiotsuka, 1987: Acute four-hour inhalation toxicity study with [3]Guthion technical in rats. Report no. 880 of 30 June 1987; study no. 87-041-11; Mobay Corporation, Corporate Toxicology Department, Stilwell, Kansas, USA. Dates of experimental work: 10 March 1987 to 1 April 1987.
Guidelines and GLP:
The study protocol complied US EPA Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals, Series 81-3, November 1984 and OECD guideline 403. The study is GLP compliant.
The study is considered acceptable.
Material and methods:
Groups of 10 male and/or 10 female Sprague-Dawley rats (strain: Sas: CD(SD)BR, source: Sasco Inc., Omaha, Nebraska, USA; body weight range for males: 188-255 g, females: 180-234 g) were exposed head-only for 4 hours to guthion technical (reference no. 79-R-225-42, purity 88.8 %) as a liquid aerosol in 9-10 % polyethylene glycol/ethanol (1:1) at analytical concentrations of 80, 105, 141, 192 and 250 mg/m^3 air. Three control groups were employed, two exposed to air alone and one to vehicle alone. All animals dying during the observation period were necropsied. The animals were observed for 14 days after exposure for mortality and clinical signs, and the survivors necropsied. Body weights recorded on days 1, 3, 7 and 14. The LC_{50} values and 95 % confidence limits were calculated by probit analysis.
Findings:
Clinical signs: Signs indicative of organophosphate poisoning (salivation, hypo-activity, tremors, dyspnea, ataxia) were observed at all dose levels with no substantial differences between the sexes. Signs occurred on the day of exposure and complete recovery was apparent by day 11. A significant reduction in weight gain was recorded at all concentrations with a trend toward recovery during the 14-day observation period. All deaths occurred between days 0 and 2 post-exposure.
Gross pathology: Compound-related gross lesions of animals that died suggested irritation of the respiratory tract (nasal and oral stains, red turbinates, red lungs).

Table B.5.2.3.2-1: Acute 4-hour LC_{50} values in rats by sex

Sex	Formulation agent	NOEL (mg/m^3 air) *	LC_{50} mg/m^3 air (95 % confidence limits)
Male	PEG/ethanol (1:1)	<105	155 (135-176)
Female	PEG/ethanol (1:1)	<80	132 (118-151)

* = maximum concentration without clinical symptoms

Conclusions:
Azinphos-methyl is of very high toxicity by the inhalative route. The LC50 values (4-hour exposure) were 0.155 mg/l for male rats and 0.132 mg/l for female rats.

B.5.2.4 INTRAPERITONEAL STUDIES

B.5.2.4.1 RAT

DuBois, Thursh and Murphy, 1957: Studies on the toxicity and pharmacologic actions of the dimethoxy ester of benzotriazine dithiophosphoric acid (DBD, Guthion). Department of Pharmacology, University of Chicago, Chicago, Illinois. Dates of experimental work: not specified. Published in: J Pharmacol Exp Ther 119:208-218, 1957.
Guidelines and GLP:
The method employed was the scientific standard at the time the study was performed. When the study was performed, GLP was not compulsory.
Main deviations from current OECD guidelines: Body weights, individual data for clinical observations, and necrospy findings were not reported.
The study is considered supplementary.
Material and methods:
Azinphos-methyl (source: Chemagro Corp., New York, purity: >97 %) was emulsified in 20 % ethanol and 80 % propylene glycol and administered once, by intraperitoneal injection (volume <0,3 % of bw), to male and female Sprague-Dawley rats (source: not specified; body weight range: 175-250 g). A total of 35 males and 43 females was used, but the number of animals per group and the dose levels were not specified. The mortality data were based on a 10-day observation period and the approximate LD_{50} values were calculated by the log probability method.
Findings:
Clinical symptoms (increased respiration, unsteadiness, lack of coordination, scattered muscular twitches, lacrimation, urination, defecation, salivation, tonic and clonic convulsions, prostration, respiratory failure) appeared in 4 to 6 minutes after intraperitoneal injection, mortality occurred usually within 10 to 30 minutes and occasionally after several hours. The LD_{50} values were calculated to be 11.6 mg/kg bw for males and 5.7 mg/kg bw for females.
Conclusions:
Azinphos-methyl is of very high toxicity by the intraperitoneal route. The LD50 values were 11.6 mg/kg bw for male rats and 5.7 mg/kg bw for female rats.

B.5.2.4.2 RAT

Nelson, 1968: The acute mammalian toxicity of two samples of Guthion technical to adult female rats. Report no. 22579 of 26 April 1968; Chemagro Corporation, Research and Development Department. Dates of experimental work: not specified.
Guidelines and GLP:
The method employed was the internal standard at the time the study was performed. When the study was performed, GLP was not compulsory.

Main deviations from current OECD guidelines: Only female rats and only 4 animals per dose group were used. Body weights, clinical observations, and necrospy findings were not reported.
The 3-page report contains also an acute oral and an acute dermal toxicity study in rats.
The study is considered supplementary.

Material and methods:
Guthion technical (samples nos. PF1966 and 31064, source and purity: not specified) as a solution in Panasol AN-2 was administered once, by intraperitoneal injection, to groups of 4 adult female Sprague-Dawley rats (source and body weight range: not specified) at dose levels of 4, 6, 7.5, 8, 9 and 10 mg/kg bw. Mortality was recorded for 14 days after dosing and the LD_{50} value estimated by an unspecified method.

Findings:
Onset and nature of clinical symptoms and time of death were not reported. Mortality occurred at dose levels of 7.5 mg/kg bw and above. The LD50 values were calculated to be 8.5 and 8.9 mg/kg bw for the samples PF1966 and 31064, respectively.

Conclusions:
Azinphos-methyl is of very high toxicity by the intraperitoneal route. The LD50 values were 8.5 and 8.9 mg/kg bw for female rats.

B.5.2.4.3 RAT

Krötlinger, 1993: E1582 (c.n.: azinphos-methyl) Study for acute intraperitoneal toxicity to rats. Report no. 22647 of 4 November 1993; study no. T 9044245. Bayer AG, Fachbereich Toxikologie, Wuppertal, Germany. Dates of experimental work: 11 January 1993 to 15 February 1993.

Guidelines and GLP:
The method employed was based on OECD guideline 401 (Acute Oral Toxicity), adopted 24 February 1987, and on US EPA Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals, Series 81-1 (Acute Oral Toxicity Study), Revised Edition, November 1984. The study is GLP compliant.
The study is considered acceptable.

Material and methods:
E1582 (batch no. 230105115, purity 91.0 %) formulated in physiological saline/Cremophor EL was administered once, by intraperitoneal injection, to groups of 5 male and 5 female non-fasting Wistar rats (strain: BOR:WISW (SPF-Cpb), source: Winkelmann, Borchen, Germany; body weight range for males 170-196 g, females 175-192 g) at dose levels of 1.0, 5.0, 6.7, 8.0, (9.0 females only) and 10 mg/kg bw. All animals dying during the observation period were necropsied. The animals were observed for 14 days after exposure for mortality and clinical signs, and the survivors necropsied. Body weights recorded on days 1, 4, 8 and 15. The LD_{50} values were calculated by the method of Bliss (1935 & 1938).

Findings:
Clinical signs: Clinical signs (apathy, piloerection, decreased motility, laboured breathing, dyspnea, palmospasms, staggering gait, salivation, swollen buccal region) occurred at all dose levels (except females 1 mg/kg bw) from one to 27 minutes after treatment and persisted up to the third day. Mortalities occurred at doses of 6.7 and 8.0 mg/kg bw and above (males and females, respectively) between 9 minutes and 2 days after treatment.
Gross pathology: Necropsy of animals that died or were killed at the end of the study revealed no consistent treatment-related findings.

Table B.5.2.4.1: Acute intraperitoneal LD_{50} values in rats by sex

Sex	Formulation agent	NOEL (mg/kg bw)*	LD_{50} mg/kg bw
Male	saline/Cremophor	<1.0	6.9
Female	saline/Cremophor	1.0	9-10

* = maximum dose level without clinical symptoms

Conclusions:
Azinphos-methyl is of very high toxicity by the intraperitoneal route. The LD50 values were 6.9 mg/kg bw for male rats and 9-10 mg/kg bw for female rats.

B.5.2.4.4 MOUSE

The acute intraperitoneal toxicity studies in mice are considered supplementary. The results of the studies are quoted in the summary table (table 5.2.1) but the studies are not described in detail.

DuBois, Thursh and Murphy, 1957: Studies on the toxicity and pharmacologic actions of the dimethoxy ester of benzotriazine dithiophosphoric acid (DBD, Guthion). Department of Pharmacology, University of Chicago, Chicago, Illinois. Dates of experimental work: not specified. Published in: J Pharmacol Exp Ther 119:208-218, 1957.
Result: The intraperitoneal LD50 values were 5.4 mg/kg bw for male mice and 3.4 mg/kg bw for female mice.

Kamienski and Murphy, 1971: Biphasic effects of methylenedioxyphenyl synergists on the action of hexobarbital and organophosphate insecticides in mice. Department of Physiology, Harvard University, School of Public Health, Boston, MA, USA. Dates of experimental work: not specified. Published in: Toxicol Appl Pharmacol 18:883-894, 1971.
Result: The intraperitoneal LD50 value was 4.2 mg/kg bw for male mice.

Benke, Cheever and Murphy, 1973: Comparative toxicology, anticholinesterase action and metabolism of methyl parathion, parathion and guthion in sunfish and mice. Department of Physiology, Harvard School of Public Health, Boston, MA, USA. Dates of experimental work: not specified. Abstract, published in: Toxicol Appl Pharmacol 25:473-474, 1973.
Result: The intraperitoneal LD50 value was 3-4.5 mg/kg bw for mice.

B.5.2.4.5 GUINEA PIG

The acute intraperitoneal toxicity study in guinea pigs is considered supplementary. The result of the study is quoted in the summary table (table 5.2.1) but the study is not described in detail.

DuBois, Thursh and Murphy, 1957: Studies on the toxicity and pharmacologic actions of the dimethoxy ester of benzotriazine dithiophosphoric acid (DBD, Guthion). Department of Pharmacology, University of Chicago, Chicago, Illinois. Dates of experimental work: not specified. Published in: J Pharmacol Exp Ther 119:208-218, 1957.
Result: The approximate intraperitoneal LD50 value for male guinea pigs was 40.0 mg/kg bw.

B.5.2.5 SKIN IRRITATION

B.5.2.5.1 RABBIT

Thyssen, 1981: R1582 (azinphos-methyl, the active ingredient of ®Guthion) Study of the irritant effect on the skin and mucous membranes (eye). Report of 19 October 1981; study nos. T 8010593 (skin) and T 9010594 (eye). Bayer AG, Institute of Toxicology, Wuppertal-Elberfeld, Germany. Dates of experimental work: July 1981.

Guidelines and GLP:
The test was performed in accordance with the guidelines recommended by the US Department of Agriculture, Federal Register, 38 (187), 27019, 1973, and OECD guideline 404. When the study was performed, GLP was not compulsory.
Main deviations from current OECD guidelines: The test conditions were not reported in detail. The 5-page report contains also an acute eye irritation study in rabbits.
The study is considered acceptable.

Material and methods:
Six New Zealand White rabbits, male and female (source: Degenfeld, Eybach, Germany; body weight range: 3-4 kg) received a single dermal application for 24 hours of R1582 (batch no. 230105019, purity 92.4 %) in an unspecified vehicle to abraded and unabraded skin. Animals were examined for signs of erythema and edema at 24 and 72 hours after application.

Findings:
No reaction to treatment in any rabbit was observed at 24 and 72 hours after application, on either intact or abraded skin.

Conclusion:
Azinphos-methyl has no dermal irritation potential.

B.5.2.5.2 RABBIT

Zorbas, 1994: Primary dermal irritation study with technical grade Guthion® in rabbits. Bayer file no. 7354 of 2 August 1994; study no. 94-325-AJ. Miles Inc., Agriculture Division, Toxicology, Stilwell, Kansas, USA. Dates of experimental work: 6 July 1994 to 9 July 1994.

Guidelines and GLP:
The study was performed in accordance with OECD guideline 404, July 1992, and corresponding US EPA and Japanese guidelines. The study is GLP compliant.
The study is considered acceptable.

Material and methods:
Guthion technical grade (0.5 g; batch no. 3030050/230205204; purity 92.2 %) was moistened with tap water and applied on a gauze pad to a shaved, non-abraded area of skin on the backs of 6 young adult male New Zealand White rabbits (source: Small Stock Industries, Pea Ridge, AR, USA). The application sites were occluded for 4 hours and then wiped with damp gauze to remove the test material. Animals were examined for signs of erythema and edema at 0.5-1, 24, 48 and 72 hours after patch removal.

Findings:
Neither erythema nor edema was evident at the dose site of any animal following exposure. As a result, a primary irritation index of 0.0 was calculated. No other lesions or toxic signs were observed.

Conclusion:
Azinphos-methyl has no dermal irritation potential.

B.5.2.6 EYE IRRITATION

B.5.2.6.1 RABBIT

Thyssen, 1981: R1582 (azinphos-methyl, the active ingredient of ®Guthion) Study of the irritant effect on the skin and mucous membranes (eye). Report of 19 October 1981; study nos. T 8010593 (skin) and T 9010594 (eye). Bayer AG, Institute of Toxicology, Wuppertal-Elberfeld, Germany. Dates of experimental work: July 1981.

Guidelines and GLP:
The test was performed in accordance with the guidelines recommended by the US Department of Health, Education & Welfare, Federal Register, 37 (83): 8535, 1972, and OECD guideline 405. When the study was performed, GLP was not compulsory.
Main deviations from current OECD guidelines: The test conditions were not reported in detail.
The 5-page report contains also an acute skin irritation study in rabbits.
The study is considered acceptable.

Material and methods:
Eight New Zealand White rabbits, male and female (source: Degenfeld, Eybach, Germany; body weight range: 3-4 kg) received a single application of R1582 (batch no. 230105019, purity 92.4 %) into one conjunctival sac, 5 animals for a contact time of 5 minutes and 3 animals for a contact time of 24 hours, after which the material was washed from the eye. Animals were examined for signs of redness, swelling and ulceration after 1, 24, 48, 72 hours and 7 days post application according to the criteria specified in the reference above.

Findings:
5 minute exposure: Grade 1 erythema of the conjunctiva was observed after 1 hour in all animals. No other reaction to treatment was observed at 1 hour, and no reaction to treatment was apparent in any animal at any of the other observation intervals.
24 hour exposure: All animals showed grade 2 erythema of the conjunctiva at 1 hour and grade 1 erythema at 24 hours. No other reaction to treatment was observed at 1 and 24 hours, and no reaction to treatment was apparent at any of the other observation intervals.

Table B.5.2.6.1-1: Primary eye irritation scores (5-minute exposure)

Animal no.	Tissue	Effect	Eye irritation scores Time after exposure and rinsing				
			1 hour	24 hours	48 hours	72 hours	7 days
6	Cornea		0	0	0	0	0
	Iris		0	0	0	0	0
	Conjunctiva	Redness	1	0	0	0	0
		Swelling	0	0	0	0	0
		Ulceration	0	0	0	0	0
8	Cornea		0	0	0	0	0
	Iris		0	0	0	0	0
	Conjunctiva	Redness	1	0	0	0	0
		Swelling	0	0	0	0	0
		Ulceration	0	0	0	0	0
9	Cornea		0	0	0	0	0
	Iris		0	0	0	0	0
	Conjunctiva	Redness	1	0	0	0	0
		Swelling	0	0	0	0	0
		Ulceration	0	0	0	0	0
10	Cornea		0	0	0	0	0
	Iris		0	0	0	0	0
	Conjunctiva	Redness	1	0	0	0	0
		Swelling	0	0	0	0	0
		Ulceration	0	0	0	0	0
11	Cornea		0	0	0	0	0
	Iris		0	0	0	0	0
	Conjunctiva	Redness	1	0	0	0	0
		Swelling	0	0	0	0	0
		Ulceration	0	0	0	0	0

Table B.5.2.6.1-2: Primary eye irritation scores (24-hour exposure)

Animal no.	Tissue	Effect	Eye irritation scores Time after exposure and rinsing				
			1 hour	24 hours	48 hours	72 hours	7 days
12	Cornea		0	0	0	0	0
	Iris		0	0	0	0	0
	Conjunctiva	Redness	2	1	0	0	0
		Swelling	0	0	0	0	0
		Ulceration	0	0	0	0	0
39	Cornea		0	0	0	0	0
	Iris		0	0	0	0	0
	Conjunctiva	Redness	2	1	0	0	0
		Swelling	0	0	0	0	0
		Ulceration	0	0	0	0	0
43	Cornea		0	0	0	0	0
	Iris		0	0	0	0	0
	Conjunctiva	Redness	2	1	0	0	0
		Swelling	0	0	0	0	0
		Ulceration	0	0	0	0	0

Conclusion:
Azinphos-methyl has a slight eye irritation potential.
According to the study author, R1582 is to be regarded as having no irritant effect on the mucous membranes.

B.5.2.6.2 RABBIT

Zorbas, 1994: Primary eye irritation study with technical grade Guthion® in rabbits. Bayer file no. 7353 of 2 August 1994; study no. 94-335-AK. Miles Inc., Agriculture Division, Toxicology, Stilwell, Kansas, USA. Dates of experimental work: 5 July 1994 to 8 July 1994.
Guidelines and GLP:
The study was performed in accordance with OECD guideline 405, July 1992, and corresponding US EPA and Japanese guidelines. The study is GLP compliant.
The study is considered acceptable.
Material and methods:
Guthion technical grade (67 mg; batch No. 3030050/230205204; purity 92.2 %), was placed in the left conjunctival sac of 6 young adult male New Zealand White rabbits (source: Small Stock Industries, Pea Ridge, AR, USA). The right eye served as a control. The treated eyes were evaluated for irritation of the cornea, iris and conjunctiva at 1, 24, 48 and 72 hours following treatment, according to the criteria of US-EPA-FIFRA, Pesticide Assessment Guidelines, Guideline 81-4, November 1984.
Findings:
The test substance caused transient iridial irritation (grade 1) as well as conjunctival chemosis (grade 1 or 2) and discharge (grade 1 or 2), but the cornea showed no lesions or other signs of irritation. All signs of irritation were present 1 hour after exposure and had resolved in all instances by 48 hours after dosing.

Table B.5.2.6.2-1: Primary eye irritation scores (non-rinsed)

Animal no.	Tissue	Effect	Eye irritation scores Time after exposure			
			1 hours	24 hours	48 hours	72 hours
15	Cornea		0	0	0	0
	Iris		0	0	0	0
	Conjunctiva	Redness	0	0	0	0
		Chemosis	2	0	0	0
		Discharge	2	0	0	0
16	Cornea		0	0	0	0
	Iris		0	0	0	0
	Conjunctiva	Redness	0	0	0	0
		Chemosis	1	1	0	0
		Discharge	2	0	0	0
19	Cornea		0	0	0	0
	Iris		0	0	0	0
	Conjunctiva	Redness	0	0	0	0
		Chemosis	2	0	0	0
		Discharge	1	0	0	0
21	Cornea		0	0	0	0
	Iris		0	0	0	0
	Conjunctiva	Redness	0	0	0	0
		Chemosis	2	1	0	0
		Discharge	1	0	0	0
23	Cornea		0	0	0	0
	Iris		0	0	0	0
	Conjunctiva	Redness	0	0	0	0
		Chemosis	1	0	0	0
		Discharge	0	0	0	0
24	Cornea		0	0	0	0
	Iris		1	0	0	0
	Conjunctiva	Redness	0	0	0	0
		Chemosis	2	2	0	0
		Discharge	2	0	0	0

Conclusion:
Azinphos-methyl has a slight eye irritation potential.

B.5.2.7 SKIN SENSITIZATION

B.5.2.7.1 GUINEA PIG

Flucke, 1986: E 1582 (c.n. azinphos-methyl) Study for skin sensitizing effect on guinea pigs (Magnusson and Klingman's maximization test). Report no. 15003 of 21 August 1986; study no. T 8021276. Bayer AG, Institute of Toxicology, Wuppertal-Elberfeld, Germany. Dates of experimental work: November 1985.
Guidelines and GLP:
The study was performed in accordance with OECD guideline 406, adopted 17.07.1992 (Guinea Pig Maximisation Test of Magnusson and Kligman). The study is GLP compliant.
The study is considered acceptable.
Material and methods:
A group of 20 (treated) and a group of 10 (control) male guinea pigs (strain: Bor:DHPW (SPF); source: Winkelmann, Borchen, Germany; body weight range 275-375 g) were given intradermal injections according to procedures described in OECD

guideline 406. The test substance (E1582, batch no. 230 505 073 = 233 596 230, purity 92.8 %) was diluted with Cremophor EL 2 % v/v in sterile physiological saline as vehicle.
1st injection site pair (cranial): Freund's complete adjuvant diluted 1:1 with vehicle, 0.1 ml.
2nd injection site pair (medial): 1 % E1582 diluted with vehicle, 0.1 ml.
3rd injection site pair (caudal): 1 % E1582 diluted with vehicle and applied 1:1 with Freund's complete adjuvant, 0.1 ml.
One week later, the application sites were shorn and irritated for 24 hours with 0.2 ml 10 % sodium lauryl sulphate in paraffin oil and covered for 48 hours with occlusive dressing containing 12.5 % formulated E1582.
1st challenge: 12.5 % formulation, 24 hours occlusive topical application 3 weeks after intradermal induction.
2nd challenge: not performed, as the results of the first challenge were clear.
Assessment of skin reactions: 24 and 48 hours after removal of the patches, the skin reactions were scored. The number of the control sites which showed signs of irritation were subtracted from the number of test article sites which showed signs of irritation (= corrected values). The corrected results formed the basis for a comparative assessment of both the treated and the control animals.
Findings:
Taking into account the corrected values after the challenge, the test article had a sensitizing effect on 19/20 (95 %) of the treated animals.

Table B.5.2.7.1: Positively reacting animals upon 1st challenge with 12.5 % E 1582

Treated group (20 animals)		Control group (10 animals)	
Test dressing	Control dressing	Test dressing	Control dressing
20	1	5	5
Corrected value: 19 (95 %)		0 (0 %)	

Conclusion:
Azinphos-methyl is potentially skin allergenic and has a skin sensitizing effect on guinea pigs in the maximisation test of Magnusson and Kligman.

B.5.2.7.2 GUINEA PIG

Porter, Craigo and Hartnagel, 1987: Dermal sensitization evaluation of Guthion® technical in the guinea pig. Report no. MTD0015 of 29 June 1987, Bayer toxicology report no. 884; Miles Laboratories, Inc., Toxicology Department, Elkhart, IN, USA. Dates of experimental work: 23 March 1987 to 23 April 1987.
Guidelines and GLP:
The study was performed in accordance with OECD guideline 406, adopted May 12, 1981 (Buehler Test) and the corresponding EC guideline. The study is GLP compliant.
The study is considered acceptable.
Material and methods:
One group of 15 (treated) and three groups of 5 (2 vehicle control groups, positive control DNCB) male Hartley outbred albino guinea pigs (source: Harlan Sprague Dawley, Indianapolis, USA, body weight range 295-359 g) received 3 topical inductions (0, 7 and 14 d) of 25 % Guthion technical (batch no. 79-R-225-42, purity 88.8 %) or 0.05 % DNCB (1-chloro-2,4-dintrobenzene), formulated in 50 % ethanol/water as vehicle, under occlusive dressing for 6 hours, followed by a two week resting period. On day 28, the above-mentioned test formulations and the vehicle were applied under occlusive dressing as the challenge dose.
Evaluation of skin reactions: 24 and 48 hours after removal of the patches. Skin reactions (erythema) were scored in animals treated with the test compounds azinphos-methyl and DNCB and compared to the controls treated with the vehicle only.

Findings:
Challenge of Guthion-induced guinea pigs resulted in a slight to moderate erythematous response in 7/15 (47 %) of the treated animals at 24 hours which persisted in 6 of the 7 animals at 48 hours. A faint reaction in 2/5 controls was observed at 24 hours only.

Table B.5.2.7.2 Dermal scores following challenge

Azinphos-methyl		DNCB	
Induced	Naive control	Induced	Naive control
7/15 = 0.47 #	2/5 = 0.40 #	4/5 = 0.80 #	0 #
15/30 = 0.50 @	2/10 = 0.20 @	17/10 = 1.70 @	0 @

Incidence: number of animals showing positive response at either 24 or 48 hours divided by the number of animals tested
@ Severity: mean of all test grades at 24 and 48 hours, corrected by subtracting the reading at vehicle site from that at test article site

Conclusion:
Azinphos-methyl is potentially skin allergenic and has a skin sensitizing effect on guinea pigs in the Buehler test.
According to the study authors, azinphos-methyl is a weak sensitizer having the potential to elicit a sensitization response in occasional susceptible individuals.

B.5.2.7.3 GUINEA PIG

Heimann, 1987: E1582 technical (common name: azinphos-methyl) Study of skin sensitisation effect on guinea pigs (Bühler patch test). Bayer report no. 16188 of 5 November 1987; Bayer AG, Toxicology Department, Wuppertal-Elberfeld, Germany. Dates of experimental work: March to May 1986.
Guidelines and GLP:
The study was performed in accordance with OECD guideline 406, adopted May 12, 1981 (Buehler Test) and corresponding US EPA and EC guidelines. The study is GLP compliant.
The study is considered acceptable.
Material and methods:
One test group and 2 control groups each of 12 male DHPW strain guinea pigs (source: Winkelmann, Borchen, Germany, body weight range: 309-373 g) received 3 topical inductions (1 per week) of 12.5 % E1582 technical (batch no. 230505073 = 233596230, purity 92.4-92.8 %) in 2 % Cremophor/saline vehicle, under occlusive dressing for 6 hours, followed by a two week resting period. First challenge dose was 6 % E1582 technical (= highest non-irritant concentration) applied for 6 hours under occlusive dressing. Second challenge dose was 0.6 % E1582 technical given 2 weeks after first challenge. Evaluation of skin reactions: 48 and 72 hours after patch removal. Skin reactions were evaluated by subtracting the number of animals with an irritant reaction on the control side from the number of animals with an irritant reaction on the test compound side for both the treated and the control group.
Findings:
After the 1st challenge, definite skin reactions were observed on the test compound side in 6 animals (50 %), compared to only 2 mildly reacting animals in the control group. After the 2nd challenge, mild reactions were observed in 1 test and 2 control animals which were of doubtful significance.

Table B.5.2.7.3-1: Number of animals with skin reactions; 1st challenge with 6 % E1582

Number of animals with skin reactions			
E1582 group		Control group 1	
Test patch	Control patch	Test patch	Control patch
6	1	2	2

Table B.5.2.7.3-2: Number of animals with skin reactions; 2nd challenge with 0.6 % E1582

Number of animals with skin reactions			
E1582 group		Control group 2*	
Test patch	Control patch	Test patch	Control patch
1	0	2	0

* 2 animals excluded in the evaluation (entire depilated area reddened)

Conclusion:
Azinphos-methyl is potentially skin allergenic and has a skin sensitizing effect on guinea pigs in the Buehler test.
According to the study authors, the skin sensitising properties induced by azinphos-methyl were elicited only after application of relatively high concentrations (6 %), suggesting a threshold for the contact-allergic reaction.

B.5.3 SHORT-TERM TOXICITY

Short-term studies were performed in rats, dogs and rabbits by oral (dietary), inhalative or dermal administration.
In rats, ChE inhibition was evident at dietary concentrations of 20 ppm and above, and there was some evidence of complete recovery within 30 days after cessation of exposure. Marked cholinergic effects and mortality occurred at 50 ppm and above following administration for more than 4 weeks. Limited histopathological examination revealed no specific organ toxicity at dose levels up to 100 ppm. The NOEL in rats was 5 ppm, equivalent to about 0.25 mg/kg bw/d.
In dogs, ChE inhibition was evident at 10 ppm and above. General clinical findings (diarrhoea, impaired general condition, reduced weight gain) and signs of cholinergic stimulation (spasms, tremors) occurred at 25 and 100 ppm and above, respectively, and mortality at 400 ppm. Comprehensive histopathological examination of dogs receiving doses up to 125 ppm for 52 weeks revealed no evidence of organ toxicity, although the activity of certain mixed function oxidase enzymes was increased at this dose level. The NOEL in dogs was 5 ppm, equivalent to 0.15-0.16 mg/kg bw/d.
Inhalative exposure of rats revealed ChE inhibition and decreased body weight gain in males at air concentraions of 50 µg/l for 1 hour/day over 10 days and 4.72 µg/l for 6 hours/day over 12 weeks. The NOEL for inhalative exposure for 6 hours/day over 12 weeks was 1.24 µg/l.
Dermal application to both abraded and non-abraded skin of rabbits over 3 weeks revealed slight reduced weight gain and ChE inhibition in RBC at 20 mg/kg bw/d. The NOEL for dermal exposure was 2 mg/kg bw/d.

Table B.5.3-1: Summary of the short-term toxicity studies

Study	Dose levels	NOEL (mg/kg bw/d)	Targets/main effects	Reference
28 day dietary; Wistar rat	0, 5, 20, 50 ppm	0.35-0.46 [5 ppm]	ChE ↓	Eiben et al., 1983
16 wk dietary SD rat	0, 2, 5, 20 ppm	0.25* [5 ppm]	ChE ↓	Doull and Rehfuss, 1956
16 wk dietary SD rat (males)	0, 50, 100 ppm	<2.5* [<50 ppm]	Clinical signs, mortality, bw ↓, ChE ↓	Doull and Anido, 1957
12 wk dietary Mongrel dog	5, 10, 20, 50 ppm	0.125** [5 ppm]	ChE ↓	Doull and Anido, 1957
19 wk dietary Beagle dog	0, 20, 50, 100, 200, 400 ppm	<0.5** [<20 ppm]	Clinical signs, mortality, bw ↓, ChE ↓	Loser and Lorke, 1967
52 wk dietary Beagle dog	0, 5, 25, 125 ppm	0.15-0.16 [5 ppm]	Clinical signs, bw ↓, ChE ↓, MFO ↑	Allen et al., 1990
10 day inhal. (1 h) Rat (females)	10, 25, 50 µg/l	25 µg/l	ChE ↓	DuBois and Flynn, 1969
12 wk inhal. (6 h) Wistar rat	0, 0.195, 1.24, 4.72 µg/l	1.24 µg/l	bw ↓, ChE ↓	Klimmerle, 1976
3 wk dermal (6 h) NZW rabbit	0, 2, 20 mg/kg bw/d	2	bw ↓, ChE ↓	Flucke and Schilde, 1980

* or ** Value calculated by means of a conversion factor of 0.05 or 0.025

B.5.3.1 ORAL STUDIES

B.5.3.1.1 RAT

Eiben, Schmidt and Löser, 1983: R1582 (common name: azinphos-methyl, the active ingredient of ®Guthion). Toxicity study on rats with particular attention to ChE activity (28-day feeding study as a range-finding test for a 2-year study). Bayer report no. 11813 of 18 May 1983; Bayer AG, Institute of Toxicology, Wuppertal, Germany. Dates of experimental work: June 1982 to July 1982.

Guidelines and GLP:
The method used was the internal standard at the time the study was performed. When the study was performed, GLP was not compulsory.
Main deviations from current OECD guidelines: Hematological and clinical biochemistry determinations (with the exception of ChE), and histopathological examinations were not performed.
The study is considered supplementary.
Material and methods:
Azinphos-methyl (batch no. 230105056; purity 93.3 %) at concentrations of 0, 5, 20 and 50 ppm in pulverized rat diet containing 1 % peanut oil was fed *ad libitum* to groups of 5 male and 5 female SPF BOR:WISW (SPF/Cpb) strain rats (source: Winkelmann, Borchen, Germany; mean body weight: 233 g for males and 158 g for females) for a period of 28 days. Observations: health status and clinical signs once or twice per day; food consumption and body weights weekly; ChE activity in RBC and plasma determined on days 1, 4, 14 and 28 by a modified Ellmann et al. (1961) method in retro-orbital blood; brain ChE activity at necropsy; gross pathology on all rats sacrificed at the end of treatment. Heart, lungs, liver, spleen, kidneys, adrenals, gonads were weighed and preserved but not examined histopathologically.
Statistics: Significance of inter-group differences with Mann-Whitney and Wilcoxon U-Test at levels of α = 5 % and 1 %.
Findings:
The average doses ingested at 5, 20 and 50 ppm were 0.35, 1.30 and 3.37 mg/kg bw/d for males and 0.46, 1.54 and 3.96 mg/kg bw/d for females, respectively. There were no effects on appearance, behavior and mortality up to and including 50 ppm, no detrimental effect on body weight gain and food consumption, and gross pathological examination gave no indications of test compound-related organ damage up to and including 50 ppm. There was a dose- and time-dependent inhibition of plasma and erythrocyte ChE activity at dose levels of 20 ppm and above. Brain ChE activity was inhibited in the female group receiving 50 ppm in the diet.

Table B.5.3.1.1-1: ChE activity (U/ml) in rats

Dose level / Sex	0 ppm M/F	5 ppm M/F	20 ppm M/F	50 ppm M/F
Plasma, 1 d	0.51/1.26	0.52/1.21	0.47/1.39	0.46/1.07
4 d	0.49/1.25	0.50/1.27	0.39/1.28	0.37/0.70*
14 d	0.48/1.34	0.48/1.41	0.38/1.42	0.32*/0.63**
28 d	0.50/1.66	0.50/1.57	0.37*/1.70	0.37*/0.65**
RBC, 1 d	2.81/2.89	3.26/3.01	3.81/3.11	3.13/3.11
4 d	2.58/2.84	2.61/3.03	2.74/3.28**	2.37/2.61
14 d	2.71/3.04	2.80/3.10	2.45/2.53*	2.13**/2.02**
28 d	2.66/3.23	2.73/2.87	2.57/2.53**	2.30**/2.11**
Brain, 28 d	1.40/1.39	1.68/1.32	1.56/1.14	1.28/0.65**

Mean values from 5 animals/sex/group; * $p < 0.05$; ** $p < 0.01$

Conclusion:
The NOEL for dietary administration of azinphos-methyl to rats over 28 days was 5 ppm, equivalent to 0.35 mg/kg bw/d in males and 0.46 mg/kg bw/d in females. A dose-related inhibition of plasma and erythrocyte ChE activity was observed at dietary concentrations of 20 and 50 ppm, whereas brain ChE activity was inhibited at 50 ppm in females only.

B.5.3.1.2 RAT

Doull and Rehfuss, 1956: The effect of diets containing Guthion (Bayer 17147) on rats (final report). Report of 3 May 1956; Department of Pharmacology,

University of Chicago, Chicago 37, Ill., USA. Dates of experimental work: not specified.
Guidelines and GLP:
The test method employed was the internal standard at the time the study was performed. When the study was performed, GLP was not compulsory.
Main deviations from current OECD guidelines: Hematological and clinical biochemistry determinations (with the exception of ChE) were not performed. Histopathology was carried out on 2 male and 2 female rats per dose level only. There is no mention of analysing diet to confirm dose levels. The 12-page report is not very detailed (missing raw data).
The study is considered supplementary.
Material and methods:
Groups of 13 Sprague-Dawley rats/sex (source: not specified, body weight range 72-84 g) were fed diets containing 0, 2, 5 and 20 ppm Guthion (25 % wettable powder, batch no. and purity not specified) for 16 weeks (in the report also designated as 120 days). At the end of the exposure period, 3-5 animals/group were used for ChE determinations in various tissues, using the method of DuBois and Mangun (1947). The remaining animals were killed at various times after cessation of exposure to plot ChE activity recovery. General appearance and cholinergic signs were recorded daily, food consumption and body weight at regular intervals. Autopsy, with gravimetry of principal organs, and histopathology of 17 organs and tissues (including femoral nerve and spinal cord) were performed on 2 males and 2 females/group at the end of the treatment period. Statistical examinations: not applied to the data.
Findings:
No mortalities occurred and food consumption, growth and general appearance were not affected by treatment. At 20 ppm, ChE activity was inhibited in the brain by about 10 % and in RBC and serum by about 30 %. When treatment at 20 ppm was discontinued, inhibition of ChE activity persisted for less than 4 days in serum, for about 10 days in brain, and for more than 20 days in RBC. There were no appreciable changes in the gross or microscopic appearance of the tissues, including central and peripheral nervous tissue.

Table B.5.3.1.2-1: ChE activity (µl CO_2/10 min/50 mg wet tissue) in rats after 16-week feeding

Dose level (ppm) Sex	0 M/F	2 M/F	5 M/F	20 M/F
Serum	6.9/24.4	6.4/22.4	6.5/22.7	4.4/18.5
RBC	10.1/10.3	9.4/10.7	9.3/9.4	6.1/6.4
Brain	99.3/98.2	99.7/96.7	95.0/97.9	90.2/84.5
Submaxillary gland	22.6/24.1	23.2/23.3	22.8/23.8	23.1/24.1

Mean values from at least 3 animals/group

Conclusion:
The NOEL for dietary administration of azinphos-methyl to rats over 16 weeks was 5 ppm, equivalent to about 0.25 mg/kg bw/d (calculated by means of a conversion factor of 0.05). At dietary concentrations of 20 ppm, inhibition of ChE activity in serum, RBC, and brain was observed.

B.5.3.1.3 RAT

Doull and Anido, 1957: Effect of high dietary levels of Guthion on rats (final report). Report of 5 June 1957; Department of Pharmacology, University of Chicago, Chicago 37, Ill., USA. Dates of experimental work: not specified.
Guidelines and GLP:
The test method employed was the internal standard at the time the study was performed. When the study was performed, GLP was not compulsory.

Main deviations from current OECD guidelines: Only male rats and only two dose levels were used. Hematological and clinical biochemistry determinations (with the exception of ChE) were not performed. There is no mention of analysing diet to confirm dose levels. The 7-page report is not very detailed (missing raw data).
The study is considered supplementary.
Material and methods:
Groups of at least 18 weanling, male Sprague-Dawley rats (source: not specified, body weight range 55-69 g) were fed diets containing 0, 50 and 100 ppm Guthion (25 % wettable powder, batch no. and purity not specified) for 16 weeks. After 8 weeks exposure, 3 animals/group were killed, necropsied and used for ChE determinations in various tissues, using the method of DuBois and Mangun (1947). Nine organs, including testis, were weighed and 13 tissues and organs preserved for histopathological evaluation. The remaining animals, except 2 or 3 rats/group from the Guthion-treated groups which were allowed a 3 week treatment-free period, were exposed for 16 weeks and subjected to the same terminal procedures. General appearance and cholinergic signs recorded daily, body weights weekly. Statistical examinations: not applicable.
Findings:
The dietary levels of both 50 and 100 ppm resulted in marked cholinergic effects (diarrhea, salivation, lacrimation, muscular tremors and fasciculations) and mortalities of 45 % and 56 %, respectively. Terminal body weights at 50 and 100 ppm were about 10 % and 18 % lower than in control animals. Inhibition of ChE was most marked in RBC and brain and the values did not fully recover during a 3-week period on control diet. There was no evidence of testicular atrophy at any dose level.

Table B.5.3.1.3: ChE activity (µl CO_2/10 min/50 mg wet tissue) in male rats

Dose level (ppm) Time (weeks)	0 8/16	50 8/16	100 8/16
Serum	5.6/5.4	3.3/3.3	2.6/2.0
RBC	9.2/10.0	2.1/2.9	1.4/2.7
Brain	92.6/98.0	37.9/51.4	21.4/24.4
Submaxillary gland	19.7/21.7	14.3/17.9	10.9/12.8

Mean values from at least 3 animals/group

Conclusion:
The NOEL for dietary administration of azinphos-methyl to male rats over 16 weeks was <50 ppm. At dietary concentrations of 50 ppm and above, clinical symptoms, mortality, reduced body weight gain and inhibition of ChE was observed.

B.5.3.1.4 DOG

Doull and Anido, 1957: Determination of the safe dietary level of Guthion for dogs. Report of 1 June 1957; Department of Pharmacology, University of Chicago, Chicago 37, Ill., USA. Dates of experimental work: not specified.
Guidelines and GLP:
The test method employed was the internal standard at the time the study was performed. When the study was performed, GLP was not compulsory.
Main deviations from current OECD guidelines: No control group and only one animal/sex/dose group were used. The dogs were not of a defined breed and differed considerably in body weight. Hematological and clinical biochemistry determinations (with the exception of ChE) as well as gross necropsy and histopathological examinations were not performed. There is no mention of analysing diet to confirm dose levels. The 5-page report is not very detailed (missing body weight and food consumption data; missing raw data).
The study is considered supplementary.

Material and methods:
Groups of one animal/sex of adult mongrel dogs (source not specified, body weight range 6-17 kg) were fed diets containing 0, 5, 10, 20 or 50 ppm Guthion (25 % wettable powder, batch no. and purity not specified) for 12 weeks. Animals were observed several times each day for signs indicating cholinergic stimulation and body weights were recorded weekly. Serum and erythrocyte ChE activities were determined weekly by the method of DuBois and Mangun (1947) and compared with control values determined twice weekly for 3 weeks before the start of treatment. Statistical examinations: not applied to the data.
Findings:
The dietary level of 50 ppm Guthion produced a decrease of about 25 % in the serum ChE activity by the end of the 12-week period. The erythrocyte ChE activity was inhibited in a dose-dependent fashion at levels of 10, 20, and 50 ppm, reaching about 50 % with the highest concentration. There were no differences between the sexes. During the whole period of treatment, there were no toxic symptoms at any concentration of Guthion administered.

Table B.5.3.1.4-1: Pre-treatment ChE activity (µl CO_2/10 min/50 mg wet tissue) in male and female dogs

Dose level (ppm)	5	10	20	50
Serum, male	11.6 ± 0.6	15.3 ± 0.8	17.7 ± 0.6	13.7 ± 0.9
RBC, male	16.6 ± 0.4	17.9 ± 0.9	13.0 ± 0.4	17.4 ± 0.7
Serum, female	12.2 ± 0.5	14.2 ± 0.8	16.0 ± 0.7	11.7 ± 0.7
RBC, female	13.9 ± 0.8	13.6 ± 0.4	12.4 ± 0.4	13.9 ± 0.2

Values obtained from at least 5 duplicate determinations expressed as mean and deviation from mean; 1 animal/sex/group

Table B.5.3.1.4-2: Approximate ChE activity (% of pre-treatment value) of male and female dogs after 12-week feeding

Dose level (ppm)	5	10	20	50
Serum	110	115	98	75
RBC	100	85	75	50

Mean values as displayed graphically; 1 animal/sex/group

Conclusion:
The NOEL for dietary administration of azinphos-methyl to dogs over 12 weeks was 5 ppm, equivalent to about 0.125 mg/kg bw/d (calculated by means of a conversion factor of 0.025). At dietary concentrations of 10 ppm and above, a dose-related inhibition of ChE activity was observed.

B.5.3.1.5 DOG

Löser and Lorke, 1967: Cholinesterase activity in dogs following administration of Gusathion in food. Report no. 292 of 13 April 1967; Bayer AG, Institute of Toxicology, Wuppertal-Elberfeld, Germany. Dates of experimental work: not specified.
Guidelines and GLP:
The test method employed was the internal standard at the time the study was performed. When the study was performed, GLP was not compulsory.
Main deviations from current OECD guidelines: Only one animal/sex/dose group were used. Hematological and clinical biochemistry determinations (with the exception of ChE) as well as gross necropsy and histopathological examinations were not performed. Body weight and food consumption data were not reported. There is no mention of analysing diet to confirm dose levels.
The study is considered supplementary.

Material and methods:
Groups of one male and one female beagle dogs (source: Appleton, England; age: approximately 10 months) were fed diets containing chemically pure Gusathion active ingredient (batch no. and purity: not specified) at dietary concentrations of 0, 20, 50, 100, 200 and 400 ppm for 19 weeks. ChE activity was determined in whole blood before treatment, after 2 days, and then weekly, according to the method of Filz et al. (1965).
Findings:
General condition was impaired at 50 ppm and above, and levels of 100 ppm and above induced signs of cholinergic stimulation (occasional muscular spasms and tremors at 100 ppm, and uncoordinated movements and intense muscular spasms at 200 and 400 ppm). Doses of 50 ppm and above caused weight loss and animals fed 400 ppm frequently refused to eat. The female animal dosed at 400 ppm died after study week 9. Dose-related blood ChE inhibition was observed in all dose groups; significant inhibition (>20 %) occurred already at the lowest dose of 20 ppm.

Table B.5.3.1.5: Whole blood ChE activity (% of pre-treatment value) in male and female dogs (mean values from 1 animal/sex/group)

Dose level	0 ppm	20 ppm	50 ppm	100 ppm	200 ppm*	400 ppm*
Pre-dose	100	100	100	100	100	100
2 days	89.0	100	98.5	84.0	88.0	50.5
1st week	100	100	90.0	85.6	49.1	35.4
2nd week	97.5	100	87.3	68.0	76.0	25.7
3rd week	100	92.5	84.2	61.6	60.0	31.0
4th week	91.6	58.4	73.0	37.6	62.0	24.8
5th week	98.4	69.1	57.1	48.0	47.2	20.4
6th week	93.2	85.8	73.7	50.4	57.4	27.4
7th week	94.9	76.7	76.0	52.0	63.0	29.2
8th week	94.9	78.3	64.7	49.6	63.8	20.4
9th week	100	90.8	70.7	54.4	47.1	18.6
10th week	100	93.4	68.4	53.7	45.4	18.6
11th week	100	84.3	71.4	49.6	50.0	35.4
12th week	100	79.2	61.7	45.6	38.9	24.8
13th week	96.8	90.0	73.7	40.0	38.9	18.6
14th week	100	71.8	63.2	56.1	62.0	40.4
15th week	100	84.1	74.4	69.6	77.8	44.2
16th week	95.0	81.8	66.2	60.8	61.1	26.6
17th week	100	86.8	75.2	73.6	57.4	31.8
18th week	100	95.0	74.5	60.8	-	-
19th week	100	93.5	82.0	50.5	-	-

* Animals given this concentration were started 2 weeks later

Conclusion:
The NOEL for dietary administration of azinphos-methyl to dogs over 19 weeks was <20 ppm. Inhibition of ChE occurred at dose levels of 20 ppm and above, clinical symptoms at 50 ppm and above, and mortality at 400 ppm.

B.5.3.1.6 DOG

Allen, Frei, Janiak, Luetkemeier, Vogel, Biedermann and Wilson, 1990: 52 week oral toxicity (feeding) study with azinphos-methyl (E1582) in the dog. Report no. R5064 of 31 May 1990; Research & Consulting Company AG, 4452 Itingen, Switzerland. Dates of experimental work: 29 March 1988 to 4 April 1989.
Guidelines and GLP:
The method employed conformed with OECD guideline 452, 1981. The study is GLP compliant.
The study is considered acceptable.

Material and methods:
Groups of 4 male and 4 female pure bred Beagle dogs (source: Laboratory Research Enterprises Inc., Kalamazoo, MI 49009, USA; age: 4-4.5 months, body weight range: 5.4-8.5 kg) were treated with azinphos-methyl (batch no. 233896032, purity 91.9 %) by admixture in the diet (pelleted) at concentrations of 0, 5, 25 and 125 ppm for 52 weeks. Stability, homogeneity and content of the test article in the diet were analyzed at regular intervals. Statistics: Univariate one-way analysis of variance; Dunnett's t-test (for normal distributed data) or steel-test (many-one rank test).
Findings:
Clinical findings:
The average doses ingested at 5, 25 and 125 ppm were 0.15, 0.69 and 3.84 mg/kg bw/d for males and 0.16, 0.78 and 4.33 mg/kg bw/d for females, respectively. All animals survived the 52 week treatment period. The only clinical sign observed was diarrhoea in animals of both sexes receiving 125 ppm, and in the males receiving 25 ppm in the diet. The food consumption was unaffected by treatment at all dose levels. Two of the 4 males receiving 125 ppm failed to gain weight, resulting in a slightly reduced group mean weight gain. There was no effect on the weight gain of females receiving 125 ppm and animals of both sexes receiving 5 or 25 ppm. Hearing tests and ophthalmoscopy did not reveal any treatment-related findings.
Laboratory findings:
Plasma ChE activity was reduced in males at 125 ppm and in females at 25 and 125 ppm from week 4. Similarly, erythrocyte ChE activity was reduced in both sexes at 25 and 125 ppm from week 4. Brain ChE activity was reduced in both sexes at 125 ppm at termination. Higher liver N-demethylase activity in both sexes and increased liver cytochrome P-450 activity in males were observed at 125 ppm. Albumin was slightly lower than in controls in males at 125 ppm. Hematological examinations and urinalysis did not reveal any treatment-related changes.
Pathology findings:
Gross pathology, organ weight analysis and histopathology did not reveal any treatment-related changes at any of the dose levels employed.

Table B.5.3.1.6: ChE (plasma, RBC: µmol-SH/ml, brain: µmol-SH/g), cytochrome P-450 (nmol/g) and N-demethylase (nmol/min/g) activity in dogs after 52-week feeding

Dose level (ppm) Sex	0 M/F	5 M/F	25 M/F	125 M/F
ChE, plasma	8.26/9.71	7.35/8.53	7.24/6.76	3.86**/4.56*
ChE, RBC	2.86/3.36	3.01/2.86	2.09/2.20*	0.41**/0.47**
ChE, brain	6.26/6.64	6.21/6.71	5.61/6.55	4.60**/5.33*
Cytochrome P-450	12.2/15.0	13.6/13.8	14.2/13.8	17.0*/17.2
N-demethylase	130.0/126.9	164.4/145.6	155.0/123.7	173.8/164.4

Mean values of 4 animals/sex/group; * $p < 0.05$; ** $p < 0.01$;

Conclusions:
The NOEL for dietary administration of azinphos-methyl to dogs over 52 weeks was 5 ppm, equivalent to 0.15 mg/kg bw/d in males and 0.16 mg/kg bw/d in females. At dietary concentrations of 25 ppm and above, clinical signs (diarrhea in males) and a dose-related inhibition of ChE activity were observed.

B.5.3.2 INHALATION STUDIES

B.5.3.2.1 RAT

DuBois and Flynn, 1969: Effects of repeated inhalation exposure of rats to Guthion. Report of 2 December 1969; Toxicity Laboratory, University of Chicago, Chicago, Illinois 60637, USA. Dates of experimental work: not specified.

Guidelines and GLP:
The test method employed was the internal standard at the time the study was performed. When the study was performed, GLP was not compulsory.
Main deviations from current OECD guidelines: Only female rats were used. The exposure time was 1 hour a day for 5 or 10 days only. Test conditions and exposure data were not reported in detail. Hematological and clinical biochemistry determinations (with the exception of ChE), gross necropsy and histopathological examinations were not performed. The 3-page report is not very detailed (missing raw data).
The study is considered supplementary.
Material and methods:
Groups of 6 adult female rats (source, strain and body weight range not specified) were exposed by inhalation to nominal Guthion (batch no. 9050026, purity not specified) concentrations of 10, 25 or 50 µg/l air as an aerosol in ethanol for 1 hour a day for 5 or 10 days. Exposure conditions for the control group were not specified. In the exposed groups, 3 animals/group were killed after 5 days exposure and 3 animals/group after 10 days exposure and ChE activity in brain, submaxillary gland and serum were determined by the method of DuBois & Mangun (1947). Statistical evaluation: not applicable.
Findings:
No inhibition of ChE activity was seen in the animals exposed to 10 and 25 µg/l (nominal) for one hour each day for 5 and 10 days. After 10 days of exposure to 50 µg/l Guthion, however, ChE activity was clearly inhibited in all the samples.

Table B.5.3.2.1: ChE activity (µl CO_2/10 min/50 mg tissue) in female rats (daily exposure: 1 h)

Dose level (µg/l air)	Time (days)	Brain	Submaxillary gland	Serum
Control	-	93.8 (92.3-94.6)	25.4 (23.6-27.8)	12.0 (11.2-12.6)
10	5	98.4 (96.1-102.4)	24.0 (23.5-24.4)	11.4 (1.9-11.7)
	10	91.3 (84.2-98.6)	23.9 (22.9-25.6)	12.8 (12.3-13.6)
25	5	85.6 (76.7-92.9)	21.5 (18.1-23.5)	13.8 (10.9-16.7)
	10	76.7 (72.8-81.9)	20.5 19.3-21.4)	12.9 (12.4-13.5)
50	5	85.8 (82.9-90.7)	21,8 20.7-23.4)	13.2 (12.0-15.3)
	10	48.2 (44.1-56.0)	17.5 15.7-20.2)	8.9 (8.2-9.7)

Values (mean and range) from 3 animals/group in the exposed groups; number of control animals not specified

Conclusions:
The NOEL for inhalative administration of azinphos-methyl to female rats over 10 days (exposure 1 h/d) was 25 µg/l. At air concentrations of 50 µg/l, inhibition of ChE activity was observed after 10 days of exposure.

B.5.3.2.2 RAT

Kimmerle, 1976: Subchronic inhalation toxicity of azinphos-methyl in rats. Bayer AG, Institute of Toxicology, Wuppertal-Elberfeld, Germany. Dates of experimental work: not specified. Published in: Arch Toxicol 35:83-89, 1976.

Guidelines and GLP:
The test method employed was the internal standard at the time the study was performed and is in general compliance with OECD guideline 413. When the study was performed, GLP was not compulsory.
The study is considered acceptable.
The 7-page report, however, is not very detailed (missing raw data).
Material and methods:
Groups of 10 male and 10 female SPF Wistar rats (source: Winkelmann, Borchen, Germany; body weight range: 150-170 g and 130-150 g, respectively) were exposed in dynamic inhalation chambers for 6 hours/day, 5 days/week for 12 weeks to azinphos-methyl (technical grade; batch no. and purity not specified) as a liquid aerosol in polyethylene glycol 400/ethanol (1:1) vehicle at mean aerosol concentrations of 0, 0.195, 1.24 and 4.72 mg/m^3 air. The control group was exposed to vehicle only. Effective concentrations were monitored by GC. The droplet size was measured with a cascade impactor: 97 % of droplets had a diameter of 1 ± 0.5 µm.
Animals were inspected daily and body weights recorded weekly. ChE activity in plasma and RBCs were determined fortnightly by the method of Pilz and Eben (1967). Laboratory examinations (haematology, ALT, AST, AP, urea, creatinine and bilirubin) were performed after 12 weeks exposure followed by necropsy, gross examination, organ weights and microscopic evaluation of principle organs. Brain ChE activity was determined using the method of Ammon (1933).
Statistical methods: not specified.
Findings:
Clinical findings: The exposure of rats to azinphos-methyl aerosol at concentrations of up to 4.72 mg/m^3 did not cause any significant changes in appearance and behaviour. Only the male rats exposed to the highest concentration showed a significant lower body weight gain.
Laboratory findings: The hematological, the clinical biochemistry (except ChE activities) and the urinalysis parameters were not altered by treatment. Plasma and erythrocyte ChE activities were inhibited by about 30-40 % at the highest concentration whereas brain ChE activity was not significantly changed (values not reported).
Pathology findings: None of the organ weights showed significant differences between groups. No morphological change or variation from normal was seen in any of the tissues examined that was considered to be associated with the treatment.

Table B.5.3.2.2-1: ChE activity (µ equivalents of acetylcholine) in male rats (daily exposure: 6 h)

Dose	0 mg/m^3		0.195 mg/m^3		1.24 mg/m^3		4.72 mg/m^3	
Time	Plasma	RBC	Plasma	RBC	Plasma	RBC	Plasma	RBC
0 wk	2.36	3.80	2.26	3.81	2.30	3.32	2.39	3.52
2 wk	2.20	3.77	2.27	3.98	2.39	3.51	1.93*	2.83
4 wk	3.26	3.64	3.12	3.90	3.09	3.36	2.96	1.90*
6 wk	2.32	3.73	2.38	3.74	2.38	3.14	2.26	2.39*
8 wk	2.37	3.73	2.31	3.66	2.49	3.26	1.92*	2.54*
10 wk	2.34	4.08	2.32	3.91	2.28	3.49	1.96*	2.89*
12 wk	2.46	4.04	2.41	3.99	2.44	3.36	2.07	2.27*

Mean values of 5 animals/group
* Inhibition of ChE more than 20 %

Table B.5.3.2.2-2: ChE activity (µ equivalents of acetylcholine) in female rats (daily exposure: 6 h)

Dose	0 mg/m^3		0.195 mg/m^3		1.24 mg/m^3		4.72 mg/m^3	
Time	Plasma	RBC	Plasma	RBC	Plasma	RBC	Plasma	RBC
0 wk	3.30	3.50	3.32	3.40	3.28	3.27	3.35	3.34
2 wk	3.32	3.44	3.35	3.65	3.31	3.55	3.09*	2.83
4 wk	4.24	3.06	4.20	3.21	4.14	3.06	3.08*	1.90*
6 wk	3.96	3.93	3.96	3.79	3.86	3.83	3.30	2.39*
8 wk	3.60	3.63	3.65	3.85	3.58	3.62	3.93*	2.54*
10 wk	4.00	3.88	4.04	4.05	3.98	4.02	3.58	2.89*
12 wk	4.05	3.55	4.02	3.56	4.00	3.97	3.45	2.27*

Mean values of 5 animals/group
* Inhibition of ChE more than 20 %

Conclusions:
The NOEL for inhalative administration of azinphos-methyl to rats over 12 weeks (exposure 6 h/d) was 1.24 mg/m^3 (1.24 µg/l). At air concentrations of 4.72 mg/m^3 (4.72 µg/l), decreased body weight gain in males and inhibition of ChE activity in plasma and RBC were observed.

B.5.3.3 DERMAL STUDIES

B.5.3.3.1 RABBIT

Flucke and Schilde, 1980: Gusathion-M active ingredient (R1582). Subacute cutaneous study of toxicity to rabbits (Study no.: Gusathion/003 = R1582/004). Report no. 8959 of 20 February 1980; Bayer AG, Institute of Toxicology, Wuppertal-Elberfeld, Germany (Dates of experimental work: 26 July 1979 to 15 August 1979).
Guidelines and GLP:
The test method employed was the internal standard at the time the study was performed and is almost in compliance with OECD guideline 410. When the study was performed, GLP was not compulsory.
Main deviations from current OECD guidelines: Only 2 dose levels and a control were used. The test groups each consisted of 3 animals/sex with intact skin and 3 animals/sex with abraded skin.
The study is considered acceptable.
Material and methods:
Groups of male and female New Zealand White rabbits (source: Hacking & Churchill, Huntingdon, UK; body weight range: 2.2-3.0 kg) received Gusathion active ingredient (batch Eg. 30.03.79 I, purity 94.1 %) at dose levels of 0, 2 and 20 mg/kg bw/d by dermal application to intact skin (3 animals/sex/group) and to abraded skin (3 animals/sex/group). The animals were treated with 0.5 ml of the test substance formulated in Cremophor EL/water for 6 hours/day, 5 days/week over 3 weeks (application on 15 days). The test sites of 7x9 cm were left uncovered and were cleaned after each exposure. General appearance, behaviour, scoring of visual skin reactions after each application according to Draize and skin fold thickness were recorded daily. Body weights were recorded weekly. Haematology, blood chemistry (ALT, AST, sugar, plasma and RBC ChE) and urinalysis were performed before and at the end of the test. Plasma and RBC ChE activities were additionally measured after application 10. Brain ChE was determined at autopsy after application 15, followed by gravimetry of principal organs and histopathology. Statistical examination: not applicable.
Findings:
Body weight was slightly retarded in females treated at 20 mg/kg bw/d. There was no skin reaction attributable to the test material and no systemic or target organ toxicity was evident.

At the high dose of 20 mg/kg bw/d, erythrocyte ChE activity was slightly inhibited without evidence of cumulation, at days 10 and 15, irrespective of whether the skin was intact or abraded. Brain and plasma ChE were unaffected at all dose levels.

Table B.5.3.3.1: Erythrocyte ChE activity (U/ml) in male and female rabbits

Dose level (mg/kg bw/d) **Sex**	0 M/F	2 M/F	20 M/F
Intact skin, 0 d	1.74/1.65	1.98/1.86	1.64/1.78
Abraded skin, 0 d	2.00/1.57	2.17/1.92	1.74/2.16
Intact skin, 10 d	1.87/1.74	1.61/1.74	1.23/1.29
Abraded skin, 10 d	1.94/1.67	1.86/1.73	1.30/1.19
Intact skin, 15 d	1.80/1.83	1.83/1.64	1.38/1.24
Abraded skin, 15 d	2.19/1.70	2.05/1.69	1.36/1.26

Mean values of 3 animals/group

Conclusion:
The NOEL for dermal administration of azinphos-methyl to rabbits over 3 weeks was 2 mg/kg bw/d. At 20 mg/kg bw/d, slight growth retardation in females and inhibition of RBC ChE activity was observed.

B.5.4 GENOTOXICITY

The genotoxic potential of azinphos-methyl was studied *in vitro* in an extensive test battery suitable to assess gene mutations, chromosome aberrations and DNA perturbations using bacteria, yeast cells and mammalian cells, as well as *in vivo* on somatic cells by means of the micronucleus test and in germinal cells by the dominant lethal test. A test for recessive lethal mutations in *Drosophila melanogaster* was also conducted, with negative result (Waters et al., 1982).

Table B.5.4-1: *In vitro* mutagenicity studies

Point mutation assays			
Reverse mutation assay	+ S-9 mix	Negative	
(S. typhimurium)	- S-9 mix	Negative[1)]	Herbold, 1978
Reverse mutation assay	+ S-9 mix	Negative	
(S. typhimurium)	- S-9 mix	Negative	Herbold, 1988
Reverse mutation assay	+ S-9 mix	Negative	
(S. typhimurium)	- S-9 mix	Negative	Waters et al., 1982[2)]
Reverse mutation assay	+ S-9 mix	Negative	
(S. typhimurium)	- S-9 mix	Negative	Lawlor, 1987
Reverse mutation assay	+ S-9 mix	Negative	
(E. coli)	- S-9 mix	Negative	Waters et al., 1982[2)]
Reverse mutation assay	+ S-9 mix	Negative	
(S. cerevisiae)	- S-9 mix	Negative	Hoorn, 1983
Reverse mutation assay	+ S-9 mix	Negative	
(S. cerevisiae)	- S-9 mix	Negative	Waters et al., 1982[2)]
Forward mutation assay	+ S-9 mix	Positive	
Mouse lymphoma (L5178Y)	- S-9 mix	Negative	Waters et al., 1982[2)]
Chromosome aberration assays			
Cytogenetic study	+ S-9 mix	Positive*	
(Human lymphocytes)	- S-9 mix	Negative	Herbold, 1986
Cytogenetic study			
(Chinese hamster ovary cells)		Positive*	Alam et al., 1974[2)]
DNA damage assays			
Rec assay			
(S. typhimurium)		Negative	Waters et al., 1982[2)]
Pol test	+ S-9 mix	Negative	
(E. coli)	- S-9 mix	Negative	Herbold, 1984
Mitotic recombination	+ S-9 mix		
(S. cerevisiae)	- S-9 mix	Positive	Waters et al., 1982[2)]
Gene conversion/crossing-over	+ S-9 mix		
(S. cerevisiae)	- S-9 mix	Negative	Waters et al., 1982[2)]
Unscheduled DNA synthesis			
(Rat primary hepatocytes)		Negative	Myhr, 1983
Unscheduled DNA synthesis	+ S-9 mix		
(Human lung fibrobl. cells)	- S-9 mix	Negative	Waters et al., 1982[2)]
Sister chromatid exch. assay	+ S-9 mix		
(Chin. hamster ovary cells)	- S-9 mix	Negative	Waters et al., 1982[2)]
Sister chromatid exch. assay	+ S-9 mix		
(V79 cells)	- S-9 mix	Negative	Chen et al., 1982a,b[2)]

* Positive only at clear cytotoxic concentration.
1) Only the highest dose and the positive controls were tested without S-9 mix.
2) Published literature.

The large majority of the *in vitro* tests, and in particular all the *in vivo* assays, revealed no evidence of mutagenic or genotoxic potential of azinphos-methyl.

It must be mentioned, that the publicated results served as supplementary information only because not all methodical details were indicated in these publications.
The different types of test systems used and the results obtained in testing for the various genotoxic endpoints are listed in tables B.5.4-1 and B.5.4-2.

Table B.5.4-2: *In vivo* mutagenicity studies

Chromosome aberration assays		
Micronucleus test (Mouse somatic cells)	Negative	Herbold, 1979b
Micronucleus test (Mouse somatic cells)	Negative	Herbold, 1995
Micronucleus test (Mouse somatic cells)	Negative	Waters et al., 1982[1)]
Dominant lethal test (Mouse germ cells)	Negative	Herbold, 1979a
Dominant lethal test (Mouse germ cells)	Negative	Waters et al., 1982[1)]
Recessive lethal test Drosophila melanogaster)	Negative	Waters et al., 1982[1)]

1) Published literature.

B.5.4.1 *IN VITRO* STUDIES

B.5.4.1.1 TESTS ON BACTERIAL SYSTEMS

B.5.4.1.1.1 REVERSE MUTATION ASSAY ON *SALMONELLA TYPHIMURIUM*

Herbold, 1978: R1582 (Gusathion M active ingredient) Salmonella/microsome test to evaluate for point mutation.Report no.: 7965 of 4 December 1978; Bayer AG, Toxicology Department, Wuppertal, Germany. Dates of experimental work: November 1978.
Guidelines and GLP:
The test method employed was according to Ames et al., 1975 and the scientific standard at the time the study was performed. In part it is in compliance with the demands of OECD Guideline 471 (adopted 26 May 1983).
Deviation: The test with the metabolizing system was performed with the highest dose level of the test substance and the positive control substances only.
When the study was performed, GLP was not compulsory. The study is considered supplementary only since it does not fully comply with current standards.
Material and methods:
Azinphos-methyl (batch no.: 230705148/201-300; purity: 92.3 %) was tested on quadruplicate cultures of *Salmonella typhimurium* strains TA1535, TA1537, TA98 and TA100 at concentrations of 0, 4, 20, 100, 500, 2500 µg/plate, in the presence of S-9 mix. Positive control substances were Endoxan, 725 µg/plate (only TA1535 and TA100) and Trypaflavine, 250 µg/plate (only TA1537 and TA98). The positive and negative control substances and the highest dose level of the test substance were also tested in the absence of S-9 mix. The test substance was formulated in DMSO, which was also used as the negative control substance. For the activation experiments, S-9 mix was derived from adult male Sprague-Dawley rats, induced 5 days before preparation with a single intraperitoneal dose of Aroclor 1254, 500 mg/kg bw dissolved in peanut oil. The liver supernatant fluid was prepared and combined with an appropriate co-factor solution according to established technique.
Evaluation criteria: A reproducible, dose-dependent increase in the number of mutants to a level about double that of the negative control, obtained with at least one strain, is considered to be a positive result.

Findings:
Cytotoxicity test: Doses of azinphos-methyl up to 2500 µg/plate had no bacteriotoxic effect. However, at the highest dose of azinphos-methyl precipitation on the plates occurred.
Reverse mutation assay: None of the strains used showed a dose-related increase of mutants in comparison to the negative control. Conversely, the positive controls clearly increased the mutant count to far over double that of the negative control count, and thus demonstrated the sensitivity of the test system and the efficacy of the S-9 mix.
Conclusion:
There was no indication of a mutagenic effect on any of the tester strains employed.

B.5.4.1.1.2 REVERSE MUTATION ASSAY ON *SALMONELLA TYPHIMURIUM*

Herbold, 1988: E1582 (common name: Azinphos-methyl) Salmonella/microsome test to evaluate for point mutagenic effects. Report no.: 16689 of 6 May 1988; Bayer AG, Toxicology Department, Wuppertal, Germany. Dates of experimental work: start and completion in January 1988.
Guidelines and GLP:
The test method employed is in compliance with the demands of OECD Guideline 471 (adopted 26 May 1983). The study is GLP compliant (formal GLP/QAU declaration of the test facility included). The study is acceptable.
Material and methods:
Azinphos-methyl (batch no.: 233 796 036; purity: 92.5 %) was tested, first, on quadruplicate cultures of *Salmonella typhimurium* strains TA1535, TA1537, TA98 and TA100 at concentrations of 0, 150, 300, 600, 1200, 2400, 4800 and 9600 µg/plate, in the presence and absence of S-9 mix, and in the repeat test at concentrations of 0, 75, 150, 300, 600, 1200 and 2400 µg/plate (strains TA1535, TA100 and TA1537) and at 0, 150, 300, 600, 1200, 2400, 4800 and 9600 µg/plate (strain TA98). Positive control substances were sodium azide (SA, 10 µg/plate, TA1535), nitrofurantoin (NF, 0.2 µg/plate, TA 100), 4-nitro-1.2-phenylenediamine (NPDA, 0,5 ug/plate, TA98; 10 µg/plate, TA1537), 2-aminoanthracene (AA, 3 µg/plate, all strains).
The positive control substances, SA, NF and NPDA were tested without S-9 and AA with S-9 mix. The test substance was formulated in DMSO, which was also used as the negative control substance.
For the activation experiments, S-9 mix was derived from adult male Sprague-Dawley rats, induced 5 days before preparation with a single intraperitoneal dose of Aroclor 1254, 500 mg/kg bw dissolved in corn oil. The liver supernatant fluid was prepared and combined with an appropriate co-factor solution according to established technique.
Evaluation criteria: A reproducible, dose-dependent increase in the number of mutants to a level about double that of the negative control, obtained with at least one strain, is considered to be a positive result.
Findings:
Cytotoxicity test: Doses of 300 µg/plate and higher showed a bacteriotoxic effect, specific to strain. Therefore the cultures could only partly be used for evaluation and the experiments were repeated to get a sufficient number of evaluable plates. At 1200 µg/plate, the substance started to precipitate.
Reverse mutation assay: With none of the four strains used the test resulted in a dose-dependent increase in mutant counts over the negative control, whether the test was conducted with or without S-9 mix. Conversely, the positive controls clearly increased the mutant count to well over double those of the negative control count, and thus demonstrated the sensitivity of the test system and the efficacy of the S-9 mix.

Conclusion:
There was no indication of a mutagenic effect on any of the tester strains employed.

B.5.4.1.1.3 REVERSE MUTATION ASSAY ON *SALMONELLA TYPHIMURIUM*

Waters et al., 1982: Study of pesticide genotoxicity. In Fleck, R. and A. Hollaender (eds.): Genetic Toxicology; an agricultural perspective. Plenum Press New York and London. p. 275-326, 1982.
Guidelines and GLP:
The test was performed according to Ames et al., 1975.
GLP: No. The information of this published literature has supplementary value only since concentrations used are not clearly given.
Material and methods:
Azinphos-methyl was tested on the *S. typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100 in the presence or absence of S-9 mix.
Findings and conclusion:
There was no mutagenic effect either in the presence or absence of S-9 mix.

B.5.4.1.1.4 REVERSE MUTATION ASSAY ON *SALMONELLA TYPHIMURIUM*

Lawlor, 1987: Salmonella/mammalian-microsome plate incorporation mutagenicity assay (Ames test)- test article Guthion. Report no.: 920 of 5 August 1987; Microbiological Associates, Inc., Bethesda and Rockville, MD, USA. Dates of experimental work: 17 June 1987 to 5 August 1987.
Guidelines and GLP:
The test method employed is in compliance with the demands of OECD Guideline 471 (adopted 26 May 1983). The study is GLP compliant. A formal QAU declaration of the test facility is included. The study is acceptable with the limitations mentioned behind.
Material and methods:
Azinphos-methyl (batch no.: 79-R-225-42; purity: 88.8 %) was tested in a dose range finding test on the *Salmonella typhimurium* strain TA100 in concentrations of 0 and 10 - 10000 µg/plate. The first main test on triplicate cultures of the *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100 included concentrations of 0, 33, 100, 333, 1000 and 2000 µg/plate, a repeat test concentrations of 100, 333, 1000, 2000, 3333 and 4000 µg/plate. The test was performed in the presence and absence of S-9 mix.
Positive control substances were sodium azide (SA, 1 µg/plate, TA100 and TA1535), 2-nitrofluorene (9-NF, 3 µg/plate, TA98 and TA1538), 9-aminoacridine (9-AA, 75 µg/plate, TA1537), 2-aminoanthracene (AA, 2 µg/plate, all strains). The positive control substances, SA, 2-NF and 9-AA were tested without S-9 and AA with S-9 mix. The test substance was formulated in DMSO, which was also used as the negative control substance.
For the activation experiments, S-9 mix was derived from adult male Sprague-Dawley rats, induced 5 days before preparation with a single intraperitoneal dose of Aroclor 1254, 500 mg/kg bw dissolved in corn oil. The liver supernatant fluid was prepared and combined with an appropriate co-factor solution according to established technique.
Evaluation criteria: A reproducible, dose-dependent increase in the number of mutants to a level about double that of the negative control, obtained with at least one strain, is considered to be a positive result.
Findings:
Cytotoxicity test: In the dose range finding test on strain TA100 doses of Azinphos-methyl from 3333 µg/plate onward were bacteriotoxic. In contrast, in the two main tests no bacteriotoxicity was observed in any strain up to the highest dose tested.

Reverse mutation assay: Azinphos-methyl did not cause a positive response in any of the tester strains with or without metabolic activation. However, in the second main test with strain TA100 the mutant counts are not assessable at the highest concentration(s) since without bacteriotoxicity and with only slight precipitation the values are far under the characteristic number of spontaneous revertants (TA100: 80-240) (Table B.5.4.1.1.4-1). The positive controls increased the mutant count in excess of double the negative control count, and thus demonstrated the sensitivity of the test system and the efficacy of the S-9 mix.

Table B.5.4.1.1.4-1: Number of colonies of strain TA100 in the two main tests

1st test Dose (µg/plate)	TA100 (- S-9 mix)	TA100 (+ S-9 mix)	2nd test Dose (µg/plate)	TA100 (- S-9 mix)	TA100 (+ S-9 mix)
0	101	102	0	138	135
33	110	117	-		
100	112	116	100	127	129
333	127	123	333	131	145
1000	169	198	1000	162	201
2000	173	154	2000	215	91
			3333	25	86
			4000	3	20

Conclusion:
In the assessable dose range the test material did not cause a positive reponse in any of the tester strains employed, neither with nor without metabolic activation.

B.5.4.1.1.5 REVERSE MUTATION ASSAY ON *ESCHERICHIA COLI*

Waters et al., 1982: Study of pesticide genotoxicity. In Fleck, R. and A. Hollaender (eds.): Genetic Toxicology; an agricultural perspective. Plenum Press New York and London. p. 275-326, 1982.
Guidelines and GLP:
The test was performed according to the method of Bridges, 1972.
GLP: No. The information of this published literature has supplementary value only since concentrations used are not clearly given.
Material and methods:
Azinphos-methyl was tested on the *Escherichia coli* strain WP2 in the presence or absence of S-9 mix.
Findings and conclusion:
Azinphos-methyl had no mutagenic effect either in the presence or absence of S-9 mix.

B.5.4.1.1.6 REC ASSAY ON *SALMONELLA TYPHIMURIUM*

Waters et al., 1982: Study of pesticide genotoxicity. In Fleck, R. and A. Hollaender (eds.): Genetic Toxicology; an agricultural perspective. Plenum Press New York and London. p. 275-326, 1982.
Guidelines and GLP:
The method employed was similar to that published by Slater et al., 1971.
GLP: No. The information of this published literature has supplementary value only since concentrations used are not given.

Material and methods:
Azinphos-methyl was tested in two concentrations on two pairs of *S. typhimurium* strains, SL4700 and SL4525, and TA1978 and TA1538 (strains with an rfa⁻ mutation).
Findings and conclusion:
Azinphos-methyl was found not to induce DNA damage in this test system.

B.5.4.1.1.7 POL TEST ON *ESCHERICHIA COLI*

Herbold, 1984: R1582 (common name: azinphos-methyl): Pol-test on *E. coli* to evaluate for potential DNA damage. Report no.: 12478 of 22 February 1984; Bayer AG, Institute of Toxicology, Wuppertal-Elberfeld, Germany. Dates of experimental work: February 1984.
Guidelines and GLP:
The method employed was that of Rosenkranz and Leifer, 1980. For this method no OECD Guideline exists. When the study was performed GLP was not compulsory.
Material and methods:
Azinphos-methyl (batch no.: 230 205 060; purity: 91.1 %) was administered, in quadruplicate cultures, to *E. coli*, strain (K12)p3478 (repair deficient) and strain W3110 (repair proficient), at dose levels of 625, 1250, 2500, 5000 and 10000 µg/plate. The cultures were incubated for 24 hours and then the inhibition zone diameters were measured. Azinphos-methyl and the positive control material, methylmethanesulphonate (MMS, 10µl/plate), were formulated in DMSO, which also served as the solvent control. The negative control substance was chloramphenicol (CAP, 30µg/plate, vehicle not given). The test was done in the presence and absence of S-9 mix.
For the activation experiments, S-9 mix was derived from adult male Sprague-Dawley rats, induced 5 days before preparation with a single intraperitoneal dose of Aroclor 1254, 500 mg/kg bw dissolved in peanut oil. The liver supernatant fluid was prepared and combined with an appropriate co-factor solution according to established technique.
Evaluation criteria: A reproducible increase in the difference in inhibition zone diameter between the two strains of more than 2 mm was considered positive.
Findings:
None of the concentrations of azinphos-methyl produced an inhibition zone, either with or without metabolic activation. The positive control substance, MMS, clearly increased the difference in inhibition zone diameters.
Conclusion:
There was no evidence of DNA damage produced by azinphos-methyl up to and including a concentration of 10000 µg/plate.

B.5.4.1.2 TESTS ON YEAST CELLS

B.5.4.1.2.1 REVERSE MUTATION ASSAY ON *SACCHAROMYCES CEREVISIAE*

Hoorn, 1983: Mutagenic evaluation of R1582 (common name: azinphos-methyl) in the reverse mutation induction assay with *Saccharomyces cerevisiae* strains S138 and S211α. Report no.: R2503 of 16 June 1983; Litton Bionetics, Veenendal, The Netherlands. Dates of experimental work: 9 March 1983 to 2 June 1983.
Guidelines and GLP:
The method used was according to Pittman and Brusick, 1971. The study can be considered to be in compliance with the requirements of OECD Guideline 480 (adopted 23 October 1983). The study is GLP compliant. A formal QAU declaration of the test facility is included. The study is acceptable.

Material and methods:
Suspension cultures of *Saccharomyces cerevisiae* strains S138 and S211α were exposed to Azinphos-methyl (batch no.: 230205060; purity: 91.1 %) for 3 hours in the presence or absence of S-9 mix. The doses employed were 0, 33.3, 100, 333.3, 1000, 3333.3 and 10000 µg/ml. The test material and the positive control materials, ethylmethanesulphonate (EMS, 1 %, S138) or quinacrine mustard (QM, 10 µg/ml, S211α) and sterigmatocystine (SC, 5 µg/ml, both strains), were formulated in DMSO, which also served as vehicle control. EMS and QM were used without S-9 mix and SC with S-9 mix.
The cytotoxicity test was performed on strain S211α using 14 concentrations of test material in the range of 1.22 to 10000 µg/ml.
The metabolic activation system was a commercially available supernatant prepared from adult Sprague-Dawley rat liver pretreated with Aroclor 1254 according to established technique.
Evaluation criteria: If the solvent control value is within the normal range, a test material that produces a positive dose-response over 3 concentrations, with the highest increase equal to twice the solvent control value, is considered to be a mutagenic agent.
Findings:
Cytotoxicity test with strain S211α: The test revealed no cytotoxicity at any of the dose levels employed.
Reverse mutation assay with strain S138 and S211α: The results of the tests conducted both with and without S-9 mix revealed no increase in the number of revertants at any of the concentrations of azinphos-methyl used. Revertant frequencies were all comparable to the solvent controls. A marked increase in revertants/survivors was noted with the control substances, demonstrating the sensitivity of the system.
Conclusion:
The test material did not exhibit mutagenic activity in *S. cerevisiae* strains S138 and S211α under the test conditions employed.

B.5.4.1.2.2 REVERSE MUTATION ASSAY ON *SACCHAROMYCES CEREVISIAE*

Waters etal., 1982: Study of pesticide genotoxicity. In Fleck, R. and A. Hollaender (eds.): Genetic Toxicology; an agricultural perspective. Plenum Press New York and London. p. 275-326, 1982.
Guidelines and GLP:
The method employed was according to that published by Zimmerman, 1975.
GLP: No. The information of this published literature has supplementary value only since concentrations used are not given.
Material and methods:
Azinphos-methyl was tested on the yeast strain S. cerevisiae D7 which requires isoleucine for growth. Five concentrations of azinphos-methyl were assayed both with and without metabolic activation.
Findings and conclusion:
No mutagenic effect was found either in the presence or absence of S-9 mix.

B.5.4.1.2.3 MITOTIC RECOMBINATION IN *SACCHAROMYCES CEREVISIAE*

Waters et al., 1982: Study of pesticide genotoxicity. In Fleck, R. and A. Hollaender (eds.): Genetic Toxicology; an agricultural perspective. Plenum Press New York and London. p. 275-326, 1982.
Guidelines and GLP:
The method employed was according to that published by Brusick and Mayer, 1973.
GLP: No. The information of this published literature has supplementary value only since concentrations used are not given.

Material and methods:
The yeast strain S. cerevisiae D3 was used. Azinphos-methyl was tested in five concentrations both with and without metabolic activation.
Findings and conclusion:
Without further information, a positive response was declared in a tabulated summary, either with and without metabolic activation.

B.5.4.1.2.4 GENE CONVERSION AND MITOTIC CROSSING-OVER ASSAY IN *SACCHAROMYCES CEREVISIAE*

Waters et al., 1982: Study of pesticide genotoxicity. In Fleck, R. and A. Hollaender (eds.): Genetic Toxicology; an agricultural perspective. Plenum Press New York and London. p. 275-326, 1982.
Guidelines and GLP:
The method employed was according to that published by Zimmerman, 1975.
GLP: No. The information of this published literature has supplementary value only since concentrations used are not given.
Material and methods:
The yeast strain S. cerevisiae D7 was used and the frequency of mitotic recombinants, gene convertants and total aberrants was determined, both in the presence and absence of metabolic activation.
Findings and conclusion:
Azinphos-methyl was not mutagenic in this test system.

B.5.4.1.3 TESTS ON MAMMALIAN CELLS

B.5.4.1.3.1 FORWARD MUTATION ASSAY IN MOUSE LYMPHOMA (L5178Y) CELLS

Waters et al., 1982: Study of pesticide genotoxicity. In Fleck, R. and A. Hollaender (eds.): Genetic Toxicology; an agricultural perspective. Plenum Press New York and London. p. 275-326, 1982.
Guidelines and GLP:
The method employed was according to that published by Clive et al., 1979.
GLP: No. The information of this published literature has supplementary value only since concentrations used are not given.
Material and methods:
L5178 mouse lymphoma cells were used to detect if azinphos-methyl produced a mutation from cells heterozygous for thymidine kinase ($TK^{+/-}$) to cells homozygous for this enzyme ($TK^{-/-}$). The test was performed with or without a metabolic system.
Findings and conclusion:
Without further information, a positive response with metabolic activation and a negative response without activation was declared in a tabulated summary.

B.5.4.1.3.2 *IN VITRO* CYTOGENETICS ON HUMAN LYMPHOCYTES

Herbold, B.A.: E1582 (common name: azinphos-methyl): cytogenetic study with human lymphocyte *in vitro* to evaluate for harmful effect on chromosomes. Report no.: 15145 of 20 October 1986; Bayer AG, Institute of Toxicology, Wuppertal-Elberfeld, Germany. Dates of experimental work: 11 February 1986 to 24 June 1986.
Guidelines and GLP:
In general, the study can be considered to be in compliance with the requirements of OECD Guideline 473 (adopted 26 May 1983). Deviation: Only one preparation time was used after a cultivation time of 72 hours. The study is GLP compliant. A formal QAU declaration of the test facility is included. The study is acceptable.

Material and methods:
Human lymphocytes were exposed in 2 separate cultures to azinphos-methyl (batch no.: 230505073 = 233596230; purity: 91.9 %) at concentrations of 0, 1, 10 and 100 µg/ml in the absence of S-9 mix, and at 0, 5, 50 and 500 µg/ml in the presence of S-9 mix. Azinphos-methyl and the positive control substances cyclophosphamide (CP, 10 µg/ml, with S-9 mix) and mitomycin C (MMC, 0.1 µg/ml, without S-9 mix) were formulated in DMSO, which also served as the negative control. After cultivation of the cells for 48 hours, in the non-activated cultures the cells were exposed to azinphos-methyl for 24 hours and in the activated cultures for 2.5 hours. All cells were prepared after a total of 72 hours. Approximately 200 metaphases (100 per donor) per concentration, with or without S-9 mix, were examined for structural chromosomal changes.
For the activation experiments, S-9 mix was derived from adult male Sprague-Dawley rats, induced 5 days before preparation with a single intraperitoneal dose of Aroclor 1254, 500 mg/kg bw dissolved in corn oil. The liver supernatant fluid was prepared and combined with an appropriate co-factor solution according to established technique.
Evaluation criteria: The test is considered positive if there is a dose dependent and statistically significant increase in the chromosome aberration rate over the negative control. A dose-dependent increase lacking statistical significance or a significantly elevated aberration frequency which is not concentration related would be assessed equivocal.
Statistics: Chi^2 test.
Findings:
Cytotoxicity test with S-9 mix: A significant reduction in mitotic index was noted at 50 µg/ml to 86.9 % and at 500 µg/ml to 43.2 % of the solvent control. The positive control, MMC, reduced the mitotic index to 86.6 %. CP was without effect on mitotic frequency.
Cytotoxicity test without S-9 mix: The mitotic index fell significantly at 100 µg/ml azinphos-methyl to 28.0 % of the solvent control value.
Chromosome aberration assay without S-9 mix: There were no significant inter-group differences between the solvent control and the cultures treated with azinphos-methyl at any concentration.
Chromosome aberration assay with S-9 mix: Statistically significant, treatment-related variations in all parameters were noted at the highest concentration of 500 µg/ml. This concentration was clearly cytotoxic, inducing a reduction in mitotic index to 43.2 % relative to the solvent control. There were no effects at concentrations up to 50 µg/ml. The positive controls, MMC and CP, both exhibited a clear clastogenic effect, thus demonstrating the sensitivity of the system.

Table B.5.4.1.3.2-1: Summary of results without metabolic activation

Treatment	Metaphases with aberrations incl. gaps		Metaphases with aberrations excl. gaps		Metaphases with exchanges		Polyploidies n/evaluated metaphases (x)	
(µg/ml)	n	%	n	%	n	%	n/x	%
DMSO (0)	6	3.0	5	2.5			0/400	0
(1)	2	1.0	1	0.5			2/400	0.5
(10)	8	4.0	5	2.5			0/400	0
(100)	11	5.5	10	5.0	1	0.5	1/400	0.3
MMC (0.1)	44**	23.0	41**	21.5	7*	3.5	1/400	0.3

** $p < 0.01$, * $p < 0.05$

Table B.5.4.1.3.2-2: Summary of results with metabolic activation

Treatment	Metaphases with aberrations incl. gaps		Metaphases with aberrations excl. gaps		Metaphases with exchanges		Polyploidies n/evaluated metaphases (x)	
(µg/ml)	n	%	n	%	n	%	n/x	%
DMSO (0)	5	2.5	5	2.5	1	0.5	0/400	0
(5)	8	4.0	5	2.5			0/400	0
(50)	11	5.5	9	4.5	1	0.5	2/400	0.5
(500)	34*	17.5	34*	17.5	20*	10.0	10/300	3.3
CP (10)	35*	17.5	33*	16.5	1	0.5	1/300	0.3

* $p < 0.05$

Conclusion:
A clastogenic effect of azinphos-methyl was indicated only at the cytotoxic concentration of 500 µg/ml. Azinphos-methyl did not show any harmful effects on the chromosomes of human lymphocytes at concentrations up to 100 µg/ml without S-9 mix and at concentrations up to 50 µg/ml with S-9 mix.

B.5.4.1.3.3 *IN VITRO* CYTOGENETICS ON CHINESE HAMSTER OVARY CELLS

Alam et al., 1974: Chromosomal anomalies induced by the organic phosphate pesticide Guthion in chinese hamster cells. Chromosoma (Ber.), 49, 77-86, 1974.
Guidelines and GLP:
No reference or guideline was mentioned for the method employed.
GLP: No. The information of this published literature has supplementary value only since not all procedural details are specified.
Material and methods:
Triplicate cultures of CHO-K1 cells were treated with azinphos-methyl (batch no.: not given, purity: ca. 90 %) in concentrations of 60, 80, 100 and 120 ug/ml for 18 hours and the chromosomal changes determined.
Findings and conclusion:
At clear cytotoxic concentrations, chromosomal anomalies were observed.

B.5.4.1.3.4 UNSCHEDULED DNA SYNTHESIS ASSAY IN RAT PRIMARY HEPATOCYTES

Myhr, 1983: Evaluation of R1582 in the primary rat hepatocyte unscheduled DNA synthesis assay. Report no.: R2686 of November 1983; Litton Bionetics, Inc., Kensington, MD, USA. Dates of experimental work: 14 June 1983 to 19 September 1983. The method employed is based on that described by Williams, 1977 and according to OECD Guideline 482 (adopted 26 May 1983). The study is GLP compliant. A formal QAU declaration of the test facility is included. The study is acceptable.
Material and methods:
Primary rat hepatocytes from an adult male Fischer 344 rat were exposed to azinphos-methyl (batch no.: 230 205 060; purity: 91.1 %) in 3 replicate cultures for 18-19 hours. Azinphos-methyl was formulated in DMSO, which also served as the negative control. The concentrations of azinphos-methyl tested for nuclear labeling were: 0.25, 0.5, 1.0, 2.5, 5.0, 10.1, 25.1 and 50.3 µg/ml. This dose range was based on a preliminary cytotoxicity study performed at 15 concentrations of azinphos-methyl from 0.025 to 1005 µg/ml. The positive control substance was 2-acetyl aminofluorene (AAF, 0.05 µg/ml). Each culture received 1 µCi/ml ^{3}H-thymidine at the time of exposure to test and control substances. Following exposure, slides were prepared for autoradiography.

Evaluation criteria: The test substance is considered active in the UDS assay at applied concentrations that cause:
1. An increase in the mean net nuclear grain count to at least 6 grains per nucleus, after subtraction of the concurrent negative control value, and/or
2. An increase in the percentage of nuclei having 6 or more net grains to at least 10 % of the analyzed population, after subtraction of the concurrent negative control value, and/or
3. The percentage of nuclei with 20 or more grains to reach or exceed 2 % of the analyzed population.
Findings:
Cytotoxicity test: Concentrations in the range of 100.5-1005 µg/ml were lethal, and at 50.3 µg/ml only 21.5 % of the cells survived.
UDS assay: The nuclear labelling of the azinphos-methyl treated cultures was equivalent to the negative control level and did not meet the criteria for a positive test result. The positive control material, AAF, induced massive UDS.
Conclusion:
The test material was evaluated as inactive in the rat primary hepatocyte UDS assay.

B.5.4.1.3.5 UNSCHEDULED DNA SYNTHESIS ASSAY IN HUMAN LUNG FIBROBLASTS

Waters et al., 1982: Study of pesticide genotoxicity. In Fleck, R. and A. Hollaender (eds.): Genetic Toxicology; an agricultural perspective. Plenum Press New York and London. p. 275-326, 1982.
Guidelines and GLP:
The method employed was a variation of the technique reported by Simmon, 197Ba.
GLP: No. The information of this published literature has supplementary value only since methodical details (e. g. concentrations used, exposure time) are not given.
Material and methods:
Human lung fibroblasts were incubated with the test chemical and tritiated-thymidine, with or without metabolic activation, for a few hours. The DNA was extracted and the tritiated-thymidine content measured by liquid scintillation counting. Testing was performed at 5 concentrations of test substance and 6 replicates of each concentration to facilitate statistical evaluation.
Findings and conclusion:
Azinphos-methyl was found not to induce unscheduled DNA synthesis.

B.5.4.1.3.6 SISTER CHROMATID EXCHANGE ASSAY IN V79 CELLS

Chen, Sirianni and Huang, 1982a: Sister chromatid exchange in chinese hamster cells treated with seventeen organophosphorus compounds in the presence of a metabolic activation system. Environm. Mutag., 621-624, 1982.
Chen, Sirianni and Huang, 1982b: Sister-chromatid exchanges and cell-cycle delay in chinese hamster V79 cells treated with 9 organophosphorus compounds (8 pesticides and 1 defoliant). Mutat. Res., 103, 307-313, 1982.
Guidelines and GLP:
GLP: No. The information of this published literature has supplementary value only since not all methodical details are given in these references.
Material and methods:
V79 cells were treated with azinphos-methyl in concentrations of 0 (DMSO), 2.5, 5.0, 10.0, 20.0 µg/ml (without S-9 mix) or 0 (DMSO), 5.0, 10.0, 20.0, 25.0 µg/ml (with S-9 mix).
Findings and conclusion:
No sister chromatid exchanges were found in these investigations.

B.5.4.2 *IN VIVO* STUDIES

B.5.4.2.1 MICRONUCLEUS TEST IN THE MOUSE, ORAL ADMINISTRATION

Herbold, 1979: R1582, micronucleus test on mouse to evaluate for potential mutagenic effects. Report no.: 8521 of 19 July 1979; Bayer AG, Institute of Toxicology, Wuppertal-Elberfeld, Germany. Dates of experimental work: March 1979 to May 1979.
Guidelines and GLP:
The method employed was based on that of Schmid (1975), the principle of whose method was taken over into OECD Guideline 474 (adopted 26 May 1983). When the study was performed, GLP was not compulsory. The study is acceptable.
Material and methods:
Groups of 5 male and 5 female NMRI mice (source: S. Ivanovas GmbH, Kisslegg/Allgäu, Germany, body weight range 22-32 g) received azinphos-methyl (batch no. 230 705 148/201-300; purity 92.3 %) as 2 oral administrations at an interval of 24 hours. The dose levels employed were 2 x 2.5 and 2 x 5.0 mg/kg bw. The test material was suspended in 0.5 % Cremophor/water, which was the negative control material. Trenimon®, the positive control material, was administered intraperitoneally as a solution in water, at a dose level of 2 x 0.125 mg/kg bw. Six hours after the second dose, the animals were killed and femoral bone marrow smears were prepared. Azinphos-methyl dose selection was based on a preliminary test at dose levels of up to 2 x 7.5 mg/kg bw, at which dose one mouse died.
Evaluation: 1000 polychromatic erythrocytes (PCE)/animal were examined by microscope for the presence of micronuclei. The number of normochromatic erythrocytes (NCE)/1000 PCE was determined at the same time in order to detect non-test substance-related bone marrow depression or general effects of the test substance on bone marrow erythropoiesis.
Statistics: Non-parametric ranking test of Wilcoxon.
Findings:
There were no effects of azinphos-methyl on the incidence of micronuclei at oral dose levels up to and including 2 x 5 mg/kg bw. There was no effect on erythrocyte production, since there was no change in the ratio of normochromatic to polychromatic erythrocytes. By contrast, Trenimon® inhibited erythropoiesis and significantly increased the incidence of polychromatic erythrocytes with micronuclei.
Conclusion:
Azinphos-methyl was not mutagenic in the micronucleus test on the bone marrow of mice at oral dose levels of up to 2 x 5 mg/kg bw.

B.5.4.2.2 MICRONUCLEUS TEST IN THE MOUSE, INTRAPERITONEAL ADMINISTRATION

Herbold, 1995: E1582 - Micronucleus test on the mouse. Report no. 24015 of 24 May 1995; Bayer AG, Institute of Toxicology, Wuppertal-Elberfeld, Germany. Dates of experimental work: January to March 1995.
Guidelines and GLP: The study was run according to OECD Guideline 474 (adopted 26 May 1983).
GLP: Yes.
Material and methods:
Azinphos-methyl (batch no. 230405033; purity 92.2 %) was intraperitoneally administered to male and female mice (Hsd/Win: NMRI; source: Harlan Winkelmann Borchen, Germany) at a dose level of 5 mg/kg bw. Each group comprised 10 mice, 5 males and 5 females. The test material was suspended in 0.5 % Cremophor/water, which was the negative control material. The positive control material cyclophosphamide (CP, 20 mg/kg bw) was administered in water. Sixteen, 24 or 48 hours after the dosage, the animals were killed and femoral bone marrow smears were prepared (control groups after 24 hours only). The selection of dose was

based on a pilot test (5 animals/group, 5, 10, 20 and 100 mg/kg bw i. p.) in which all animals died from the dose of 10 mg/kg bw onward.
Evaluation: 1000 polychromatic erythrocytes (PCE)/animal were examined by microscope for the presence of micronuclei. The number of normochromatic erythrocytes (NCE)/1000 PCE was determined at the same time in order to detect non-test substance-related bone marrow depression or general effects of the test substance on bone marrow erythropoiesis.
Statistics: Non-parametric ranking test of Wilcoxon.
Findings:
General tolerance: Symptoms like apathy, spasm, difficulty in breathing, and lethality occured in the substance treated animals.
Assessment of clastogenic potential: No effect of azinphos-methyl on the incidence of micronuclei and on the erythrocyte production was noted in the substance treated animals. By contrast, CP significantly increased the incidence of polychromatic erythrocytes with micronuclei.
Conclusion:
Azinphos-methyl showed no clastogenic effect in this micronucleus test on the mouse.

B.5.4.2.3 MICRONUCLEUS TEST IN THE MOUSE

Waters et al., 1982: Study of pesticide genotoxicity. In Fleck, R. and A. Hollaender (eds.): Genetic Toxicology; an agricultural perspective. Plenum Press New York and London. p. 275-326, 1982.
Guidelines and GLP:
The method employed was that of Schmid, 1976.
GLP: No. The information of this published literature has supplementary value only since methodical details (e. g. used concentrations, solvent) are not given.
Material and methods:
Test substance was administered by oral gavage or intraperitoneal injection to male Swiss-Webster mice. Eight mice per group were used. 500 polychromatic erythrocytes from cardiac blood and 500 from bone marrow were examined.
Findings and conclusion:
Azinphos-methyl was not mutagenic in that micronucleus test in mice.

B.5.4.2.4 DOMINANT LETHAL TEST IN THE MOUSE

Herbold, 1979: R1582-Dominant lethal study on the male mouse to test for mutagenic effects. Report no.: 8425 of 7 June 1979; Bayer AG, Institute of Toxicology, Wuppertal-Elberfeld, Germany. Dates of experimental work: not specified.
Guidelines and GLP:
When the study was performed, no specific method was compulsory. In principle, the method used has been taken over into OECD Guideline 478 (adopted 4 April 1984). Deviations from this guideline: No information on a positive control group or historical data were given, the females were not inspected to check mating (see below), only one dose was used. When the study was performed, GLP was not compulsory. The study is acceptable.
Material and methods:
Azinphos-methyl (batch no. 230705148/201-300; purity 92.3 %) was administered as a single oral dose of 4 mg/kg bw, in Cremophor/water, to a group of 50 male NMRI mice (source: S. Ivanovas GmbH, Kisslegg/Allgäu, Germany, body weight range 31-43 g). A similar group of control mice received an equivalent volume of vehicle alone. The dose level of azinphos-methyl was selected on the basis of a preliminary experiment in which groups of 5 female mice received single oral doses of azinphos-methyl at levels of 2.5, 5.0 and 10.0 mg/kg bw. A dose of 2.5 mg/kg bw was tolerated without inducing symptoms of intoxication. The number of

untreated female mice used was 598 per experimental group (i. e. 50 females/mating).
Starting on the day of test substance administration, a series of 12 matings was performed, each mating interval lasting for 4 days. Each male was caged for 4 days with an untreated virgin female after which time the female was removed and replaced by another female. During the period of 12 matings, all germ cell stages present in the testes at the time of test substance administration were theoretically capable of effecting insemination and fertilizing eggs. The females were not inspected for the presence of a vaginal plug. In place of this, an interval of about 14 days from the mid-point of the mating period to the inspection of the female was established.
Evaluation: The dominant lethal test is capable of detecting artificially induced mutations (lethal factors) in the male germ cell by determining the early death of affected embryos. Thus, the uterus of each female was examined to determine pre-implantation and post-implantation losses, the criteria for assessment. The total implants, viable and dead implants (sum of the deciduomata, resorptions and dead embryos) and the corpora lutea were counted.
Statistics: Analysis of variance, Dunnett test, Kolmogorov-Smirnov test.
Findings:
General tolerance of the test substance: The male mice showed no symptoms of damage. Their food consumption, physical appearance and motor activity were unaffected and did not differ from the controls. There were no deaths.
Fertility: The fertility of the test group was unaffected by test substance administration.
Pre-implantation loss: There were no statistically significant differences in this parameter between the control and test groups.
Post-implantation loss: There were no statistically significant differences in this parameter between the control and test groups, either in terms of absolute numbers of dead implants or the ratio of dead implants to total implants.
Conclusion:
The dominant lethal test on male mice provided no evidence of a mutagenic effect of azinphos-methyl on any of the various stages of male germ cell development.

B.5.4.2.5 DOMINANT LETHAL TEST IN THE MOUSE

Waters et al., 1982: Study of pesticide genotoxicity. In Fleck, R. and A. Hollaender (eds.): Genetic Toxicology; an agricultural perspective. Plenum Press New York and London. p. 275-326, 1982.
Guidelines and GLP:
GLP: No. The information of this published literature has supplementary value only since methodical details (e. g. doses used) are not given.
Material and methods:
Test and vehicle control groups of 20 adult male ICR/SIM mice were used. The test substance was administered at three dose levels, the MTD and one half and one quarter of that. The test substance was dissolved in corn oil, added to ground diet and administered for a period of 7 weeks. At the end of the treatment period, each male was caged with 2 virgin females for a period of 7 days. Females were replaced weekly for a total of 8 weeks. Females were sacrificed mid-term of gestation and scored for early and late foetal deaths and living foetuses.
Findings and conclusion:
This dominant lethal test on male mice provided no evidence of a mutagenic effect of azinphos-methyl.

B.5.4.2.6 RECESSIVE LETHAL TEST IN *DROSOPHILA MELANOGASTER*

Waters et al., 1982: Study of pesticide genotoxicity. In Fleck, R. and A. Hollaender (eds.): Genetic Toxicology; an agricultural perspective. Plenum Press New York and London. p. 275-326, 1982.
Guidelines and GLP:
GLP: No. The information of this published literature has supplementary value only since methodical details (e. g. doses used) are not given.
Material and methods:
The sex-linked recessive lethal test can detect lethal point mutations and small deletions on the X-chromosomes which constitute about 20 % of the Drosophila genome. Male fruit flys were exposed to azinphos-methyl and crossed to untreated females. The male and female progeny of this mating were in turn mated with each other and the following progeny (F_2 generation and, if required, F_3 generation) was examined for mutation defects.
Findings and conclusion:
This sex-linked recessive lethal test on *Drosophila melanogaster* provided no evidence of a mutagenic effect of azinphos-methyl.

B.5.5 LONG-TERM TOXICITY AND CARCINOGENICITY

Long-term feeding studies in rats, mice and dogs revealed no specific toxicity to organs and tissues, including the nervous system. When compared with the short-term studies, no additional effects were revealed in either rats or dogs. No evidence of oncogenic potential of azinphos-methyl was obtained in long-term feeding studies in rats and mice. However, in one study in Osborne-Mendel rats (NCI, 1978), the incidence of pancreatic tumors (islet-cell adenomas and carcinomas) in the high-dose males (13 %) appeared to be elevated when compared to the pooled controls (2 %). Since the spontaneous incidence of this lesion varied from 0 % to 22 % in the performing laboratory, the incidence found in the high-dose males cannot be clearly implicated as a chemically induced effect. Similarly, in the same study, a significant higher incidence of thyroid follicular-cell neoplasms was found in the male low (32 %) and high (33 %) dose groups when compared with the matched control (11 %) and pooled control (8 %) groups. However, since the spontaneous incidence of these neoplasms in male Osborne-Mendel rats varied from 0 % to 43 % at the performing laboratory, the incidence found in azinphos-methyl-treated male rats cannot be clearly implicated as a chemically induced effect. Thus, the results of this study suggest but do not provide sufficient evidence for the carcinogenicity of azinphos-methyl in male Osborne-Mendel rats (NCI, 1978). The NOEL in rats, dogs and mice is 5 ppm in the diet, equivalent to dose levels of 0.25-0.31, 0.15-0.26 and 0.79-0.98 mg/kg bw/d, respectively.

Table B.5.5: Summary of the long-term toxicity and carcinogenicity studies

Study	Dose levels	NOEL (mg/kg bw/d)	Target/main effects	Reference
2 yr feeding; Cocker spaniel dogs	0, 5, 20/50, 50/100/150/300 ppm	5 ppm (0.15-0.26)	Clinical signs, ChE ↓	Worden et al., 1973
2 yr feeding; Wistar rat	0, 2.5, 5, 20, 50/100 ppm	5 ppm (0.25*)	Clinical signs, ChE ↓	Worden et al., 1973
80 wk feeding/115 wk observation; Osborne-Mendel rat	m: 0, 78, 156 ppm f: 0, 62.5, 125 ppm	m: <78 ppm (<3.9*) f: 62.5 ppm (3.1*)	Clinical signs, bw ↓, mortality ↑	NCI, 1978
2 yr feeding; Wistar rat	0, 5, 15, 45 ppm	5 ppm (0.25-0.31)	Clinical signs, bw ↓, ChE ↓	Schmidt, 1987
80 wk feeding/92 wk observation; B6C3F1 mice	m: 0, 31.3, 62.5 ppm f: 0, 62.5, 125 ppm	62.5 ppm (9.4**)	Clinical signs, bw ↓	NCI, 1978
104 wk feeding; CD-1 mice	0, 5, 20, 40 ppm	5 ppm (0.79-0.98)	ChE ↓	Hayes, 1985

* or ** Value calculated by means of a conversion factor of 0.05 or 0.15

B.5.5.1 DOG

Worden, Wheldon, Noel and Mawdesley-Thomas, 1973: Toxicity of Gusathion for the rat and dog. Huntingdon Research Centre, Huntingdon PE18 6ES, England. Dates of experimental work: not specified in detail (start of acclimatisation: February 1964). Published in: Tox Appl Pharmacol 24:405-412, 1973.

Guidelines and GLP:
The test method employed was the internal standard at the time the study was performed and is in general compliance with OECD guideline 452. When the study was performed, GLP was not compulsory.
Main deviations from current OECD guidelines: The test procedures are not described in detail. The 8-page report (publication) is not very detailed (misssing data for food consumption and body weight, for clinical, laboratory and pathological examinations; missing raw data).
The study is considered supplementary.

Material and methods:
Groups of 4 male and 4 female young pure-bred Cocker spaniels (source, age and body weight not specified) were administered gusathion (source and purity not specified) mixed with their diet over a period of two years. The initial concentrations were 0, 5, 20 or 50 ppm. The concentration in the high-dose group was increased from 50 to 100 ppm, and that in the mid-dose group from 20 to 50 ppm starting at week 37. Two further increases of the test substance concentration in the high-dose group took place; one after week 57 (from 100 to 150 ppm) and the other after week 84 (from 150 to 300 ppm). Blood and urine samples were taken at regular intervals and examined almost according to OECD guideline 452. The pathological examinations were not described.

Findings:
The average doses ingested at 5, 20-50 and 50-300 ppm were 0.15-0.24, 0.72-1.77 and 1.64-8.65 mg/kg bw/d for males and 0.16-0.26, 0.73-1.79 and 2.06-9.42 mg/kg bw/d for females, respectively.
In the high-dose group, decreased motility, tremor of the body musculature, muscular weakness and abnormal sitting posture were registered starting at week 85 (after increase from 150 to 300 ppm). A slight body weight loss was observed in 3 animals of this group, and one male dog died as a result of cholangitis.
A depression of plasma ChE activity was observed in the mid and high-dose groups, starting at week 37. Erythrocyte ChE activity was depressed to about 50 % of the control values at 100 ppm and to about 25 % of the control values at 150 ppm.
The results of the other examinations conducted (hematology, clinical biochemistry, organ weights, and histopathology including that of the central and peripheral nervous systems) showed no treatment-related changes.

Conclusion:
The NOEL for dietary administration of azinphos-methyl to dogs over 2 years was 5 ppm, equivalent to 0.15-0.24 mg/kg bw/d in males and 0.16-0.26 mg/kg bw/d in females. A depression of plasma and erythrocyte ChE activity was observed at 50 ppm and above, and clinical signs occurred at 300 ppm.

B.5.5.2 RAT

Worden, Wheldon, Noel and Mawdesley-Thomas, 1973: Toxicity of Gusathion for the rat and dog. Huntingdon Research Centre, Huntingdon PE18 6ES, England. Dates of experimental work: not specified in detail (start of acclimatisation: February 1964). Published in: Tox Appl Pharmacol 24:405-412, 1973.

Guidelines and GLP:
The test method employed was the internal standard at the time the study was performed and is in general compliance with OECD guideline 452. When the study was performed, GLP was not compulsory.
Main deviations from current OECD guidelines: The test procedures are not described in detail. The 8-page report (publication) is not very detailed (misssing data for food consumption and body weight, for clinical, laboratory and pathological examinations; missing raw data).
The study is considered supplementary.

Material and methods:
Groups of 40 male and 40 female Wistar rats (source: Manor Farm Breeding Laboratories, Staatsburg, NY) were administered gusathion (source and purity not specified) in the diet over a period of two years. The initial concentrations were 0, 5, 20 or 50 ppm. A supplementary group receiving 2.5 ppm and an additional control were introduced into the study at week 23. In the highest dose group, the concentration in the diet was increased from 50 to 100 ppm after 47 weeks. Blood and urine samples were taken at regular intervals and examined almost according to OECD guideline 452. The pathological examinations were not described.

Findings:
Several females exhibited signs of toxicity (convulsions) at the 100 ppm dose. There was a significant inhibition of plasma, erythrocyte and brain ChE activity at the highest dose group, more marked in females than in males. An initial inhibition of plasma and erythrocyte ChE activity was observed at 20 ppm. There was no evidence that the test substance had any effect on tumor incidence.
Conclusion:
The NOEL for dietary administration of azinphos-methyl to rats over 2 years was 5 ppm, equivalent to about 0.25 mg/kg bw/d (calculated by means of a conversion factor of 0.05). A depression of plasma and erythrocyte ChE activity was observed at 20 ppm and above, and clinical signs occurred at 100 ppm.

B.5.5.3 RAT

Schmidt, 1987: R1582 (common name: azinphos-methyl). Study of chronic toxicity and carcinogenicity to Wistar rats (Administration in the feed for up to 2 years). Report no. 16290 of 10 December 1987; study no. T 2015169; Bayer AG, Toxicology Department, Wuppertal, Germany. Dates of experimental work: December 1982 to December 1984.
Guidelines and GLP:
The method employed conformed with OECD guideline 453, adopted 12 May 1981. The study is GLP compliant.
The study is considered acceptable.
Material and methods:
Groups of 60 male and 60 female 5-6 week old Wistar rats (strain: Bor:WISW (SPF Cpb), source: Winkelmann, Borchen, FRG, body weight range: 73-89 g for males and 69-91 g for females) received R1582 technical ai (batch no. 79-R-225-42, purity 87.2 %) in the diet (with 1 % peanut oil) for 24 months at concentrations of 0, 5, 15 and 45 ppm. The dose levels were established on the basis of a previous 4-week range-finding study (Eiben et al., 1983). Ten rats/sex/group were killed and necropsied after 12 months. All animals on study, whether dying or being killed in a moribund condition during the study, or killed at scheduled sacrifice, were subjected to a thorough gross examination and their tissues preserved.
Statistical methods: Intergroup differences by U-test of Mann & Whitney and Wilcoxon, at the significance level $\alpha = 5$ % and $\alpha = 1$ % (two-tailed). In the case of remarkable differences, Fisher's exact test was used at the same levels of significance. Neoplastic lesions were evaluated according to Peto et. al. (1980) using the test for positive trend with respect to dose rates.
Findings:
General examinations:
The average doses ingested at 5, 15 and 45 ppm were 0.25, 0.75 and 2.33 mg/kg bw/d for males and 0.31, 0.96 and 3.11 mg/kg bw/d for females, respectively. Specific clinical signs were not observed, but animals (particularly females) receiving 45 ppm exhibited a higher incidence of alopecia than the controls. Mortality was unaffected by treatment at all dose levels. The feed consumption of females receiving 45 ppm was slightly increased (about 10 %), but there was no effect on the water consumption of any group. The body weights of males receiving 45 ppm were markedly lower during the entire period of the study (up to approximately 10 %).

Table: B.5.5.3-1: General observations in rats

Dose level (ppm) Sex	0 M/F	5 M/F	15 M/F	45 M/F
Mortality (%), 0-24 mo	18/22	14/20	12/26	14/26
Alopecia (%), 0-24 mo	13/30	7/37	8/43	25/83
Feed intake (g/kg bw/d), 0-24 mo	49.0/61.7	50.1/62.3	49.8/63.7	51.7/69.0
Body weight (g), 12 mo	401/242	389*/239	387*/239	367**/229**
Body weight (g), 24 mo	429/274	421/275	424/279	407**/273

* $p < 0.05$; ** $p < 0.01$

Clinical laboratory tests:
Clinical chemistry, hematology and urinalysis at 3, 6, 12, 18 and 24 months revealed no evidence of any treatment-related impairment or effect on functions of the organs and metabolism, or of any impairment of the blood or hemopoietic tissues, with the exception of ChE. At 45 ppm, there was marked inhibition of ChE in plasma and RBC (at 1, 3, 6, 12, 18 and 24 months) and in the brain (at 12 and 24 months). At 15 ppm, ChE activity in RBC was decreased in both sexes whereas ChE activity in plasma and brain was decreased significantly in females only. No treatment-related changes occurred at 5 ppm.

Table B.5.5.3-2: ChE activity (% of control) in rats

Dose level (ppm) Sex	0 M/F	5 M/F	15 M/F	45 M/F
Plasma, 3 mo	100/100	91/88	102/65**	60**/35**
Plasma, 1 yr	100/100	84/90	87/65**	54**/33**
Plasma, 2 yr	100/100	113/102	88/81	51**/38**
RBC, 3 mo	100/100	101/110**	88**/88**	77**/72**
RBC, 1 yr	100/100	102/101	82*/81**	73**/69**
RBC, 2 yr	100/100	88**/98	78**/84**	63**/71**
Brain, 1 yr	100/100	130**/112	137**/90	109/50**
Brain, 2 yr	100/100	117/102	112/79**	68**/45**

* $p < 0.05$; ** $p < 0.01$

Pathological examinations:
Gross pathology and organ weight analysis revealed no treatment-related changes after 12 and 24 months of treatment. Histopathological examination of animals from the control and treated groups revealed predominantly inflammatory or degenerative changes after 12 and 24 months that were typical spontaneous lesions of conventionally housed rats of the age and strain employed. The nature, incidence and distribution of these non-neoplastic lesions did not suggest an effect of treatment.
The nature, incidence and time of appearance of benign, malignant and multiple neoplastic changes in all test groups showed only a slight variation, and there was no shift in the normal spectrum of neoplasms in any of the R1582-treated groups. Thus, there was no indication of a carcinogenic effect.

Table B.5.3.3-3: Summary of the incidence of neoplasms in rats

Dose level (ppm)	0	5	15	45
Sex	M/F	M/F	M/F	M/F
Animals examined (no.)	50/50	49/50	50/50	50/49
Total neoplasms (no.)	40/48	38/50	26/42	38/41
Benign neoplasms (no.)	34/40	30/40	20/34	33/33
Malignant neoplasms (no.)	6/8	8/10	6/8	5/8
Animals with neoplasms (no.)	28/34	26/34	20/32	23/29
Animals with multiple neoplasms (no.)	7/10	6/13	4/9	11/9

Table B.5.3.3-4: Incidence of neoplasms in rats

Dose level (ppm)		0	5	15	45
Sex		M/F	M/F	M/F	M/F
Brain (#)		40/39	39/40	40/39	38/38
- Granular cell tumor	(b)	0/0	0/0	1/0	1/0
- Sarcoma, unclassified	(m)	1/0	0/0	0/0	0/0
- Neurinoma	(m)	0/0	1/0	0/0	0/0
- Astrocytoma	(m)	0/0	2/0	0/0	0/0
- Schwannoma	(m)	0/0	1/0	0/0	0/0
Eyes (#)		50/47	47/49	47/48	49/46
- Iridic leiomyoma	(b)	0/1	0/0	0/0	1/0
- Neurinoma	(b)	0/0	0/0	0/0	1/1
Lungs (#)		50/49	49/50	50/49	48/48
- Metastasis/carcinoma	(m)	1/0	0/4	0/1	0/0
- Metastasis/sarcoma	(m)	0/0	1/0	0/0	0/0
Pituitary gland (#)		48/49	48/47	50/48	47/47
- Adenoma	(b)	13/15	9/18	4/19	7/15
Adrenal glands (#)		50/49	48/50	50/48	48/48
- Pheochromocytoma	(b)	6/1	3/1	3/2	7/0
- Pheochromocytoma	(m)	0/0	1/0	1/0	0/0
- Ganglioneuroma	(b)	0/0	0/0	0/1	0/0
Thyroid gland (#)		49/49	48/49	49/47	48/45
- C-cell adenoma	(b)	8/7	9/9	9/3	10/4
- Follicular adenoma	(b)	0/0	0/0	1/0	0/0
- Medullary carcinoma	(m)	0/0	2/0	0/0	1/0
Liver (#)		50/49	49/50	50/49	48/48
- Hepatocellular adenoma	(b)	0/1	0/0	0/1	0/0
- Hepatocellular carcinoma	(m)	0/0	0/0	0/0	1/0
- Colangiocarcinoma	m)	0/0	0/0	0/0	1/0
Pancreas (#)		49/48	49/49	48/49	48/47
- Islet cell carcinoma	m)	0/0	0/0	1/0	0/1
Stomach (#)		49/48	49/49	48/48	47/45
- Adenocarcinoma	(m)	0/0	0/1	0/0	0/0
Hemolymphoret. syst. (no. exam.)		50/49	49/50	50/49	48/48
- Histiocytoma	(m)	2/0	0/1	0/0	0/1
- Malignant lymphoma	(m)	0/0	0/1	1/1	0/0
Lymph nodes (#)		49/49	48/50	49/46	47/46
- Hemangioma	(b)	0/0	1/0	0/1	0/0
- Hemangioendothelioma	(m)	0/0	0/0	1/0	1/0
Spleen (#)		50/49	49/49	50/49	46/48
- Hemangioendothelioma	(m)	0/0	0/0	0/0	0/1
- Sarcoma, unclassified	(m)	0/0	0/0	1/0	0/0
Kidneys (#)		50/49	49/50	50/49	48/48
- Adenocarcinoma	(m)	0/0	0/0	0/1	0/0
Urinary bladder (#)		50/48	49/49	50/48	48/48
- Carcinoma	(m)	0/0	0/0	1/0	0/0
Testes (#)		50/-	49/-	50/-	48/-
- Leydig cell tumor	(b)	4/-	5/-	1/-	3/-
- Mesothelioma	(m)	1/-	0/-	0/-	0/-
Ovaries (#)		-/49	-/50	-/48	-/47
- Granul. theca cell tumor	(b)	-/0	-/0	-/1	-/0
Uterus (#)		-/49	-/50	-/48	-/47
- Adenocarcinoma	(m)	-/4	-/7	-/2	-/3
- Squamous cell papilloma	(b)	-/0	-/1	-/0	-/0
- Granular cell tumor	(m)	-/1	-/0	-/0	-/0
- Endometrial polyp	(b)	-/9	-/6	-/3	-/10
- Leiomyosarcoma	(m)	-/0	-/0	-/1	-/0
Other locations (@)					
Adipose tissue (#)		0/0	0/1	0/0	0/1
- Lipoma	(b)	0/0	0/0	0/0	0/1
Body cavities (#)		0/1	0/2	0/0	1/2
- Hemangioendothelioma	(m)	0/0	0/0	0/0	1/0
Mammary glands (#)		1/8	0/6	0/12	0/13
- Fibroadenoma	(b)	0/3	0/3	0/3	0/2
- Adenocarcinoma	(m)	0/2	0/1	0/1	0/2
Skin (#)		4/9	1/5	1/5	6/5
- Lipoma	(b)	0/0	0/1	0/0	0/0
- Fibroma	(b)	0/0	0/0	0/0	1/0
- Squamous cell papilloma	(b)	0/0	0/0	0/0	1/0
- Trichoepithelioma	(b)	1/1	0/0	0/0	0/0
- Neurinoma	(m)	0/0	0/0	0/1	0/0
- Squamous cell carcinoma	(m)	1/1	0/0	0/1	0/0
Clitoral glands (#)		0/1	0/0	0/0	0/0
- Adenoma	(b)	0/1	0/0	0/0	0/0

No. of examined organs; @ tissues not systematically examined
(b) benign; (m) malignant

Conclusions:
The NOEL for dietary administration of azinphos-methyl to rats over 2 years was 5 ppm, equivalent to 0.25 mg/kg bw/d in males and 0.31 mg/kg bw/d in females. A dose related depression of ChE activity was observed at 15 ppm and above, and decreased body weight gain, increased food consumption and alopecia occurred at 45 ppm. There was no evidence that azinphos-methyl is tumorigenic in rats.

B.5.5.4 RAT

NCI, 1978: Bioassay of azinphosmethyl for possible carcinogenicity. National Cancer Institute (NCI), National Institutes of Health, Bethesda, Maryland 20014, USA. National Cancer Institute Carcinogenesis Technical Report Series No. 69, 1978. Dates of experimental work: not specified.
Kimmerle, 1980: Comments on the bioassay of azinphosmethyl for possible carcinogenicity (National Cancer Institute Carcinogenesis Technical Report Series No. 69, 1978). Mobay Chemical Corporation, Stanley Research Center, Stilwell, Kansas, USA; letter of 9 December 1980 to Dr. Reuver, Bayer AG, Leverkusen, Germany.
Guidelines and GLP:
The method employed was that of the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health. When the study was performed, GLP was not compulsory.
Main deviations from current OECD guidelines: Only two dose levels were used. The animals were exposed for 80 weeks only and not for the entire lifetime (or at least 24 months). In low- and high-dose males and high-dose females, the selected dose levels caused marked depression of body weight gain and seemed to

reach the maximum tolerated dose. The concurrent control group consisted of 10 male and 10 female rats only.
The study is considered supplementary.

Material and methods:
Groups of 50 male and 50 female 35 days old Osborne-Mendel rats (source: Battelle Memorial Institute, Columbus, Ohio, USA; body weight range not specified) received azinphos-methyl (Guthion®, Mobay Chemical Corp., Kansas City, Missouri, USA; batch no. not specified, purity 90 %) for 80 weeks by admixture in the diet (with 2 % corn oil) at concentrations of 125 and 250 ppm for males and 62.5 and 125 ppm for females. The concentrations offerred to male rats were reduced to 62.5 and 125 ppm as from week 21, resulting in time-weighted average doses of 78 and 156 ppm. The dose levels were selected on the basis of a previous subchronic feeding study conducted to estimate maximum tolerated doses of azinphos-methyl. A group of 10 male and 10 female rats served as matched controls; the pooled controls for statistical tests consisted of the matched controls combined with 95 male and 95 female untreated rats from similar bioassays of 10 other test chemicals. Following 80 weeks of exposure, the animals were retained untreated but under observation for a further 34 or 35 weeks. All animals were observed twice daily for signs of toxicity, weighed at regular intervals, and palpated for masses at each weighing. All animals found dead, killed in a moribund condition or killed at scheduled sacrifice were subjected to a gross and microscopic examination of major tissues, major organs, and all gross lesions.
Statistical methods: Survival analysis, Kaplan and Meier (1958), Cox (1972), and Tarone's (1975) extension of Cox's methods. Incidences of lesions, one-tailed Fisher exact test at significance level of 0.05, with Bonferroni inequality correction, and Cochran-Armitage test for linear trend in proportions, as well as time-adjusted analysis when appropriate. Statistical comparisons were made against the matched and pooled control groups.

Findings:
General examinations:
The mean body weights of low- and high-dose male and high-dose female rats were consistently lower than those of matched controls throughout the study, and the depression of body weight gain in males and high-dose females exceeded 10 % at most periods of time. Clinical symptoms (body tremors) were observed in high-dose animals after one week on study. In females, a significant positive dose-related trend in mortality (p=0.041) was observed.

Table B.5.5.4-1: Mortality and body weights in rats

Dose group	Control	Low dose	High dose
Sex	M/F	M/F	M/F
No. of animals initially in study	10/10	50/49#	50/50
Natural death	2/1	4/5	9/9
Moribund sacrifice	2/2	11/10	14/16
Terminal sacrifice	6/7	35/34	27/25
Survival rate (%)	60/70	70/68	54/50*
Body weight [g] (week 0) @	155/125	150/125	145/125
Body weight [g] (week 53) @	610/370	540/390	530/330
Body weight [g] (week 110) @	650/440	615/460	595/380

50 animals initially, but 1 animal was found to be a male animal
@ Approximate mean body weight, derived from graphically presented data
* p=0.041 for positive dose-related trend in mortality in females

Pathological examinations: Numerous tumors of the endocrine organs were observed in both dosed male and dosed female rats. Those of the adrenals in dosed males and females, the follicular cells of the thyroid in dosed females, the anterior pituitary in dosed males, and the parathyroid in dosed males occurred at statistically significant incidences when compared with the pooled controls, but

not with the matched controls, and they were not considered to be related to administration of the test compound.
In male rats, the results of the Cochran-Armitage test for positive dose-related trend on the combined incidence of islet-cell adenomas or carcinomas of the pancreas (pooled controls 2/92 [2 %], matched controls 0/9, low-dose 1/47 [2 %], high-dose 6/45 [13 %]) is significant, using either the pooled (p=0.008) or matched (p=0.033) controls. The result of the Fisher exact test comparing the incidence in the high-dose group with that in the pooled controls was also significant (p=0.015). Time-adjusted tests, eliminating animals that died before week 52 on study (pooled control 2/88 [2 %], matched controls 0/9, low-dose 1/47 [2 %], high-dose 6/44 [14 %]) resulted in essentially the same statistics as those of the non-adjusted tests. Since, however, the spontaneous incidence of this lesion in male Osborne-Mendel rats at the performing laboratory varied from 0 % to 22 %, with a mean of 2 %, the incidence found in the high-dose male rats can not be clearly implicated as a treatment-induced effect.

Table B.5.5.4-2: Summary of the incidence of neoplasms in rats

Dose group	Control	Low dose	High dose
Sex	M/F	M/F	M/F
No. of animals examined microscopically	10/9	49/48	49/46
No. of animals with primary tumors	7/7	40/37	41/26
No. of animals with benign (b) tumors	6/7	32/32	33/24
No. of animals with malignant (m) tumors	4/2	15/13	15/6
No. of animals with tumors uncertain b or m	1/0	1/0	0/0
No. of animals with secondary tumors	1/0	3/1	3/0

Table B.5.5.4-3: Incidence of neoplasms in rats

Dose group	Control	Low dose	High dose
Sex	M/F	M/F	M/F
Skin (@)	10/10	50/49	49/49
- Squamous cell carcinoma	0/0	1/0	0/0
- Fibroma	0/0	1/0	0/0
- Fibrosarcoma	0/0	1/0	1/0
- Keratoacanthoma	0/0	0/0	0/1
- Liposarcoma	0/1	0/0	0/0
- Mast cell sarcoma	0/0	0/0	1/0
Subcutaneous tissue (@)	10/10	50/49	49/49
- Liposarcoma	1/0	0/0	0/0
Lung (#)	10/9	49/48	48/46
- Alveolar/bronchiolar adenoma	0/0	1/1	0/0
- Lymphoma, metastatic	0/0	0/0	1/0
Bone marrow (#)	10/9	49/48	46/46
- Lymphoma, metastatic	0/0	1/0	0/0
Spleen (#)	9/9	49/43	47/41
- Leiomyoma	0/0	1/0	0/0
- Hemangioma	0/0	1/1	0/1
- Hemangiosarcoma	2/0	0/1	4/0
- Malignant lymphoma, NOS	0/0	2/0	1/0
Lymph node (#)	8/9	49/48	44/46
- Leiomyosarcoma, metastatic	0/0	0/0	1/0
- Malignant lymphoma, NOS	0/0	1/0	0/0
Skeletal muscle (@)	10/10	50/49	49/49
- Malignant lymphoma, histiocytic	1/0	0/0	0/0
- Rhabdomyosarcoma	0/0	1/0	0/0

Liver (#)	9/9	49/47	46/45
- Lymphoma, metastatic	0/0	1/0	1/0
- Adenoma, NOS	0/0	1/0	0/0
- Hepatocellular adenoma	1/2	3/2	5/4
- Adenocarcinoma, NOS	0/0	0/0	0/1
- Hepatocellular carcinoma	0/0	0/0	0/1
- Hemangiosarcoma, metastatic	0/0	0/1	0/0
Pancreas (#)	9/9	47/47	45/45
- Acinar cell adenoma	0/0	1/0	0/0
- Liposarcoma, metastatic	0/0	1/0	0/0
- Lymphoma, metastatic	0/0	1/0	0/0
Stomach (#)	9/9	47/46	47/44
- Leiomyosarcoma	0/0	0/0	1/0
- Hemangiosarcoma	0/0	0/1	0/0
Small intestine (#)	9/9	47/48	48/46
- Leiomyosarcoma, metastatic	0/0	0/0	1/0
Kidney (#)	10/9	49/48	47/45
- Transitional cell carcinoma	0/0	1/0	0/0
- Liposarcoma	0/0	2/0	0/0
- Multiple polyposis	0/1	0/0	0/0
Heart (#)	10/9	48/48	47/46
- Lymphoma, metastatic	0/0	0/0	1/0
- Fibrosarcoma, metastatic	0/0	1/0	0/0
- Hemangiosarcoma, metastatic	0/0	0/0	1/0
Pituitary (#)	9/8	46/44	43/41
- Adenoma, NOS	0/0	0/0	3/1
- Chromophobe adenoma	4/2	21/14	13/12
- Chromophobe carcinoma	0/0	0/0	2/0
- Cystadenoma, NOS	0/0	0/0	2/1
- Adenocarcinoma, NOS	0/0	0/8	0/1
Adrenal (#)	9/9	45/45	46/41
- Adenocarcinoma, NOS	0/0	1/0	3/0
- Cortical adenoma	1/1	3/4	7/8
- Pheochromocytoma	0/0	0/0	1/2
- Lymphoma, metastatic	0/0	0/0	1/0
Thyroid (#)	9/9	44/45	43/38
- Adenoma, NOS	0/1	2/2	2/1
- Adenocarcinoma, NOS	0/0	3/1	3/0
- Follicular cell adenoma	1/0	1/0	0/0
- Cystadenoma, NOS	0/0	7/4	10/3
- Cystadenocarcinoma, NOS	0/0	1/0	0/0
- Papillary adenocarcinoma	0/0	0/0	0/1
- Papillary cystadenocarcinoma, NOS	0/1	0/1	1/1
Parathyroid (#)	5/7	26/31	24/19
- Adenoma, NOS	1/0	0/0	4/1
Pancreatic islets (#)	9/7	47/41	45/39
- Islet cell adenoma	0/2	1/1	4/1
- Islet cell carcinoma	0/0	0/0	2/0
Mammary gland (@)	10/10	50/49	49/49
- Cystadenocarcinoma, NOS	0/0	0/1	1/0
- Fibroma	0/0	0/0	2/0
- Cystfibroadenoma	1/0	0/0	0/0
- Adenoma, NOS	0/0	0/1	0/0
- Adenocarcinoma, NOS	0/0	0/2	0/0
- Papillary cystadenocarcinoma, NOS	0/0	0/0	0/1
- Lipoma	0/0	0/1	0/0
- Leiomyosarcoma	0/0	0/1	0/0
- Fibroadenoma	0/2	0/9	0/9

Prostate(#)	10	47	45
- Papillary adenoma	0	1	0
Testis (#)	10	49	48
- Interstitial cell tumor	0	0	1
Uterus (#)	9	43	41
- Endometrial stromal polyp	1	3	0
- Hemangioma	0	1	0
Ovary (#)	9	47	42
- Adenocarcinoma, NOS	0	1	0
- Papillary adenocarcinoma	0	1	0
Brain (#)	10/9	49/48	48/46
- Glioblastoma, multiforme	0/0	1/0	0/0
Rib (@)	10/10	50/49	49/49
- Hemangiosarcoma	0/0	0/0	1/0
Abdominal cavities (@)	10/10	50/49	49/49
- Hemangioma	0/0	0/0	1/0
Parietal peritoneum (@)	10/10	50/49	49/49
- Leiomyosarcoma, metastatic	0/0	0/0	1/0
Tunica vaginalis (@)	10	50	49
- Mesothelioma, NOS	0	1	0
Multiple organs (@)	10/10	50/49	49/49
- Liposarcoma, metastatic	1/0	0/0	0/0

# No. of animals examined microscopically; @ No. of animals necropsied
NOS = not otherwise specified

Table B.5.5.4-4: Statistical analysis of the incidence of neoplasms of the pancreas and the thyroid in male rats

Dose group	Pooled control	Matched control	Low dose	High dose
Pancreas (islet-cells): adenoma or carcinoma	2/92 (2 %) [p=0.008#]	0/9 (0 %) [p=0.033#]	1/47 (2 %) [N.S.@]	6/45 (13 %) [p=0.015@]
Thyroid (follicular cells): benign tumors	7/86 (8 %) [p=0.002#]	1/9 (11 %) [N.S.#]	10/44 (23 %) [p=0.022@]	12/43 (28 %) [p=0.004@]
Thyroid (follicular cells): malignant tumors	0/86 (0 %) [p=0.008#]	0/9 (0 %) [N.S.#]	4/44 (9 %) [p=0.012@]	4/43 (9 %) [p=0.011@]
Thyroid (follicular cells): benign and malignant tumors	7/86 (8 %) [p=<0.001#]	1/9 (11 %) [N.S.#]	14/44 (32 %) [p=0.001@]	14/43 (33 %) [p=0.001@]

N.S. = not significant (when p>0.05)
# Cochran-Armitage test for linear trend
@ Fisher exact test (comparison of the dosed group with the pooled control)

In male rats, the results of statistical tests using the pooled-control animals on the incidences of benign thyroid tumors (follicular-cell adenomas, adenomas, or cystadenomas), malignant thyroid tumors (adenocarcinomas, cystadenocarcinomas, or papillary cystadenocarcinomas), or the combined benign and malignant follicular-cell tumors are all significant. In each analysis, the result of the Cochran-Armitage test is significant using the pooled controls, and the results of the Fisher exact comparisons of the incidences in any of the dosed groups with the pooled-control group show probability levels less than 0.025. The results of the Fisher exact test comparing the incidence in the matched-control group with that in each dosed group are not significant. Time-adjusted analyses on the incidences of all follicular-cell tumors tumors, eliminating animals that died before week 52 on study (pooled controls 7/82 [9 %], matched controls 1/9 [11 %], low-dose 14/44 [32 %], high-dose 14/43 [33 %]),

resulted in essentially the same statistics as those of the non-adjusted tests. Since, however, the spontaneous incidence of these neoplasms varied in male Osborne-Mendel rats at the performing laboratory from 0 % to 43 %, with a mean of 7 %, the incidence found in the high-dose male rats can not be clearly implicated as a treatment-induced effect.

Conclusion:

The NOEL for dietary administration of azinphos-methyl to Osborne-Mendel rats over 80 weeks (then observed for 34-35 weeks) was <78 ppm in males and 62.5 ppm in females. Clinical symptoms, decreased body weight gain, and increased mortality were observed at higher dose levels. Azinphos-methyl was not shown to be carcinogenic in female rats. In male rats, neoplasms of the thyroid follicular cells and pancreatic islet cells occurred at significant incidences when compared with pooled controls. Since, however, the incidences were within the historical control ranges, they can not be clearly implicated as a treatment-induced effect.

The study authors concluded that under the conditions of this bioassay, neoplasms of the thyroid and pancreatic islets suggest but do not provide sufficient evidence for the carcinogenicity of azinphos-methyl in male Osborne-Mendel rats.

B.5.5.5 MOUSE

NCI, 1978: Bioassay of azinphosmethyl for possible carcinogenicity. National Cancer Institute (NCI), National Institutes of Health, Bethesda, Maryland 20014, USA. National Cancer Institute Carcinogenesis Technical Report Series No. 69, 1978. Dates of experimental work: not specified.

Guidelines and GLP:

The method employed was that of the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health. When the study was performed, GLP was not compulsory.

Main deviations from current OECD guidelines: Only two dose levels were used. The concurrent control group consisted of 10 male and 10 female mice only.

The study is considered supplementary.

Material and methods:

Groups of 50 male and 50 female 35 days old B6C3F1 mice (source: Charles River Breeding Laboratories, Inc., Wilmington, MA, USA; body weight range not specified) received azinphos-methyl (Guthion®, Mobay Chemical Corp., Kansas City, MO., batch no. not specified, purity 90 %) for 80 weeks by admixture in the diet (with 2 % corn oil) at concentrations of 0, 31.3 and 62.5 ppm active ingredient (males) and 0, 62.5 and 125 ppm active ingredient (females). The dose levels were selected on the basis of a previous subchronic feeding study conducted to estimate maximum tolerated doses of azinphos-methyl. A group of 10 male and 10 female mice served as matched controls; the pooled controls for statistical tests consisted of the matched controls combined with 130 male and 120 female untreated mice from similar bioassays of 11 other test chemicals. Following 80 weeks of exposure, the animals were retained untreated but under observation for a further 12 or 13 weeks. All animals were observed twice daily for signs of toxicity, weighed at regular intervals, and palpated for masses at each weighing. All animals found dead, killed in a moribund condition or killed at scheduled sacrifice were subjected to a gross and microscopic examination of major tissues, major organs, and all gross lesions.

Statistical methods: Survival analysis, Kaplan and Meier (1958), Cox (1972), and Tarone's (1975) extension of Cox's methods. Incidences of lesions, one-tailed Fisher exact test at significance level of 0.05, with Bonferroni inequality correction, and Cochran-Armitage test for linear trend in proportions, as well as time-adjusted analysis when appropriate. Statistical comparisons were made against the matched and pooled control groups.

Findings:

General examinations:

Clinical signs, including hyperactivity and convulsions, were observed in treated animals but also in control animals. The body weights of male mice and low-dose female mice were comparable to the controls throughout the study. The mean body weight of the high-dose female group was up to approximately 17 % lower than the control group throughout the treatment period, but was comparable to the controls after week 80. There were no treatment-related differences in the incidences of mortality in any of the experimental groups.

Table B.5.5.5-1: Mortality and body weights in mice

Dose group	Control	Low dose	High dose
Sex	M/F	M/F	M/F
No. of animals initially in study	10/10	50/50	50/50
Natural death	1/0	1/3	1/2
Moribund sacrifice	1/3	4/3	7/6
Terminal sacrifice	8/7	45/44	42/42
Survival rate (%)	80/70	90/88	84/84
Body weight [g] (week 0) @	21/18	22/18	21/18
Body weight [g] (week 52) @	35/31	35/31	36/28

@ Approximate mean body weight, derived from graphically presented data

Table B.5.5.5-2: Summary of the incidence of neoplasms in mice

Dose group	Control	Low dose	High dose
Sex	M/F	M/F	M/F
No. of animals examined microscopically	10/10	49/49	50/49
No. of animals with primary tumors	4/5	23/10	23/17
No. of animals with benign (b) tumors	5/1	16/5	10/6
No. of animals with malignant (m) tumors	1/5	8/7	15/11
No. of animals with tumors uncertain b or m	0/0	0/0	0/1
No. of animals with secondary tumors	0/3	1/2	0/4

Table B.5.5.5-3: Incidence of neoplasms in mice

Dose group	Control	Low dose	High dose
Sex	M/F	M/F	M/F
Skin (@)	10/10	50/50	50/50
- Fibrosarcoma	0/0	0/0	1/0
- Leiomyosarcoma	0/0	1/0	0/0
Subcutaneous tissue (@)	10/10	50/50	50/50
- Leiomyosarcoma	0/0	0/0	1/0
- Fibrosarcoma	0/0	0/0	1/0
- Malig. lymphoma, lymphocytic type	0/0	0/0	0/1
Lung (#)	10/10	49/50	50/50
- Alveolar/bronchiolar adenoma	1/0	6/1	4/3
- Alveolar/bronchiolar carcinoma	1/0	2/0	0/0
- Papillary cystadenocarcinoma, MET	0/1	0/0	0/0
- Lymphoma, metastatic	0/1	0/0	0/1
- Granulocytic leukemia	0/0	0/1	0/0
Bone marrow (#)	10/10	49/47	50/50
- Lymphoma, metastatic	0/1	0/0	0/0
- Hemangioma	0/0	1/0	0/0
- Hemangiosarcoma, metastatic	0/0	1/0	0/0

Spleen (#)	8/9	46/49	46/50
- Malig. lymphoma, lymphocytic type	0/0	1/0	0/3
- Hemangioma	0/0	1/0	0/0
- Hemangiosarcoma	0/0	1/1	0/1
- Malig. lymphoma, NOS	0/1	0/0	0/0
- Malig. lymphoma, histiocytic type	0/0	0/2	0/0
Lymph node (#)	9/9	46/40	46/45
- Hemangioma	1/0	0/0	0/0
- Malig. lymphoma, NOS	0/0	0/1	1/2
- Malig. lymphoma, histiocytic type	0/0	0/1	1/0
- Lymphoma, metastatic	0/1	0/0	0/0
- Lymphoma, lymphocytic, metastatic	0/0	0/0	0/1
Salivary gland capsule (#)	10/10	49/49	50/49
- Hemangioma	0/0	0/1	0/0
Liver (#)	8/10	49/49	50/50
- Hepatocellular adenoma	2/0	8/0	7/1
- Hepatocellular carcinoma	0/1	3/0	12/0
- Hemangiosarcoma	0/0	2/0	0/0
- Lymphoma, metastatic	0/1	0/0	0/1
- Lymphoma, lymphocytic, metastatic	0/0	0/0	0/1
Small intestine (#)	10/10	49/48	50/50
- Malig. lymphoma, lymphocytic type	0/1	0/0	0/0
- Lymphoma, lymphocytic, metastatic	0/0	0/0	0/1
Kidney (#)	10/10	49/49	50/50
- Malig. lymphoma, lymphocytic type	0/0	0/0	0/1
- Malig. lymphoma, histio-type metas	0/0	0/1	0/0
- Cortex: lymphoma, metastatic	0/1	0/0	0/0
Pituitary (#)	10/7	49/39	50/40
- Chromophobe adenoma	0/0	0/1	0/0
Adrenal (#)	10/10	49/47	50/49
- Lymphoma, metastatic	0/1	0/0	0/0
Thyroid (#)	10/9	49/42	50/46
- Cystadenoma, NOS	0/1	0/0	0/0
- Papillary cystadenoma, NOS	0/0	0/0	0/1
- Papillary cystadenocarcinoma, NOS	0/1	0/0	0/0
Mammary gland (@)	10/10	50/50	50/50
- Papillary cystadenocarcinoma, NOS	0/0	0/0	0/1
- Fibroadenoma	0/0	0/0	0/1
- Lymphoma, metastatic	0/0	0/0	0/1
Uterus (#)	7	48	48
- Endometrial stromal polyp	0	2	0
- Leiomyosarcoma	0	0	1
Cervix uteri (#)	7	48	48
- Leiomyosarcoma	0	1	0
Ovary (#)	9	47	41
- Granulosa-cell tumor	0	0	1
Pelvic cavity (@)	10/10	50/50	50/50
- Liposarcoma	0/0	0/0	0/1
Eye/lacrimal gland (@)	10/10	50/50	50/50
- Papillary cystadenoma, NOS	1/0	0/0	0/0
Multiple organs (@)	10/10	50/50	50/50
- Malig. lymphoma, NOS	0/1	0/0	0/0
- Malig. lymphoma, lymphocytic type	0/0	0/1	0/0
- Lymphoma, lymphocytic, metastatic	0/0	0/0	0/1
- Malig. lymphoma, histio-type metas.	0/0	0/1	0/0
- Granulocytic leukemia	0/0	0/0	0/1

# No. of animals examined microscopically; @ No. of animals necropsied
NOS = not otherwise specified

Pathological examinations:
No treatment-related gross pathological changes were reported and all the tumor types observed during the study had been encountered as a spontaneous lesion in the mouse strain employed. There was no statistical evidence of an association of tumors with the administration of azinphos-methyl at either dose level in either sex of mouse.

Conclusion:
The NOEL for dietary administration of azinphos-methyl to B6C3F1 mice over 80 weeks (then observed for 12-13 weeks) was 62.5 ppm. Clinical symptoms and decreased body weight gain were observed at higher dose levels. Azinphos-methyl was not shown to be carcinogenic in B6C3F1 mice of either sex.

B.5.5.6 MOUSE

Hayes, 1985: Oncogenicity study of azinphos-methyl (®Guthion) in mice. Report no. 612 of 10 April 1985; study no. 80-271-02; Mobay Chemical Corp., Corporate Toxicology Department, Stilwell, Kansas, USA. Dates of experimental work: 17 March 1980 to 19 March 1982.

Guidelines and GLP:
The study was performed according to OECD guideline 451, with additional hematological and clinical chemistry determinations. The study is GLP compliant. The study is considered acceptable.

Material and methods:
Groups of 50 male and 50 female 38 day old CD1 outbred strain mice (source: Charles River Breeding Laboratories, Wilmington, MA., USA) received azinphos-methyl (batch no. 79-R-225-42, purity 88.6 %) in the diet (with 1 % corn oil) for 104 weeks at nominal concentrations of 0, 5, 20 and 40 ppm. The study was initially started with 80 ppm as the high dietary level, but this was reduced to 40 ppm after one week, due to severe reaction to treatment, including mortality, at 80 ppm. Hematology values and ChE activities were determined in 10 animals/sex/group at 6, 12 and 24 months. All animals found dead, killed in a moribund condition or killed at scheduled sacrifice were subjected to a gross pathological examination and tissues preserved for histopathology.
Statistical methods: Body weight, feed consumption, hematological parameters, and organ weights were subjected to an analysis of variance followed by Duncan's new multiple range test. All significant differences were reported at the 95 % confidence level.

Findings:
General examinations:
The average doses ingested at 5, 20 and 40 ppm were 0.79, 3.49 and 11.33 mg/kg bw/d for males and 0.98, 4.12 and 14.30 mg/kg bw/d for females, repectively. At the initial high dietary level of 80 ppm, 4 females were found dead after 4 days, and a decrease in body weights of 10 to 13 % was noted in males and females after one week. Following the reduction in the high dietary level from 80 to 40 ppm, there were no clinical signs of reaction to treatment and mortality remained unaffected by treatment. Body weight gain and feed intake of both sexes were unaffected by treatment throughout the study at dose levels up to and including 40 ppm.

Table B.5.5.6-1: Mortality and body weights in mice

Dose level (ppm) / **Sex**	**0** **M/F**	**5** **M/F**	**20** **M/F**	**40** **M/F**
No. of animals	50/50	50/50	50/50	50/50
Mortality, 104 wk (%)	44/60	34/40	48/68	44/46
Body weight, 104 wk (g)	38.6/35.4	39.0/34.4	38.8/36.5	38.9/35.3

Clinical laboratory examinations:
There were no biologically significant changes in the hematological profile at any of the dose levels of either male or female animals at 6, 12 and 24 months. A toxicologically relevant inhibition (up to 80 %) of ChE activity occurred in plasma, RBC and brain at 20 and 40 ppm. At 5 ppm there was no biologically significant inhibition of ChE activity, except for female RBC at 2 years which was 22 % lower than the control value. At a subsequent analysis one week later, erythrocyte ChE activities in different females from the control and 5 ppm were almost identical in both groups (0.84 and 0.83 µmol/ml/min, respectively).

Table B.5.5.6-2: ChE activities (plasma, RBC: µmol/ml/min; brain: µmol/g/min) in mice

Dose level (ppm) Sex	0 M/F	5 M/F	20 M/F	40 M/F
Plasma, 6 mo	3.11/5.76	3.33/5.45	2.57/3.08	1.62/1.48
Plasma, 1 yr	3.88/6.51	4.81/5.44	2.63/3.27	1.32/1.50
Plasma, 2 yr	4.33/4.98	3.95/4.93	2.97/3.86	1.89/1.65
RBC, 6 mo	1.33/1.16	1.11/1.03	0.88/0.67	0.67/0.63
RBC, 1 yr	1.04/0.87	0.99/0.81	0.45/0.39	0.20/0.20
RBC, 2 yr	0.95/0.79	0.80/0.62	0.54/0.40	0.35/0.32
Brain, 2 yr	14.7/14.4	12.9/13.6	12.3/10.6	5.4/4.7

Result of statistical analysis not given in the report.

Pathological examinations:
No treatment-related effects were revealed on organ weights and on gross or microscopic examinations. The statistically significant increased absolute heart weight in females at 20 ppm is considered not biologically significant since the effect was not seen at 40 ppm.
There was increased frequency of adenomatous hyperplasia in the lungs with increased dosage. Although the incidence in male mice at 40 ppm was statistically significant increased, the severity of hyperplasia was less than in controls. In contrast to the increased frequency of adenomatous hyperplasia the incidence of alveolar/bronchiolar adenoma in the lungs of the 40 ppm males was lower than in controls. Thus, there was no dose-related increase in the combined incidence of of adenomatous hyperplasia and alveolar/bronchiolar adenoma in the lungs of male mice.

Table B.5.5.6-3: Incidence of adenomatous hyperplasia and alveolar/bronchiolar adenoma in the lungs of mice

Dose level (ppm) Sex	0 M/F	5 M/F	20 M/F	40 M/F
Number of animals	50/50	50/50	50/50	50/50
Adenomatous hyperplasia: incidence	7/6	10/16	14/7	15*/11
severity	2.6/2.2	1.7/1.9	2.0/1.4	2.2/2.5
Alveolar/bronchiolar adenoma	14/7	8/6	4/6	8/9

* p<0.05

Table B.5.5.6-4: Summary of the incidence of neoplasms in mice

Dose level (ppm) Sex	0 M/F	5 M/F	20 M/F	40 M/F
Total no. of animals	50/50	50/50	50/50	50/50
Animals with tumors	32/28	32/28	23/31	35/30
Animals with only benign tumors	19/12	17/10	12/9	16/13
Animals with only malignant tumors	7/11	8/13	7/13	14/12
Total no. of tumors	116/224	86/172	117/177	170*/179
Total no. of malignant tumors	86/204	56/154	99/153	143*/157

* p<0.05

Neoplasms were similar in type, localisation, time of occurrence and incidence in control mice and mice receiving azinphos-methyl up to and including 40 ppm in the diet. Although there was no dose-related increase in the number of animals with tumors, at the 40 ppm level, there was an increased frequency of total tumors found and of total malignant tumors in males which was statistically significantly different from the controls (p<0.05). However, the tabulation of malignant tumors is based on the number of malignant designations per tissue per animal and, thus, hematopoietic neoplasms are counted in each tissue rather than once per animal. In males fed 40 ppm, the slight increase in malignant lymphoma bearers markedly increases the the total tumor and total malignant tumor categories due to multiple site involvement.

Table B.5.5.6-5: Incidence of neoplasms in mice

Dose level (ppm)	0		5		20		40	
Sex	male	female	male	female	male	female	male	female
Total no. of animals	50	50	50	50	50	50	50	50
Skin (#)	50	50	50	50	49	50	49	50
- Lymphoma (m) all types	1	4	3		3	1	1	1
- Adenocarcinoma NOS(m)				1				
- Fibrosarcoma (m)				1				
- Metastasis NOS (m)						1		
- Sarcoma NOS (m)						1		
Mammary gland (#)	30	50	20	50	23	50	19	50
- Lymphoma (m) all types	1	7	2	1	2	4	1	2
- Fibrosarcoma (m)		1		1				
- Adenoacanthoma (m)		1						
- Sarcoma NOS(m)		1						
- Adenocarcinoma NOS(m)				3		2		
Salivary gland, submax.(#)	50	50	50	50	50	50	50	49
- Lymphoma (m) all types	3	4	2	4	3	2	2	4
Lymph node, cervical (#)	45	48	47	47	42	49	44	47
- Lymphoma (m) all types	3	6	3	7	4	6	5	6
- Metastasis NOS (m)						1		
Lymph node, mesenteric (#)	50	46	50	48	48	49	50	49
- Lymphoma (m) all types	3	7	3	8	4	8	7	7
- Metastasis NOS (m)		1				2		1
Trachea (#)	50	48	50	50	50	48	50	49
- Lymphoma (m) all types		1			1		1	2
Larynx (#)	50	49	50	50	50	49	50	49
- Lymphoma (m) all types		3		1	1		2	2
- Myelo. neoplasia NOS (m)				1				
Esophagus (#)	50	47	50	50	50	48	50	50
- Lymphoma (m) all types		1					1	1
Thyroid (#)	48	48	50	47	50	48	49	48
- Lymphoma (m) all types		1		3			2	2
- Adenoma NOS (b)				1				
- Adenocarcinoma NOS(m)								1
Parathyroids (#)	20	22	21	24	19	29	24	27
- Lymphoma (m) all types							1	
Sternum (#)	49	49	47	50	50	50	49	49
- Lymphoma (m) all types	3	6			3		3	3
- Hemangiosarcoma (m)			1					
Ribs costochondral jct.(#)	50	50	49	48	50	50	49	50
- Lymphoma (m) all types	2	6	2		3		4	3
- Alveol/bronchiol. ca.(m)		1				2	1	
- Sarcoma NOS (m)				1				
Thymus (#)	46	46	44	44	43	46	47	48
- Lymphoma (m) all types	3	7	1	6	4	7	5	5
- Alveol/bronchiol. ca.(m)							1	
- Metastasis NOS (m)						1		
Heart (#)	50	50	50	50	50	50	50	50
- Lymphoma (m) all types		4	3	3	3		3	3
- Alveol/bronchiol. ca.(m)		1					1	
- Metastasis NOS (m)						1		
Aorta (#)	48	45	49	47	49	48	50	47
- Lymphoma (m) all types	2	3		1	2		4	3
- Alveol/bronchiol. ca.(m)		1					1	

Lung (#)	50	50	50	50	50	50	50	50
- Alveol/bronchiol.ca.(m)	2	2	3	2	5	3	6	2
- Alveol/bronchiol. ad.(b)	14	7	8	6	4	6	8	9
- Lymphoma (m) all types	3	7	2	5	4	8	5	6
- Leukemia NOS (m)				1				1
- Metastasis NOS (m)						2		2
Liver (#)	50	50	50	50	50	50	50	50
- Hepatocellular ca. (m)	5		6		2		4	
- Hepatocellular ad. (b)	8	2	10	1	8	3	8	2
- Hemangiosarcoma (m)	3	1				2		
- Hemangioma (b)		1		2	1	1	1	
- Lymphoma (m) all types	3	6	2	6	3	5	7	6
- Sarcoma NOS (m)		1				1		1
- Myeloprol. neoplasia (m)				1				
- Metastasis NOS (m)						2		2
Gall bladder (#)	48	43	45	45	41	40	46	40
- Lymphoma (m) all types	2	3		3	2		1	2
Spleen (#)	49	50	50	50	50	50	50	50
- Lymphoma (m) all types	3	6	2	7	4	8	6	6
- Hemangiosarcoma (m)			1			1		
- Hemangioma (b)			1	1		2		
- Myelo. neoplasia NOS (m)				1				
- Metastasis NOS (m)		1						
Pancreas (#)	50	50	50	50	50	50	50	50
- Lymphoma (m) all types	3	6		6	3	5	4	7
- Hemangiosarcoma (m)	1							
- Metastasis NOS (m)						2		
Stomach (#)	50	50	50	50	50	50	50	50
- Lymphoma (m) all types	2	6	1	4	1	1	1	4
Small intestine (#)	50	50	49	50	50	50	50	50
- Adenocarcinoma (m)			2				1	
- Lymphoma (m) all types	2	1		4	1	1	3	2
Caecum (#)	49	49	48	47	50	50	49	50
- Lymphoma (m) all types	1	4		2		1	1	3
Large intestine (#)	50	48	50	49	50	48	50	50
- Lymphoma (m) all types	1	4		4	2	1		1
Adrenals (#)	50	50	50	50	50	50	50	49
- Cortical adenoma NOS(b)	2		1				1	1
- Pheochromocytoma (b)						1		
- Lymphoma (m) all types	3	5	2	6	2	3	3	4
- Metastasis NOS (m)						2		
Kidneys (#)	50	50	50	50	50	50	50	50
- Adenoma NOS (b)	1							
- Lymphoma (m) all types	3	6	1	8	4	6	7	6
- Endometr. strom.sarc.(m)		2				2		1
- Metastasis NOS (m)						1		
Testes (#)	50		50		50		50	
- Interstit. c. tumor (b)			1				2	
- Hemangioma					1			
- Lymphoma (m) all types							1	
Urinary bladder (#)	49	48	50	50	50	49	50	50
- Carcinoma (m)				1				
- Lymphoma (m) all types	2	6	1	4	3	4	3	5
- Metastasis NOS (m)						1		
Seminal vesicles (#)	50		50		49		50	
- Lymphoma (m) all types	3		1		3		5	
Prostate (#)	50		50		50		50	
- Lymphoma (m) all types	3		1		4		2	
Muscle, gastrocnemius (#)	50	50	49	50	49	50	50	50
- Lymphoma (m) all types	1	1	1		1		2	3
Sciatic nerve (#)	49	49	48	48	50	50	50	50
- Lymphoma (m) all types			1		1		1	
Bone (#)	50	49	49	48	49	49	49	50
- Osteosarcoma (m)							1	
- Lymphoma (m) all types	2	1			1		1	1
Bone marrow (#)	50	50	50	50	50	50	50	50
- Lymphoma (m) all types	1	5	1	1	3	5	5	3
- Myelo. neoplasia NOS (m)				1				
- Metastasis NOS (m)								1

Spinal cord (#)	49	48	50	48	50	50	50	50
- Neurofibroma (b)		1						
- Lymphoma (m) all types	2	3		1	1	1	3	1
Eyes (#)	50	50	50	50	50	50	49	50
- Lymphoma (m) all types	1	2	1		2	2	1	1
- Metastasis NOS (m)						1		
Optic nerve (#)	44	44	38	42	44	33	42	47
- Lymphoma (m) all types	1	1		1	1		3	1
Harderian glands (#)	50	48	50	50	50	50	50	50
- Adenom (b)	4	2	8		3	3	7	2
- Adenocarcinoma (m)								1
- Lymphoma (m) all types	2	3	1	1	3	3	3	2
Pituitary (#)	49	50	50	48	50	50	49	49
- Adenoma (b)	1	1		2		3		
- Carcinoma NOS (m)						1		
- Lymphoma (m) all types	1	4			1	1	1	2
Skull (#)	50	50	50	49	50	50	50	50
- Osteoma NOS (b)			1					
- Lymphoma (m) all types	3	3	1		1	2	3	2
- Metastasis NOS (m)						1		
Brain (#)	50	49	50	50	50	50	50	50
- Glioma NOS (b)				1		1		
- Lymphoma (m) all types	1	2		1	1			1
- Metastasis NOS (m)						1		
Ovaries (#)		49		49		48		50
- Granulosa c. tumor (b)				4		1		3
- Hemangioma (b)		1						1
- Hemangiosarcoma (m)		1						
- Adenoma NOS (b)								1
- Lymphoma (m) all types		7		6		3		3
- Metastasis NOS (m)						1		2
Uterus (#)		48		50		50		49
- Endom. stromal polyp (b)		1						2
- Endom. stromal sarc. (m)		5		3		6		4
- Leiomyoma (b)		1		1				
- Hemangioma (b)		2		1		3		2
- Hemangiosarcoma (m)				1				1
- Adenocarcin. NOS (m)						1		1
- Carcinoma NOS (m)				2		1		2
- Sarcoma NOS (m)						1		
- Lymphoma (m) all types		4		4		2		3
- Metastasis NOS (m)				1				
Cervix (#)		38		49		50		46
- Endom. stromal sarc. (m)		3				1		3
- Sarcoma NOS (m)		1						
- Leiomyoma (b)		1						1
- Lymphoma (m) all types		3		2		3		3

Total no. of organs examined; NOS - not otherwise specified
(b) benign; (m) malignant

Conclusion:

The NOEL for dietary administration of azinphos-methyl to mice over 2 years was 5 ppm, equivalent to 0.79 mg/kg bw/d in males and 0.98 mg/kg bw/d in females. A dose-related depression of ChE activity was observed at 20 ppm and above, and mortality and a decrease in body weight occurred at 80 ppm. There was no evidence that azinphos-methyl is tumorigenic in mice.

B.5.6 REPRODUCTIVE TOXICITY

In two reproduction toxicity studies with azinphos-methyl in rats there was evidence of reduced fertility and pup viability at 15 ppm and above and, additionally, at 45 ppm, reduced birth weight and subsequent growth retardation of the offspring. One study also demonstrated inhibition of ChE activity in the brain of pups at 45 ppm, whereas in the parents brain ChE activity was inhibited at 15 ppm and above in females and at 45 ppm in males. No malformations were observed and histological evaluation of major organs showed no treatment-related changes at dietary concentrations up to and including 45 ppm. Treatment of F0 male animals and subsequent mating with untreated females elicited no effects on reproduction parameters or on the progeny. The NOEL for reproduction toxicity is 5 ppm, equivalent to a dose level of 0.33-0.42 mg/kg bw/d in male rats and 0.48-0.67 mg/kg bw/d in female rats.
In developmental toxicity studies, no embryotoxic, fetotoxic or teratogenic effects were observed in rats and rabbits at dose levels up to 5.0 and 6.0 mg/kg bw/d, respectively, which elicited overt signs of maternal toxicity (clinical signs of ChE inhibition, reduced weight gain and feed consumption, reduced ChE activity). Treatment of female rats up to the end of the lactation period (peri-/postnatal toxicity study) indicated that dams were more sensitive to azinphos-methyl later in gestation. Treatment-related mortalities, a reduced gestation index, and reduced survival and weight gain of the pups were observed at 5 mg/kg bw/d. In mice, there was a slight increase in naturally occuring anomalies at a dose level causing maternal toxicity (5 mg/kg bw/d), but there were no specific (teratogenic) defects.

Table B.5.6: Summary of reproductive toxicity studies (NOEL: in mg/kg bw/d [or ppm])

Study	Dose levels	NOEL, parental or maternal	NOEL, reproduction	Reference
2-generation, # Wistar rats	0, 5, 15, 45 ppm	m: 0.33-0.42 [5 ppm] f: 0.48-0.67 [5 ppm]	0.33-0.67 [5 ppm]	Eiben and Janda, 1987
1-generation, Wistar rats	0, 5, 15, 45 ppm	m: <0.43-0.44 [<5 ppm] f:<0.55 [<5 ppm]	0.43-0.55 [5 ppm]	Holzum, 1990
Teratogenicity, @ CD rats	0, 1.25, 2.5, 5.0 mg/kg bw/d	2.5 2.5 @	5.0 2.5 @	Short et al., 1978, 1980
Teratogenicity, CD rats	0, 0.5, 1.0, 2.0 mg/kg bw/d	1.0	2.0	Kowalski et al., 1987
Teratogenicity, Himalayan rabbits	0, 0.3, 1.0, 3.0 mg/kg bw/d	3.0	3.0	Machemer, 1975
Teratogenicity, Dutch rabbits	0, 1.0, 2.5, 6.0 mg/kg bw/d	1.0	6.0	Clemens et al., 1988
Teratogenicity, CD-1 mice	0, 1.25, 2.5, 5.0 mg/kg bw/d	2.5	2.5	Short et al., 1978, 1980

Basis for the proposed ADI
@ Including a study for peri-/postnatal toxicity

B.5.6.1 MULTIGENERATION STUDIES

B.5.6.1.1 RAT

Eiben and Janda, 1987: R1582 (common name: azinphos-methyl, the active ingredient of Guthion): Two-generation study on rats. Report no. R3956 of 10 March 1987; study no. T 6006415; Bayer AG, Toxicology Department, Wuppertal, Germany. Dates of experimental work: September 1982 to February 1984.
Guidelines and GLP:
The test method employed conformed with OECD guideline 416, adopted 26 May 1983.
The study is GLP compliant.
The study is considered acceptable.

Material and methods:
Groups of 12 male and 24 female 5-6 week old Wistar rats (strain: Bor:WISW (SPF-Cpb); source, Winkelmann, Borchen, FRG; body weight range: 78-87 g) received R1582 (batch no. 79/R225/42, purity 87.2 %) admixed in the diet at concentrations of 0, 5, 15 and 45 ppm, throughout the entire test period, including mating, gestation, and raising of the pups (2 generations, 2 litters per generation). The dose levels were selected based on the results of a 4-week toxicity study (Eiben et al., 1983). During the 3-week mating period, two females were caged together with one male. The male rats were interchanged each week so that essentially each female was together with 3 different males.
Additional pathology: The livers, kidneys, testes, and ovaries of the F1B parental rats were weighed. The histopathological examinations were performed for the brain, pituitary, liver, kidneys, and the organs of the reproductive system of all F0 and F1B parental rats of the 0 ppm, 15 ppm, and 45 ppm groups and all fixed organs of the rats of the 5 ppm group that died during the experiment.
Statistical methods: Comparisons of test population with controls by means of U-test of Mann, Whitney, and Wilcoxon at the significance level of alpha = 5 %, and alpha = 1 %. Comparison of indices at 95 % and 99 % confidence limits calculated according to Clopper & Pearson, and intergroup differences compared by Fisher's exact test at the significance level of alpha = 5 %, and alpha = 1 %. The mean pup weights per dose level were calculated from the mean pup weight of each individual litter. Each calculation of litter size was based on the number of females that had been pregnant.
Findings:
The average doses ingested at 5, 15 and 45 ppm were 0.33/0.42, 1.02/1.22 and 3.46/7.37 mg/kg bw/d for males (F0/F1B) and 0.48/0.67, 1.48/2.02 and 4.84/10.27 mg/kg bw/d for females (F0/F1B), respectively.
F0 (F1A/F1B) generation:
Increased alopecia, unpreened hair coat, poor general physical condition, inflamed areas of the eye, sporadic convulsions and increased mortality of dams were observed at 45 ppm. Feed intake was increased and body weight gain was decreased in females at 45 ppm.
There was a decrease in fertility, particularly at the 2nd mating, and a decrease of the number of pups born at 15 and 45 ppm.
The mean litter size was reduced at 45 ppm (F1B). The viability index was markedly decreased at 45 ppm and slightly at 15 ppm in the F1A generation. [The study authors, however, state that the viability index of the 15 ppm group (F1A) was within the range of normal laboratory variation (range for viability index: 78.7-100 %; historical control values 1978-1984) and this deviation is considered of no relevance.] The lactation index was markedly decreased at 45 ppm and slightly at 15 ppm in the F1B generation. [The study authors, however, state that the lactation index of the 15 ppm group (F1B) was within the range of normal laboratory variation (range for lactation index: 79.8-100 %; historical control values 1978-1984) and this deviation is considered of no relevance.] As a consequence of these effects, at 45 ppm only 5 females were available for mating in the F1B generation.
At birth, pups of the 45 ppm group were in some cases significantly lighter and smaller (F1B). During the 4-week lactation period, significantly lower pup body weights were recorded at 45 ppm.
No malformations were observed up to and including 45 ppm.
F1B (F2A/F2B) generation:
Impairment of general physical condition, unpreened hair coat, and, in some rats, convulsions were observed at 45 ppm. Body weights of males at 15 ppm and both sexes at 45 ppm were significantly lower than in controls. Feed intake was increased at 45 ppm [but this is not evaluated as a toxic effect by the study authors].
After the 2nd mating, the indices for fertility and gestation were slightly lower at 45 ppm. [According to the study authors, the relevance of these differences from control is questionable, however, because of the small number

of rats in this group. In addition, all females of the 45 ppm group had live pups at least one time. The study authors state that no effect on fertility can be inferred from these results.]
There was a decrease of the number of pups born at 15 and 45 ppm and a decrease of the mean litter size at 45 ppm (F2A).
The viability index was decreased at 45 ppm in the F2A generation and at 5 and 15 ppm in the F2B generation. [The study authors, however, state that the reduction in viability of F2A pups at 45 ppm is evaluated as a random occurrence, since all F2B pups at 45 ppm survived the first 5 days. The viability indices at 5 and 15 ppm (F2B) were within the range of normal laboratory variation (range for viability index: 78.7-100 %; historical control values 1978-1984) and the deviations were considered of no relevance by the study authors.] The lactation index was significantly decreased at 15 and 45 ppm in the F2A generation. [The study authors, however, state that the lactation indices up to and including 15 ppm were within the range of normal laboratory variation (range for lactation index: 79.8-100 %; historical control values 1978-1984).]
During the 4-week lactation period, significantly lower pup body weights were recorded at 45 ppm.
No malformations were observed up to and including 45 ppm.

Table B.5.6.1.1-1: Mortality, feed intake (Fi) and body weight (Bw) in parental animals

Dose level (ppm)	0	5	15	45
F0	**M/F**	**M/F**	**M/F**	**M/F**
Mortality (no.)	0/0	0/2	0/1	1/5
Fi (g/animal)	2298/1877	2200/1971	2091/2017	2430/2118
Fi (g/animal/d)	19/16	18/17	18/17	20/18
Bw at 0 wk (g)	84/78	86/79	86/80	87/79
Bw at 13 wk (g)	323/197	337/199	309/197	315/188*
F1B	**M/F**	**M/F**	**M/F**	**M/F**
Mortality (no.)	0/1	0/0	0/2	0/0
Fi (g/animal)	1438/1372	1338/1360	1191/1386	2214/2019
Fi (g/animal/d)	21/20	19/19	17/20	32/29
Bw at 5 wk (g)	78/69	76/69	69/67	73/52*
Bw at 13 wk (g)	318/182	319/184	287*/190	265**/166*

* p<0.05; ** p<0.01

Table B.5.6.1.1-2: Fertility parameters

Dose level (ppm)	0	5	15	45
F0	**Mating 1/2**	**Mating 1/2**	**Mating 1/2**	**Mating 1/2**
Mated females (no.)	24/24	24/24	24/23	23/19
Insemination index (%)	100/100	95.8/100	91.6/91.3	95.6/94.7
Fertility index (%)	91.7/91.7	95.7/95.8	90.0/85.7	86.4/83.3
Gestation index (%)	100/100	100/100	100/100	100/93.3
Gestation period (d)	22.5/22.3	22.5/22.7	22.5/22.6	22.8/22.8
F1B	**Mating 1/2**	**Mating 1/2**	**Mating 1/2**	**Mating 1/2**
Mated females (no.)	24/24	24/24	24/23	5/5
Insemination index (%)	100/100	100/100	100/100	100/100
Fertility index (%)	91.7/87.5	100/91.7	87.5/95.7	100/80.0
Gestation index (%)	100/95.2	100/100	100/95.5	100/75.0
Gestation period (d)	22.1/22.2	22.3/22.6	22.6/22.2	22.4/22.3

Table B.5.6.1.1-3: Pup parameters

Dose level (ppm)	0	5	15	45
F1A				
Pups at birth/dead (no.)	252/1	247/0	204/8	197/9
Pups m/f (%)	52/48	53/47	49/51	52/48
Litter size at 0/5 d (no.)	11.5/11.1	11.2/10.5	10.1/8.7*	10.1/3.9**
Viability index (%)	96.8	93.9	86.6**	38.7**
Pups at 5 d#/4 wk (no.)	175/169	167/156	139/134	62/17
Lactation index (%)	96.6	93.4	96.4	27.4**
Body weight at 0 d/3 wk (g)	5.8/36.7	5.7/37.5	5.9/35.9	5.4/25.8**
F1B				
Pups at birth/dead (no.)	235/1	236/11	175/1	133/0
Pups m/f (%)	52/48	50/50	51/49	55/45
Litter size at 0/5 d (no.)	10.6/10.5	9.8/9.5	9.7/9.7	8.9/2.8**
Viability index (%)	98.3	97.3	98.9	31.6**
Pups at 5 d#/4 wk (no.)	165/161	164/162	128/117	39/18
Lactation index (%)	97.6	98.8	91.4*	46.2**
Body weight at 0 d/3 wk (g)	5.7/39.9	5.8/39.2	5.9/37.8	5.2**/27.2**
F2A				
Pups at birth/dead (no.)	259/3	270/0	230/0	43/0
Pups m/f (%)	55/45	55/45	54/46	51/49
Litter size at 0/5 d (no.)	11.7/11.5	11.2/10.8	11.0/10.7	8.6*/7.0*
Viability index (%)	98.1	95.9	97.8	81.4**
Pups at 5 d#/4 wk (no.)	176/173	185/174	152/134	29/21
Lactation index (%)	98.3	94.1	88.7**	72.4**
Body weight at 0 d/3 wk (g)	5.7/37.3	5.7/35.6	5.7/36.0	5.4/22.4**
F2B				
Pups at birth/dead (no.)	223/1	244/2	214/3	25/0
Pups m/f (%)	51/49	55/45	49/51	56/44
Litter size at 0/5 d (no.)	10.6/10.1	11.0/10.0	9.6/8.5	6.2/6.2
Viability index (%)	95.5	90.1*	88.6*	100
Pups at 5 d#/4 wk (no.)	143/133	165/138	137/123	22/20
Lactation index (%)	93.0	83.6*	89.8	90.9
Body weight at 0 d/3 wk (g)	5.8/40.2	5.9/39.6	5.6/37.8	5.8/27.0**

After reduction; * p<0.05; ** p<0.01

Gross pathology, histopathology:
The majority of the F0 rats at 45 ppm that died had dark red discoloration of the lungs. Necropsy and organ weight determinations of the F1B rats indicated no specific treatment-related organ damage up to and including 45 ppm (but males at 45 ppm that exhibited marked delay in body weight gain had also significant reductions in absolute organ weights). Microscopic examination of the F0 and F1B rats revealed no evidence of treatment-related organ changes.
Conclusion:
The NOEL for dietary administration of azinphos-methyl to rats over 2 generations was 5 ppm with respect to parental toxicity and reproduction, equivalent to a dose level of 0.33-0.42 mg/kg bw/d in males and 0.48-0.67 mg/kg bw/d in females. At dietary concentrations of 15 ppm and above, body weight gain, fertility (fertility index, number of delivered pups) and pup viability were affected.
The proposed ADI is based on the NOEL in this study.

B.5.6.1.2 RAT

Holzum, 1990: E1582 (R1582) (c.n. azinphos-methyl): Investigation of inhibition of cholinesterase activity in plasma, RBC and brain in a 1-generation study.

Report no. 19594 of 8 October 1990; study no. T0027362; Bayer AG, Toxicology Department, Wuppertal, Germany. Dates of experimental work: 18 February 1988 to 8 August 1988.

Guidelines and GLP:

Since the objective of this study was to investigate whether the slight effect on fertility at 15 ppm in a previous 2-generation study (Eiben and Janda, 1987) could be confirmed, and, if reproducible, to determine whether the effect was attributable to treatment of the males or females, no particular method is applicable. The study is GLP compliant.

The study is considered acceptable.

Material and methods:

Groups of 18 male and 46 female 6-8 weeks old Wistar rats (strain: Bor:WISW (SPF-Cpb); source: Winkelmann, Borchen, FRG; body weight range: 92-146 g) received E1582 technical (batch no. 233796036, purity 92.0 % or 91.7 %) admixed in the diet at concentrations of 0, 5, 15 and 45 ppm. Additional groups of 10 treated males (5, 15 and 45 ppm) were paired with groups of 20 untreated females. After 14 weeks of treatment females were mated to males on a 2:1 basis for a period of 16 days. Five days after birth, F1 litters were reduced, where necessary, to 8 pups. Rearing of the F1 pups ended on day 28 p.p. for groups with both sexes dosed and on day 5 p.p. for groups with males only treated. ChE activities were determined in all groups with both sexes dosed, in F0 males at the end of the mating period (10 animals), and in F0 females at the end of pretreatment phase, on day 11 p.c., and on days 5 and 28 p.p (10 animals each). In F1 animals brain ChE activity was determined in 5 and 28 day old pups (5 animals each). Macroscopically changed organs of F0 animals and pups were fixed for microscopic examination.

Statistical methods: U-test after Mann & Whitney, or after Wilcoxon for parental and litter data; Fisher's exact test for reproduction indices at the significance levels alpha = 5 % and alpha = 1 %; F-test and t-test after Welch for ChE values, the significance levels corresponding to the results of the t-test.

Findings:

Analysis of deposit feed samples showed inhomogenous distribution of the test compound in the 45 ppm group in the 3rd, 4th and 6th week of the study. As a result of modifying the mixing process at the end of the 6th week, the homogeneity of the test substance was within the permitted tolerances thereafter.

The mean test compound intake at the dose levels of 0, 5, 15 and 45 ppm was estimated to be 0, 0.43/0.44, 1.30/1.32 and 3.73/3.83 mg/kg bw/d in the males (respectively) and 0, 0.55, 1.54 and 4.87 mg/kg bw/d in the females (respectively).

The 45 ppm level led to non-specific symptoms (poor general condition, bloody nose, inertia, stumbling gait) and mortality in the F0 females, whereas males of the same dose level were not affected. Five F0 females died between weeks 3 and 6, and 2 were killed in a moribund condition in weeks 3 and 10. These effects were probably due to inhomogeneous distribution of the test substance in the diet. Body weights and feed efficiency were marginally lower at 45 ppm during the pretreatment phase, whereas the feed intake in females was reduced during lactation at 15 ppm and above (marginal at 15 ppm, statistically significant at 45 ppm).

The activity of ChE was inhibited in the F0 males at 5 ppm in RBC, at 15 ppm in plasma and at 45 ppm in the brain. In F0 females, inhibition of ChE activity was noted at 5 ppm and above in plasma and RBC and at 15 ppm and above in the brain. In the pups, brain ChE activity was inhibited at 45 ppm.

The reproduction parameters investigated were not affected by treatment of male and female parent animals with 5 ppm. At 15 ppm and higher, when males and females were treated, the viability index and the body weight of the pups during the rearing period were reduced. However, after treatment of male parental animals only, reproduction parameters remained unaffected up to and including 45 ppm.

Table B.5.6.1.2-1: ChE activity (plasma, RBC: kU/l, brain: U/g) in F0 animals and F1 pups

	F0 males	F0 females				F1 pups	
	End of mating	End of pretreat.	Day 11 p.c.	Day 5 p.p.	Day 28 p.p.	Day 5	Day 28
Plasma							
0 ppm	0.44	1.84	1.44	1.34	0.80	-	-
5 ppm	0.43	1.84	1.55	0.99*	0.76	-	-
15 ppm	0.38***	1.39*	1.18	0.72***	0.49**	-	-
45 ppm	0.25***	0.70***	0.57***	0.45***	0.30***	-	-
RBC							
0 ppm	0.48	0.24	0.21	0.67	0.62	-	-
5 ppm	0.39**	0.25	0.21	0.50**	0.33***	-	-
15 ppm	0.15***	0.13***	0.10***	0.17***	0.10***	-	-
45 ppm	0.03***	0.07***	0.04***	0.06***	0.07***	-	-
Brain							
0 ppm	1.72	1.79	2.39	2.11	2.04	1.79	2.49
5 ppm	1.71	1.62*	2.49	2.12	1.80	1.92	2.84
15 ppm	1.89*	1.72	1.88**	1.30**	1.06***	1.77	2.14
45 ppm	1.40**	0.80***	0.74***	0.72***	0.66***	1.49*	1.34***

* p<0.05 ; ** p<0.01 ; *** p<0.001

Table B.5.6.1.2-2: Fertility parameters

Dose level, m-f (ppm)	0-0	5-5/5-0	15-15/15-0	45-45/45-0
Mated females (no.)	36	36/20	36/20	31/20
Insemination index (%)	100	100/100	100/100	100/100
Fertility index (%)	97.2	94.4/100	100/95.0	96.8/100
Gestation index (%)	88.5	100/100	100/100	95.5/95.0
Gestation period (d)	22.8	22.4/22.6	22.6/22.7	22.7/22.5

Table B.5.6.1.2-3: Pup parameters

Dose level, m-f (ppm)	0-0	5-5/5-0	15-15/15-0	45-45/45-0
Pups at birth (no.)	240	293/216	275/196	214/211
Pups dead at birth (no.)	13	4/2	3/1	3/3
Live birth index (%)	94.6	98.6*/99.1*	98.9**/99.5**	98.6*/98.6*
Male pups (%)	45	48.8/52.3	48.7/48.5	49.5/65.4
Litter size (no.)	9.9	11.1/10.7	10.5/10.3	10.0/10.9
Viability index (%)	93.4	92.4/98.1*	86.0*/90.8	48.3**/95.7
Lactation index (%)	62.1	74.2/-	69.8/-	57.7/-
Body weight, 0 d (g)	5.9	5.7/5.8	5.8/5.9	5.9/5.7
Body weight, 5 d (g)	9.2	8.7/8.9	9.0/9.3	7.8*/8.7
Body weight, 14 d (g)	24.4	22.5/-	21.4/-	19.9/-
Body weight, 28 d (g)	52.4	52.7/-	49.5/-	46.3/-

* p<0.05; ** p<0.01

No treatment-related pathological changes were observed in routinely sacrificed male and female F0 animals and in the pups at doses up to and including 45 ppm. Some female F0 rats which died or were sacrificed moribund were found to have dark red or red-brown lungs, clear demarcation of the liver lobules or a pale spleen, but microscopic examination revealed no indication of test compound induced organ damage.

Conclusion:

The NOEL for dietary administration of azinphos-methyl to rats over 1 generation was <5 ppm with respect to parental toxicity, equivalent to a dose level of <0.43-0.44 mg/kg bw/d in males and <0.55 mg/kg bw/d in females. Plasma and RBC

ChE activity was inhibited at all dose levels, whereas brain ChE activity was depressed at 15 ppm and above in females and at 45 ppm in males. The NOEL with respect to reproduction was 5 ppm, based on reduced viability index and retardation of growth in F1 pups at 15 ppm and above. At 45 ppm, brain ChE activity in pups was also inhibited. Treatment of F0 male animals and subsequent mating with untreated females elicited no effects on reproduction parameters or on the progeny.
The slight effect on fertility (fertility index, number of delivered pups) observed in the previous 2-generation study (Eiben and Janda, 1987) at and above 15 ppm was not confirmed in this study.

B.5.6.2 DEVELOPMENTAL TOXICITY

B.5.6.2.1 RAT

Short, Minor, Unger and Lee, 1978: Teratology of Guthion. Report no. EPA-600/1-78-056 of August 1978; Midwest Research Institute, Kansas City, MO 64110, USA, contracted by US Environmental Protection Agency. Dates of experimental work: not specified.
Short, Minor, Lee, Chernoff and Baron, 1980: Developmental toxicity of guthion in rats and mice. Midwest Research Institute, Kansas City, MO 64110, USA, and US Environmental Protection Agency, Research Triangle Park, NC 27711, USA.
Published in: Arch Toxicol 43:177-186, 1980.
Guidelines and GLP:
The test method employed was the internal standard at the time the study was performed and is, for the teratology study, in general compliance with OECD guideline 414, adopted 12 May 1981. When the study was performed, GLP was not compulsory. Main deviations from current OECD guidelines: The report is not very detailed (missing raw data). The study is considered supplementary.
Material and methods:
Groups of inseminated CD rats (source: Charles River Breeding Laboratories, North Wilmington, MA, USA) received azinphos-methyl (lot no. M007, purity: 90.6 %) orally in a vehicle of corn oil at dose levels of 0, 1.25, 2.5 and 5.0 mg/kg bw/d. The dose levels were selected on the basis of a range finding study.
a) In the 1st study (teratology study), groups of 21 rats were treated from day 6 to day 15 of gestation and sacrificed on day 20 of gestation for examination of the fetuses.
b) In the 2nd study (peri-/postnatal toxicity study), groups of 14 or 15 rats were treated from day 6 of gestation until the pups were weaned, 21 days after birth. After weaning, surviving pups of the control, intermediate and high dose group were sacrificed at 30-40 days of age and preserved.
Statistical methods: Homogenous data were analyzed by Dunnett's procedure, heterogenous data by a nonparametric rank test.
Findings:
a) In the teratology study, reduced weight gain and feed consumption during the treatment period, signs of ChE inhibition (salivation, urination, lacrimation and tremors) and one death were observed at 5 mg/kg bw/d. There was no effect of treatment on the reproduction parameters and no evidence of embryotoxicity, fetotoxicity or teratogenicity was obtained.
b) Treatment of the dams up to the end of the lactation period resulted in reduced weight gain and feed consumption at 5 mg/kg bw/d. At this dose level, dams were more sensitive to treatment later in gestation with the results that deaths and clinical signs of ChE inhibition increased during this time. The fertility index was not altered by the treatment, however, there was a trend towards a reduced gestation index in the high dose group.
Pup weight and pup survival were reduced at 5 mg/kg bw/d. One day after weaning, pups in the single surviving litter of the high dose group were observed to maintain their rear legs at right angles to the body and to have muscular incoordination in the use of these legs, muscular tremors in the tail, and

upturned snouts. In this litter of 5 pups, these effects were noticeable in 2 pups and of questionable incidence in 2 pups. However, similar symptoms were also observed in one pup from the control group, which complicated attempts to correlate these observations with azinphos-methyl treatment.

Table B.5.6.2.1-1: Maternal and developmental toxicity parameters in rats treated during gestation and lactation

Dose level (mg/kg bw/d)	0	1.25	2.5	5.0
Dams treated/pregnant	14/13	14/13	14/12	15/13
Mortality, total/days 6-16	0/0	0/0	0/0	8/3
Mortality, days 16-23/postpartum	0/0	0/0	0/0	4/1
Fertility/gestation index (%)	93/100	93/100	86/100	87/46
Viable litters, birth/day 4	13/13	13/13	12/11	6/3
Viable litters, day 7/21	13/13	13/13	11/11	1/1
Pup weight, birth/day 4 (g)	7.1/9.0	6.4/8.8	6.5/8.6	5.7*/5.4*
Pup weight, day 7/21 (g)	12.0/37.0	12.5/37.0	12.2/34.4	7.8/24.4
Pup survival, days 0-4/4-21 (%)	100/96	86/95	87/98	46*/14*

* Significantly different from control (two-sample rank test)

Conclusion:
The NOEL for oral administration of azinphos-methyl to pregnant rats from day 6 to day 15 of gestation was 2.5 mg/kg bw/d with respect to maternal toxicity, based on reduced weight gain and feed consumption and clinical signs of ChE inhibition at 5.0 mg/kg bw/d. The NOEL for developmental toxicity was 5.0 mg/kg bw/d, the highest dose tested.
The NOEL for oral administration of azinphos-methyl to pregnant rats from gestational day 6 to postpartum day 21 was 2.5 mg/kg bw/d with respect to maternal toxicity, based on deaths, clinical signs of ChE inhibition and reduced weight gain and feed consumption at 5.0 mg/kg bw/d. The NOEL for reproduction toxicity was 2.5 mg/kg bw/d, based on a reduced gestation index and reduced survival and weight gain of pups at 5 mg/kg bw/d.

B.5.6.2.2 RAT

Kowalski, Clemens, Bare and Hartnagel Jr., 1987: A teratology study with azinphos-methyl (Guthion® technical) in the rat. Report no. MTD0043 and 94987 of 22 December 1987; Bayer toxicology report no. 973 and 1074; Miles Inc., Toxicology Department, Elkhart, IN 46515, USA. Dates of experimental work: 7 July 1987 to 7 August 1987.
Guidelines and GLP:
The study was performed according to OECD guideline 414, adopted 12 May 1981, with additional ChE determinations. The study is GLP compliant.
The study is considered acceptable.
Material and methods:
Groups of 33 inseminated Crl:CD BR rats (source: Charles River Breeding Laboratories, Portage, MI, USA) received azinphos-methyl (batch no. 79-R-225-42/5FEB87, purity 87.7 %) orally, by intubation in a 6 % aqueous Emulphor emulsion from day 6 to day 15 of gestation at dose levels of 0, 0.5, 1.0 and 2.0 mg/kg bw/d. The dose levels were selected on the basis of a range finding study. From each group, 5 dams were sacrificed on gestation day 16 and the remaining dams on day 20. Plasma, RBC and brain ChE activities in dams were determined on days 16 and 20. Brain ChE activity was also determined in 20 fetuses/group on gestation day 20.
Statistical methods: Dunnett's test (bw, feed consumption, ChE); Fisher's exact test, Kruskall-Wallis & Dunn's test (reproductive data); Chi-square test, Fisher's exact test (fetal skeletal data).

Findings:
Appearance, behaviour, feed consumption, and body weight gain of the dams were not adversely affected by treatment at any of the dose levels employed.

Table B.5.6.2.2-1: ChE activity (plasma, RBC: kU/l, brain: U/g, and % of control) in dams and fetuses

Dose level	0 mg/kg bw/d	0.5 mg/kg bw/d	1.0 mg/kg bw/d	2.0 mg/kg bw/d
Plasma, day 16	1.73 (100 %)	1.56 (90 %)	1.64 (95 %)	1.08 (63 %)
RBC, day 16	0.38 (100 %)	0.34 (90 %)	0.34 (90 %)	0.08* (21 %)
Brain, day 16	2.53 (100 %)	2.70 (107 %)	2.55 (101 %)	1.55* (61 %)
Plasma, day 20	1.44 (100 %)	1.47 (102 %)	1.40 (97 %)	1.32 (92 %)
RBC, day 20	0.58 (100 %)	0.59 (103 %)	0.61 (106 %)	0.44 (77 %)
Brain, day 20	2.67 (100 %)	2.72 (102 %)	2.46 (92 %)	1.91* (72 %)
Fetal brain	1.04 (100 %)	0.96 (91 %)	1.04 (100 %)	1.00 (96 %)

* $p<0.05$

Statistically significant ($p \leq 0.05$) and/or biologically relevant (>20 %) inhibition of ChE in all three compartments occurred in the high dose group at day 16. Practically complete restitution of the plasma ChE activity was found at day 20; recovery of erythrocyte and brain activities was incomplete. The fetal brain ChE activity was not inhibited by the treatment of the dams. There was no effect of treatment on the reproduction parameters and no evidence of embryotoxicity, fetotoxicity or teratogenicity was obtained.
Conclusion:
The NOEL for oral administration of azinphos-methyl to pregnant rats was 1.0 mg/kg bw/d with respect to maternal toxicity, based on inhibition of RBC and brain ChE activity at 2.0 mg/kg bw/d. The NOEL for developmental toxicity was 2.0 mg/kg bw/d, the highest dose tested.

B.5.6.2.3 RABBIT

Machemer, 1975: R1582 (active ingredient of Gusathion®). Studies for embryotoxic and teratogenic effects on rabbits following oral administration. Report no. 5455 of 3 June 1975; Bayer AG, Toxicology Department, Wuppertal-Elberfeld, Germany. Dates of experimental work: January 1975 to June 1975.
Guidelines and GLP:
The test method employed was the internal standard at the time the study was performed. The study is in general compliance with OECD guideline 414, adopted 12 May 1981. When the study was performed, GLP was not compulsory.
Main deviations from current OECD guidelines: Only 11 pregnant animals were used in the control group. The selected dose levels were too low to induce some overt maternal toxicity.
The study is considered supplementary.
Material and methods:
Groups of 11 or 12 inseminated Himalayan rabbits (source: Dr. Karl Thomae GmbH, Biberach, Germany) received azinphos-methyl (batch no. not specified, rcvd. 1/73, purity 92.4 %) orally, by intubation in a 0.5 % aqueous Cremophor emulsion from day 6 to day 18 of gestation at dose levels of 0, 0.3, 1.0 or 3.0 mg/kg bw/d. The dose levels were selected on the basis of a range finding study. Caesarian section was carried out on day 29 of gestation.
Statistical methods: U test of Wilcoxon, Mann and Whitney (weight gain, no. of implantaions, fetuses and resorptions, fetus weight, placenta weight), Chi-square test (no. of fetuses with alterations or malformations), Chi-square test or Fisher's exact test (quotas of fertilized and pregnant does).
Findings:
Azinphos-methyl had no adverse effect on the appearance, behavior and body weight gain of the animals at any of the dose levels employed, and all does survived to caesarian section. At 1 mg/kg bw/d, 2 does resorbed all fetuses. At

3 mg/kg bw/d, one doe also resorbed the fetuses and another doe aborted. The observed frequency is within the normal range for the rabbit strain used in this study. There were no detectable effects on the number of fetuses, number of resorptions, fetal weight, placental weight, number of stunted fetuses, and number of fetuses with slight alterations in bone development or with malformations.
Conclusion:
The NOEL for oral administration of azinphos-methyl to pregnant rabbits was 3.0 mg/kg bw/d. The tested dose levels did not induce maternal toxicity and had no detectable effects on embryonic nor fetal development.
However, the study can not be regarded as satisfactory by OECD standards since the selected dose levels were too low to induce some overt maternal toxicity.

B.5.6.2.4 RABBIT

Clemens, Bare and Hartnagel, 1988: A teratology study in the rabbit with azinphos-methyl (Guthion® technical). Report no. MTD0070 and 97406 of 27 June 1988, Bayer toxicology report no. 1030; Miles Inc., Toxicology Department, Elkhart, IN 46515, USA. Dates of experimental work: 22 September 1987 to 23 October 1987.
Guidelines and GLP:
The study was performed according to OECD guideline 414, adopted 12 May 1981, with additional ChE determinations. The study is GLP compliant.
The study is considered acceptable.
Material and methods:
Groups of 20 inseminated American Dutch rabbits (source: Langshaw Farms, Augusta, MI, USA) received azinphos-methyl (batch no. 79-R-225-42/5FEB87, purity 87.7 %) orally, by gavage in a 7 % aqueous Emulphor emulsion from day 6 to day 18 of gestation at dose levels of 0, 1, 2.5 and 6 mg/kg bw/d. The dose levels were selected on the basis of a range finding study. Caesarian section was performed on day 28 of gestation. Plasma and RBC ChE activities were determined on day 19 of gestation, and in brain on day 28 of gestation.
Statistical methods: Dunnett's test (bw, feed consumption, ChE); Fisher's exact test, Kruskall-Wallis & Dunn's test (reproductive data); Chi-square test, Fisher's exact test (fetal skeletal data), Healy's test (fetal weight).
Findings:
At 6 mg/kg bw/d, ataxia was noted in 4 does and tremors in 2 of these same animals. Body weight and feed consumption were unaffected by treatment at all dose levels.

Table B.5.6.2.4-1: ChE activity (plasma, RBC: U/l, brain: U/g, and % of control)

Dose level	0 mg/kg bw/d	1 mg/kg bw/d	2.5 mg/kg bw/d	6 mg/kg bw/d
Plasma, day 19	396 (100 %)	404 (102 %)	345* (87 %)	307* (78 %)
RBC, day 19	615 (100 %)	530 (86 %)	489 (80 %)	307* (50 %)
Plasma, day 28	200 (100 %)	240 (120 %)	212 (106 %)	229 (115 %)
RBC, day 28	564 (100 %)	592 (105 %)	595 (105 %)	493 (87 %)
Brain, day 28	2.21 (100 %)	2.11 (96 %)	2.08 (94 %)	1.94* (88 %)

* $p<0.05$
At both the intermediate and high dose levels, statistically significant ($p<0.05$) and/or toxicologically meaningful (>20 %) inhibition of plasma and erythrocyte ChE activity was noted on day 19 of gestation. The values returned to normal near term at day 28 of pregnancy. Brain ChE activity was inhibited to a statistically significant extent at 6 mg/kg bw/d.

Administration of azinphos-methyl produced no meaningful adverse effect on any maternal reproductive or fetal parameter studied. There was, however, a statistically significant increase in pre-implantation loss for all 3 treatment groups, when compared with the control. The median values for the low- and mid-

dose group fell within the laboratory's historical control range (0-13.3 %). According to the study authors, the increased pre-implantation loss in the high-dose group is not believed to be treatment-related but, rather, an incidental finding, resulting from a decrease in number of implantations compared with the number of corpora lutea for this group. In the range-finding study, at a dose level of 7 mg/kg bw/d, and in a prior study in rabbits, at a dose level of 6 mg/kg bw/d, pre-implantion loss was not increased. Due to a slight but statistically significant reduction in litter size (within the laboratory's historical control range), fetal and placental weights were increased in the high-dose group.

Table B.5.6.2.4-2: Reproductive efficiency and fetal data

Dose level (mg/kg bw/d)	0	1	2.5	6
Pregnant/total does	18/20	18/20	20/20	20/20
Litter size, mean/median	7.4/7.0	6.2/7.0	7.0/7.0	5.5/6.0*
Pre-implantation loss, mean/median (%)	1.5/0.0	23.0/11.3**	14.8/12.5*	28.0/30.3**
Post-implantation loss, mean/median (%)	2.4/0.0	3.0/0.0	4.3/0.0	7.2/0.0
Weight of viable fetuses, median (g)	37.1	38.2	36.1	39.4**
Placenta wt, median (g)	5.4	5.4	5.1	6.0*

* p<0.05; ** p<0.01

Conclusion:
The NOEL for oral administration of azinphos-methyl to pregnant rabbits was 1.0 mg/kg bw/d with respect to maternal toxicity, based on inhibition of RBC and plasma ChE activity at 2.5 mg/kg bw/d and above and clinical signs and inhibition of brain ChE activity at 6 mg/kg bw/d. The NOEL for developmental toxicity was 6 mg/kg bw/d, the highest dose tested.

B.5.6.2.5 MOUSE

Short, Minor, Unger and Lee, 1978: Teratology of Guthion. Report no. EPA-600/1-78-056 of August 1978; Midwest Research Institute, Kansas City, MO 64110, USA, contracted by US Environmental Protection Agency. Dates of experimental work: not specified.
Short, Minor, Lee, Chernoff and Baron, 1980: Developmental toxicity of guthion in rats and mice. Midwest Research Institute, Kansas City, MO 64110, USA, and US Environmental Protection Agency, Research Triangle Park, NC 27711, USA. Published in: Arch. Toxicol. 43, 177-186.
Guidelines and GLP:
The test method employed was the internal standard at the time the study was performed and is in general compliance with OECD guideline 414, adopted 12 May 1981. When the study was performed, GLP was not compulsory.
Main deviations from current OECD guidelines: The report is not very detailed (missing raw data).
The study is considered supplementary.
Material and methods:
Groups of 22 or 23 inseminated CD-1 mice (source: Charles River Breeding Laboratories, North Wilmington, MA, USA) received azinphos-methyl (lot no. M007, purity: 90.6 %) orally in a vehicle of corn oil at dose levels of 0, 1.25, 2.5 and 5.0 mg/kg bw/d from day 6 to day 15 of gestation. The dose levels were selected on the basis of a range finding study. The animals were sacrificed on day 18 of gestation for examination of the fetuses.
Statistical methods: Homogenous data were analyzed by Dunnett's procedure, heterogenous data by a nonparametric rank test.

Findings:
Signs of ChE inhibition (salivation, urination, lacrimation and tremors) and one death were observed at 5 mg/kg bw/d. There was no effect on litter size, incidence of resorptions or fetal body weights at any of the doses tested. No external anomalies were observed and none of the individual soft-tissue anomalies increased in a dose-related fashion. However, when all of the anomalies were combined by rank there was a significant increase in soft-tissue anomalies with a rank of 2 (anomalies with intermediate value in assessing teratogenic potential) or 3 (anomalies most valuable in assessing teratogenic potential) in the groups that received 2.5 and 5 mg/kg bw/d. In addition, skeletal anomalies with a rank of 2 and the incidence of malaligned sternebrae were significantly increased at 5 mg/kg bw/d. However, there was no pattern of characteristic defects. According to the study authors, this observation suggests that azinphos-methyl increased the incidence of naturally occuring anomalies without producing specific defects.

Table B.5.6.2.5-1: Incidence of soft tissue and skeletal anomalies in mice

Dose level (mg/kg bw/d)	0	1.25	2.5	5.0
Litters/fetuses inspected	19/80	18/82	17/80	14/64
Hemorrhage in pericardium [2#]	0	1.1	3.5	3.0
Duodenum enlarged [3#]	0	0	0	1.0
Hydronephrosis, marked [2#]	0	1.9	1.0	1.0
Small kidney [3#]	0	0	0	1.8
Ectopic kidney [3#]	0	0	1.2	0
Hydroureter [2#]	0	0	1.5	0
Gastroschisis [3#]	0	1.1	0	0
Summary by rank (rank 2 and 3)	0	4.1	7.2*	6.8*
Litters/fetuses inspected	19/87	18/93	17/85	14/67
Interparietal medially curved [2#]	0	0	0	1.4
Sternebrae: unossified [1#]	0	0	0	1.4
incompletely ossif. [1#]	19.4	9.5	7.3	8.7
lobed [2#]	0	2.0	2.0	2.6
malaligned [2#]	6.4	12.5	19.0	24.3*
extra ossif. between [2#]	0	1.1	0	0
Summary by rank (rank 2)	6.4	15.6	21.4	26.9*

Rank of anomaly
* Significantly different from control (two-sample rank test)

Conclusion:
The NOEL for oral administration of azinphos-methyl to pregnant mice from day 6 to day 15 of gestation was 2.5 mg/kg bw/d with respect to maternal toxicity, based on clinical signs of ChE inhibition at 5.0 mg/kg bw/d. The NOEL for developmental toxicity was 2.5 mg/kg bw/d, based on an increased incidence of naturally occuring anomalies (malaligned sternebrae) at 5.0 mg/kg bw/d, a dose overtly toxic to the dams. There was no evidence of teratogenicity.

B.5.7 DELAYED NEUROTOXICITY

Administration of azinphos-methyl to hens did not elicit any evidence of delayed neurotoxicity, neither after single nor after continuous oral administration.

B.5.7.1 HEN

Kimmerle, 1965: Neurotoxicity study with Gusathion active ingredient. Report of 20 May 1965; Bayer AG, Institute of Toxicology, Wuppertal-Elberfeld, Germany. Dates of experimental work not specified.

Guidelines and GLP:
The test method employed was the internal standard at the time the study was performed. Main deviations from the later introduced OECD Guideline 418 (adopted 04 April 1984, revised form 27 July 1995): No mention of a positive control, no measurement of neuropathy target esterase (NTE), deficiencies in reporting. When the study was performed, GLP was not compulsory. According to current standards of neurotoxicity testing, the study is supplementary only.

Material and methods:
Five groups of eight 18-20 month old NHL strain hens (source not specified, body weight range 1.48-2.17 kg) received azinphos-methyl (batch no. and purity not specified), as an 80 % premix in Silkasil S, in the diet for 30 days at concentrations of 0, 900, 1200, 1500 and 1800 ppm followed by a period of 4 weeks with offering of untreated diet. During the post-treatment period, the hens were housed in groups in hen stalls with runs. The hens were carefully observed for signs of neurotoxicity. The animals were weighed weekly and their food consumption was measured at the same intervals.
Blood cholinesterase activity was determined before the study start, at the end of the exposure period and at the end of the post-treatment period. Two hens/group were sacrificed one day after completion of dosing and the remaining animals one day after the end of the post-treatment period. Samples of spinal cord (medulla, thorax and lumbar portions) and sciatic nerve were processed for histopathological examination.

Findings:
The general health of the treated animals was slightly impaired during the feeding period, but there were no overt signs of neurotoxicity at any dose level. Both body weight and food consumption were reduced in all the treated groups in comparison to the controls. Recovery was evident during the post-treatment period but the hens had not achieved their pre-treatment body weight at the end of the study. Feeding of azinphos-methyl for 30 days had no effect on blood cholinesterase activity in any of the treated groups. Histopathology revealed no evidence of a neurotoxic effect of azinphos-methyl (Grundmann, 1965).

Conclusion:
After administration of azinphos-methyl in the diet at concentrations of 900, 1200, 1500 and 1800 ppm no delayed neurotoxic effects occurred in the hen.

Grundmann, 1965: Neurotoxicity study with Gusathion active ingredient - histological findings. Report of 12 November 1965; Bayer AG, Wuppertal-Elberfeld, Germany. Dates of experimental work not specified.

Guidelines and GLP:
Appropriate standard staining techniques were employed. When the study was performed, GLP was not compulsory. The investigation is acceptable.

Material and methods:
Samples of sciatic nerve and spinal medullae (3 stages from the neck, thorax and sacrum) of 10 hens which had received azinphos-methyl in their diet as well as of 6 untreated control hens were histologically examined (see above Kimmerle, 1965). Four of these animals received 1500 ppm and 6 animals 1800 ppm. One animal from each group was sacrificed at the end of the exposure period and the remaining animals were sacrificed at the end of the post-treatment period.

Stains for the sciatic nerve and myelin sheath were prepared using hematoxylin & eosin and van Gieson solution. From the 3 stages of the spinal medulla, hematoxylin & eosin, lipid and myelin sheath stains were prepared. Ganglion cells were stained using cresyl violet.

Findings:
Several interstitial infiltrations were found in the sciatic nerve in both test and control animals. Interstitial infiltrations were also apparent in the spinal medulla of individual animals. These infiltrations are related to non-specific accompanying infections. Changes in the ganglion cells, glia or nerve fibres due to the test substance were not observed.

Conclusion:
There was no histological evidence of a neurotoxic effect by azinphos-methyl at the doses administered.

B.5.7.2 HEN

Kimmerle, 1964: Neurotoxicity study with Gusathion active ingredient. Report of 30 November 1964; Bayer AG, Institute of Toxicology, Wuppertal-Elberfeld, Germany. Dates of experimental work not specified.

Guidelines and GLP:
The test method employed was the internal standard at the time the study was performed. Main deviations from the later introduced OECD Guideline 418 (adopted 04 April 1984, revised form 27 July 1995): No mention of a positive control, no measurement of neuropathy target esterase (NTE), deficiencies in reporting. When the study was performed, GLP was not compulsory. According to current standards of neurotoxicity testing, the study is supplementary only.

Material and methods:
Five groups of eight 18-20 month old NHL strain hens (source not specified, body weight range 1.53-2.15 kg) received azinphos-methyl (batch no. 352, purity not specified), as an 80 % premix in Silkasil S, in the diet for 30 days at concentrations of 0, 75, 150, 300 and 600 ppm followed by a period of 4 weeks with offering of untreated diet. During the post-treatment period, the hens were housed in groups in hen stalls with runs. The hens were carefully observed for signs of neurotoxicity. The animals were weighed weekly and their food consumption was measured at the same intervals.
Blood cholinesterase activity was determined before the study start, at the end of the exposure period and at the end of the post-treatment period. Two hens/group were sacrificed one day after completion of dosing and the remaining animals one day after the end of the post-treatment period. Samples of spinal cord (medulla, thorax and lumbar portions) and sciatic nerve were processed for histopathological examination.

Findings:
There were no overt signs of neurotoxicity at any dose level. The body weight of hens dosed at 600 ppm was slightly reduced, but food consumption was unaffected in all dosed groups. Feeding of azinphos-methyl for 30 days had a slight effect on blood cholinesterase activity in the groups treated with 300 or 600 ppm.

Table B.5.7.2-1: Blood cholinesterase activity

Dose group (ppm)	Cholinesterase activity (%)		
	Pre-treatment	1 day post-treatment	4 weeks post-treatment
0	100	100	100
75	100	89.1	100
150	100	100	100
300	100	84.5	100
600	100	72.8	100

Conclusion:
The slight inhibition of blood cholinesterase seems doubtful, since no inhibition was found at higher dose levels (B.5.7.1.1).
After administration of azinphos-methyl in the diet at concentrations of 75, 150, 300 and 600 ppm no delayed neurotoxic effects occurred in the hen.

B.5.7.3 HEN

Kimmerle and Löser, 1974: Delayed neurotoxicity of organophosphorus compounds and copper concentration in the serum of hens. In Coulston, F. and F. Korte (eds.): Environmental quality and safety; global aspects of chemistry, toxicology and technology as applied to the environment. Vol. 3, Georg Thieme Verlag Stuttgart, Academic Press New York and London. p. 173-178, 1974.
Guidelines and GLP:
GLP: No. The information of this published literature has supplementary value only since the methodical details (e. g. doses used and route of application) are not clearly given in this publication.
Material and methods:
White leghorn hens (16-18 month old, source not specified, body weight range 1.5-2.0 kg) received azinphos-methyl (batch no. and purity not specified):
group 1: one oral dose (1-500 mg/kg bw, not further specified)
group 2: one intraperitonal dose (30 mg/kg bw, obviously incorrectly published as oral)
group 3: repeated doses (0, 900, 1200, 1500 or 1800 ppm in the diet) for 30 days. Group 1 and 2 were observed for signs of delayed neurotoxicity for 42 days. Group 3 was observed during the period of repeated dosing and for 28 days thereafter. The hens were housed individually but could move freely.
Findings and conclusion:
No signs of delayed neurotoxicity were found, neither after single nor after repeated dosing.

B.5.7.4 HEN

Glaza, 1988: Guthion technical - acute delayed neurotoxicity study in the domestic fowl. Report no.: 1067 of 22 September 1988; Hazleton Laboratories America, Inc., Madison, Wisconsin, USA. Dates of experimental work: 21 October 1987 to 4 December 1987.
Guidelines and GLP:
The method employed was according to EPA Guideline 81-7. The study is compliant with the EPA Pesticide Program GLP Standards (40 CFR 160), with the exception that analyses of test article formulations for stability, homogeneity and concentration were omitted. A formal QAU declaration of the test facility is included. The study is acceptable.
Material and methods:
A group of thirty nonfed ca. 12 months old White Leghorn hens (source: Peck's Feed and Grain, Spring Green, Wisconsin, USA) were given a single oral dose by intubation of 330 mg/kg bw azinphos-methyl technical (batch no.: 79R-225-42, purity: 85 %) in corn oil vehicle. The level of 330 mg/kg bw was determined to be the LD50 value in unprotected hens in a preliminary test. Three groups of 10 hens were either left untreated, dosed once orally with vehicle alone or dosed once with 600 mg/kg bw tri-ortho-tolyl-phosphate (TOTP, positive control). Surviving test and vehicle control animals, and 6 positive control animals (without definitive neurotoxic effects) received a second dose on day 21. All animals received 15 mg/kg bw atropine sulfate 15 minutes before the first dose and as needed up to ca. 24 hours postdose. The animals were observed hourly for 4 hours after dosing and then daily until termination. Assessment of ataxia, based on forced locomotor activity, was performed. Body weights were recorded before initiation, at weekly intervals and at termination.

Four positive control animals (with neurotoxic symptoms) were sacrificed on day 21 and all other survivors were killed on day 43 or 44. Animals that died during the study were necropsied but not perfused. All moribund animals and all animals surviving till the end of the observation period were anesthetized and perfused with 10 % phosphate buffered formalin. Sections from brain (medulla/pons, cerebellar and cerebral cortex), spinal cord (cervical, midthoracic and lumbar-sacral region) and peripheral nerves (sciatic, fibular and tibial nerves) were prepared for histological examination.
Statistics: Levene's test for variance homogeneity, standard one-way analysis of variance (ANOVA), Dunnett's t-test.
Findings:
Test substance group: Hypoactivity, prostration, liquid-like feces, impaired locomotor activity, predominantly during first week after treatment were found. One hen was killed in a moribund condition and 17 hens died during the first 2 study days, and one further animal died on day 22. The 11 surviving animals appeared clinically unexceptional at termination although body weight gain was impaired. There was no increase in the incidence or severity of neurodegenerative lesions.
Positive control group: Varying degrees of impaired locomotor activity were evident, becoming more severe as the study progressed. Body weight gain was impaired. All animals survived to termination on day 21. There was an increase in the incidence and/or degree of severity of degenerative digestive chambers, macrophage accumulation, axonal degeneration and demyelination typical of delayed neurotoxicity.
Conclusion:
Azinphos-methyl technical, when given orally to White Leghorn hens at the toxic dose level of 330 mg/kg bw, does not cause acute delayed neurotoxicity.

B.5.8 FURTHER TOXICOLOGICAL STUDIES

Major metabolites of azinphos-methyl have a substantially lower acute oral toxicity to the rat than the parent compound. Similarly, the acute inhalation toxicity is lower for benzazimide than for the parent compound.
An additive effect was observed after simultaneous acute oral administration of azinphos-methyl with methamidophos, azinphos-ethyl or propoxur and a slight super-additive (potentiating) effect with chlorpyrifos and azinphos-methyl.
Oximes such as toxogonin and pralidoxime, alone or in combination with atropine, were found to represent effective antidotes in cases of acute poisoning.
Subacute/subchronic studies on cattle demonstrated that azinphos-methyl is highly acutely toxic. The highest no-effect-level established in cattle for cholinesterase inhibition was 15 ppm for 30 days, equivalent to a daily dose level of 0.45 mg/kg bw. No reduction in milk yield resulted from the administration of 17 ppm to dairy cows for 28 days. In horses the dose of 100 ppm led to inhibition of plasma and erythrocytic cholinesterase activity by about 50 % without signs of toxicity.

B.5.8.1 SUMMARY OF TOXICITY STUDIES ON METABOLITES

Crawford and Anderson, 1974: The acute oral toxicity of Guthion technical, benzazimide and methyl-benzazimide to rats. Report no. 41190 of 23 July 1974; Chemagro Division of Baychem Corporation, Research and Development, USA. Dates of experimental work: not specified.
The method employed was the scientific standard at the time the study was performed. When the study was performed, GLP was not compulsory. The study is considered supplementary. For more details see B.5.2.1.5.

Lamb and Anderson, 1974: The acute oral toxicity of Guthion, benzazimide and methyl-benzazimide to fasted and non-fasted rats using CMC as the vehicle. Report no.: 41621 of 4 September 1974; Chemagro Division of Baychem Corporation, Research and Development, USA. Dates of experimental work: not specified.
The method employed was the scientific standard at the time the study was performed. When the study was performed, GLP was not compulsory. The study is considered supplementary. For more details see B.5.2.1.6.

Sheets, 1988: Acute dermal toxicity of technical grade benzazimide in rabbits. Report no.: 1077 of 28 October 1988; Mobay Corporation, Corporate Toxicology Department, Stilwell, Kansas, USA. Dates of experimental work: 11 November 1986 to 25 November 1986.
The test method employed is in compliance with the demands of the OECD Guideline 402 (adopted 24 February 1987). The study is GLP compliant. A formal GLP/QAU declaration of the test facility is included. The study is acceptable.

Shiotsuka, 1987b: Acute inhalation toxicity study with benzazimide technical in rats. Report no.: 911 of 29 July 1987; Mobay Corporation, Corporate Toxicology Department, Stilwell, Kansas, USA. Dates of experimental work: 30 October 1986 to 20 November 1986.
The test method employed is in compliance with the demands of the OECD Guideline 403 (adopted 12 May 1981). The study is GLP compliant. A formal GLP/QAU declaration of the test facility is included. The study is acceptable.

Eigenberg, 1987: Primary dermal irritation of benzazimide in albino rabbits. Report no.: 854 of 20 May 1987; Mobay Corporation, Corporate Toxicology Department, Stilwell, Kansas, USA. Dates of experimental work: 10 November 1986 to 14 November 1986.
In general, the test method employed is in compliance with the demands of the current OECD Guideline 404 (adopted 17 July 1992). The study is GLP compliant. A

formal GLP/QAU declaration of the test facility is included. The study is acceptable.

Like it is pointed out in table B.5.8-1, the major metabolites of azinphos-methyl have a substantially lower acute oral toxicity to the rat than the parent compound. Similarly, the acute inhalation toxicity is lower for benzazimide than for the parent compound. No skin irritant properties were seen in rabbits.

Table B.5.8-1: Metabolites of azinphos-methyl - acute oral, dermal and inhalation toxicity as well as dermal irritancy

Metabolite	$LD_{50,\ oral}$ (mg/kg bw) Male	$LD_{50,\ oral}$ (mg/kg bw) Female	Reference
Benzazimide*	412 (369-461) in DMSO, fasted rat	269 (213-340) in DMSO, fasted rat	Crawford & Anderson, 1974
	576 (528-628) in CMC, fasted rat	368 (314-433) in CMC, fasted rat	Lamb & Anderson, 1974
	576 (528-628) in CMC, unfasted rat	487 (371-639) in CMC, unfasted rat	Lamb & Anderson, 1974
	LD_{50}, dermal (mg/kg bw)	LD_{50}, dermal (mg/kg bw)	
	>2000 rabbit	>2000 rabbit	Sheets, 1988
	LC_{50} (mg dust/m^3 air, 4 h)		
	>1760 rat	>1760 rat	Shiotsuka, 1987b
	No skin irritant properties rabbit		Eigenberg, 1987
	$LD_{50,\ oral}$ (mg/kg bw)	$LD_{50,\ oral}$ (mg/kg bw)	
Methylbenz-azimide	330 (286-381) in DMSO, fasted rat	330 (286-381) in DMSO, fasted rat	Crawford & Anderson, 1974
	412 (347-488) in CMC), fasted rat	390 (343-444) in CMC), fasted rat	Lamb & Anderson, 1974
	524 (483-570) in CMC, unfasted rat	460 (413-515) in CMC), unfasted rat	Lamb & Anderson, 1974

* 4-oxo-3,4-dihydrobenzo-[1,2,3-triazine]
( ) 95 % confidence limit

B.5.8.2 ACUTE ORAL COMBINATION TOXICITY

The following studies were performed to investigate the combination toxicity of azinphos-methyl and other insecticides. The results are summarized in this chapter. All the studies were performed at Bayer AG, Institute of Toxicology, Wuppertal-Elberfeld, Germany, to the prevailing scientific standard. When the studies were performed, GLP was not compulsory. The studies are acceptable as further information on the active ingredient.
Material and methods:
With the exceptions of animal numbers and doses used (see below), the experimental design was comparable in all studies (for other methodical details see B.5.2.1.8-11). To determine the LD_{50} value of the combination, the individual substances were mixed in the percentage ratio of their LD_{50} values (equitoxic

quantities of both substances) to form a new sample. The anticipated LD_{50} value of this sample was calculated. The ratio of the anticipated to the determined LD_{50} resulted in a factor (F) for combination toxicity (F 0.8-1.2 = additive effect, F < 0.8 = sub-additive effect, F > 1.2 = super-additive effect).
Statistics: LD_{50} according to Litchfield & Wilcoxon, 1949; expected LD_{50} according to Finney, 1952.

Thyssen, 1976: Studies to determine the toxic effects of the simultaneous application of azinphos-methyl or azinphos-ethyl and methamidophos. Report no.: 6354 of 24 September 1976; Dates of experimental work: not specified in report.
Substances: azinphos-methyl, technical purity (batch no.: not supplied) and methamidophos, technical purity (batch no.: not specified).
Number of rats: 10 or 20 males/group.
Doses of the mixed sample: 15, 17.5, 20, 22.5, 25 mg/kg bw.

Thyssen, 1977a: Study for the combination toxicity of azinphos-methyl and propoxur. Report no.: 7174 of 14 December 1977; Dates of experimental work: not specified in report.
Substances: azinphos-methyl (batch no.: 6/05010, purity: 93.0 %) and propoxur (batch no.: 75/40, purity: 99.1 %).
Number of rats: 15 or 30 males/group.
Doses of the mixed sample: 25, 30, 32.5, 35 mg/kg bw.

Thyssen, 1977b: Study for the combination toxicity of azinphos-methyl and azinphos-ethyl. Report no.: 7178 (14 December 1977); Dates of experimental work: not specified in report.
Substances: azinphos-methyl (batch no.: 6/05010, purity: 93.0 %) and azinphos-ethyl (batch no.: not specified, purity: 97.5 %).
Number of rats: 15 males/group.
Doses of the mixed sample: 9, 10, 11, 13, 15 mg/kg bw.

Thyssen, 1977c: Study for combination toxicity of chlorpyrifos, cytrolane, cyolane, tamaron, gusathion-ethyl and gusathion-methyl active ingredient. Report no.: 7179 of 14 December 1977; Dates of experimental work: not specified in report.
Substances: azinphos-methyl (batch no.: 6/05010, purity: 93.0 %) and
1.) chlorpyrifos (batch no. and purity: not specified, LD50: 81 mg/kg bw), 2.) cytrolane (batch no.: 372, purity: 88.5 %, LD50: 5.1 mg/kg bw),
3.) cyolane (batch no.: 3380, purity: 78 %, LD50: 3.2 mg/kg bw), respectively.
Number of rats: 15 males/group.
Doses of the mixed samples: 1.) 20, 25, 35, 50 mg/kg bw; 2.) 4, 5, 6.5, 7.5 mg/kg bw; 3.) 2.5, 3.5, 5 mg/kg bw.

Findings:
The animals showed the typical cholinergic signs. The LD_{50} values are summarized in table B.5.8.2-1.

Table B.5.8.2-1: LD_{50} (mg/kg bw) of azinphos-methyl, the combination ingredient and the combination of both ingredients

LD_{50} Azinphos-methyl	LD_{50} Combination compound	LD_{50} Combination (factor F)	Reference
16.75	31.9 methamidophos	19.5 (1.2)	Thyssen, 1976
9.7	39 propoxur	29.3 (0.84)	Thyssen, 1977a
9.7	11.8 azinphos-ethyl	11.1 (0.96)	Thyssen, 1977b
5.3	81 chlorpyrifos	26 (1.7)	Thyssen, 1977c
5.3	5.1 cytrolane	5.2 (1.0)	Thyssen, 1977c
5.3	3.2 cyolane	3.9 (1.1)	Thyssen, 1977c

Conclusion:
Following acute oral administration a slight super-additive effect was observed with the combination of azinphos-methyl and chlorpyrifos. With all other compounds additive effects were evident.

B.5.8.3 ANTIDOTE STUDIES

B.5.8.3.1 RAT

Sanderson, 1961: Treatment of poisoning by anticholinesterase insecticides in the rat. J. Pharm. Pharmacol. 13, 435-442, 1961. This publication provides supplementary information.
Material and methods:
Azinphos-methyl (12 mg/kg bw) as commercial liquid formulation was administered undiluted once to Wistar rats (150-250 g), followed immediately by intraperitoneal injection of atropine or pyridine-2-aldoxime (2-PAM) alone or in combination.
Findings and conclusion:
Atropine and 2-PAM alone were effective against azinphos-methyl poisoning in rats. The combination was less effective.

B.5.8.3.2 RAT

Lorke and Kimmerle, 1969: Effect of reactivators on organophosphate poisoning. Reprint from Naunyn-Schmiedeberg's Arch. Exp. Path., 263, 237, 1969, abstract. This abstract provides supplementary information.
Material and methods:
Rats were poisoned with azinphos-methyl and treated by reactivators (pyridine-2-aldoxime (2-PAM), toxogonin) alone or in combination with atropine after initial signs of poisoning had appeared.
Findings and conclusion:
The therapeutic effect was good (LD_{50} increased by 50-200 %), when the reactivator given without atropine. In combination with atropine, an enhanced effect was observed.

B.5.8.3.3 RAT AND MOUSE

Crawford, and Doull, 1970: Antagonism of the lethal effects of Dipterex and Guthion with atropine and related drugs. Fed. Proc. 29, 349, 1970, abstract no. 589. This abstract provides supplementary information.
Material and methods:
Azinphos-methyl in polypropylene glycol was administered once, orally, at unspecified dose levels to non-fasted adult male and female CF1 mice and Charles river rats. When the symptoms were well established, the following antidotes were given parenterally to antagonise poisoning: atropine, scopalamine, methantheline or propantheline, alone or in combination with pyridine-2-aldoxime (2-PAM).
Findings and conclusion:
Atropine was effective against azinphos-methyl poisoning in rats and mice but the best results were obtained with scopalamine and propantheline in rats. 2-PAM alone did not protect against azinphos-methyl and its effect in combination was dependent on both the species and antidote used.

B.5.8.3.4 RAT AND MOUSE

Edery, Soroker and Kuhnberg, 1970: Antidotal action of new oximes in experimental organophosphate intoxication. Israel J. Med. Sci. 6, 209-218, 1970. This publication provides supplementary information.
Material and methods:
Rats and mice were treated i. p. with toxogonine or 4-hydroxyiminomethyl-1-(3-N,N-dimethylaminopropyl)pyridinium chloride hydrochloride (a synthetized oxime in this laboratory) each together with atropine. Five minutes later azinphos-methyl was administered by subcutaneous injection (multiples of LD_{50}).
Findings and conclusion:
The treatment with the oxime/atropine combination was successful in order to reduce the toxic action of azinphos-methyl.

B.5.8.3.5 MOUSE

Sterri et al., 1979: Effect of toxogonin and P2S on the toxicity of carbamates and organophosphorus compounds. Acta Pharmacol. et Toxicol., 45, 9-15, 1979. This publication provides supplementary information.
Material and methods:
Toxogonin (80 mg/kg bw) was given i. p. to mice 15 min prior to the oral administration of azinphos-methyl (10, 20, 40 mg/kg bw). 20 hours thereafter the LD50 was determined and the animals were taken for the analysis of acetylcholinesterase activity in erythrocytes, cerebrum and diaphragm. (For further methodical details see B.5.2.1.17.)
Findings and conclusion:
The treatment with toxogonin increased 2-fold the LD50 of azinphos-methyl, but had no influence on the cholinesterase activities.

B.5.8.4 DERMAL ABSORPTION

Franklin, Muir and Moody, 1986: The use of biological monitoring in the estimation of exposure during the application of pesticides. Toxicology Letters, 33, 127-136, 1986. This publication (review of studies on animals and humans) provides supplementary information on the active ingredient (see also B.5.9.3.6).

Material and methods:
Rats were treated with azinphos-methyl. It was dermally applied to the shaved intrascapular skin or intramuscularly injected. The doses were 100, 200, 400, 800 µg/rat. The excretion rate of dimethylthiophosphate (DMTP), a urinary metabolite, was determined.
Findings and conclusion:
A very strong correlation (r=0.94) was found between the dermally applied doses and the levels of DMTP excreted. Over the used dose range, the proportion of azinphos-methyl applied to the amount of DMTP in urine was 10 to 1. After i. m. dosing approx. twice as much DMTP was excreted as after dermal application.

B.5.8.5 STUDIES ON FARM ANIMALS

B.5.8.5.1 CATTLE

B.5.8.5.1.1 HEIFERS

White, Nelson and Allen, 1968: The toxicity of Guthion to cattle. Report no. 23065 of 26 August 1968; Chemagro Corporation, Research and Development Department. Dates of experimental work: not specified.
Guidelines and GLP:
The test method employed was the scientific standard at the time the study was performed.
When the study was performed, GLP was not compulsory. The study is acceptable.
Material and methods:
A group of 12 mixed breed heifers (average body weight 335 kg) were dosed orally for 6 days with azinphos-methyl (batch nos. 670 and 679, purity not specified) in gelatine capsules at a dose level of 3.6 mg/kg bw (calculated to be equivalent to a concentration of 33 ppm in succulent grass feed). One half dose was given in the morning, the other half in the evening. Two heifers were used as controls. At the end of the dosing period, the animals were observed for 7 days. Blood samples for cholinesterase determinations were taken on 3 consecutive days before the start of dosing, on days 2 and 5 of dosing, and on days 2 and 7 of the post-treatment observation period. When animals showed cholinergic symptoms, atropine sulphate (0.25-1.0 mg/kg bw) and pyridine-2-aldoxime (2-PAM, 10-15 mg/kg bw) were administered to the animals intravenously or intramuscularly. Three of the five animals that died during the study were necropsied.
Findings:
On the sixth and last day the cattle were dosed, all animals showed moderate to severe cholinergic symptoms that persisted for 5 days after the cessation of treatment. Treatment with atropine sulphate and 2-PAM did not appreciably alter the severity of the symptoms. Blood cholinesterase activity was reduced to approximately 40 % of the pre-dose level by the fifth day of treatment and fell further to approximately 25 % by day 2 post-treatment. Thereafter, slight recovery occurred. One animal died on day 7, two died on day 8, and two on day 10. Necropsy of 3 of the animals that died revealed hemorrhages from mechanical trauma to the alimentary canal.
Conclusion:
An oral dose of 3.6 mg/kg bw azinphos-methyl for 6 days is acutely toxic resulting in the death of 5/12 animals.

B.5.8.5.1.2 BULL CALVES

Crawford and Anderson, 1973: The effect of daily oral administration of ®Guthion to cattle at doses of 5 and 15 ppm for 30 days. Report no. 35408 of 8 January

1973; Chemagro Corporation, Research and Development Department. Dates of experimental work: not specified.
Guidelines and GLP:
The method employed was the scientific standard at the time the study was performed. When the study was performed, GLP was not compulsory. The study is acceptable.
Material and methods:
Three groups of 3 Holstein bull calves (source: not specified, body weight approximately 124 kg) were dosed orally for 30 days with azinphos-methyl (54.1 % wettable powder, batch no. 2090269), in gelatine capsules, at dose levels of 0, 5 and 15 ppm, equivalent to daily dose levels of 0, 0.15 and 0.45 mg a.i./kg bw, respectively. The animals were observed periodically for signs of toxicity. Food consumption and body weights were recorded weekly. Whole blood cholinesterase activity was determined (Michel 1949) in blood samples taken before the start of dosing, on day 1, and at weekly intervals thereafter.
Findings and conclusion:
Treatment at either 5 or 15 ppm azinphos-methyl, equivalent to daily dose levels of 0.15 and 0.45 mg/kg bw respectively, for 30 days, had no effect on growth, food consumption or cholinesterase activity in blood.

B.5.8.5.1.3 DAIRY COWS

Anderson, 1965: The effect of feeding Guthion on the blood cholinesterase activity and milk production of dairy cows. Report no. 16959 of 18 October 1965 (revision of report no. 10440 of 8 January 1963); Chemagro Corporation, Research and Development Department. Dates of experimental work: not specified.
Guidelines and GLP:
The method employed was the scientific standard at the time the study was performed. When the study was performed, GLP was not compulsory. The study is acceptable.
Material and methods:
Two groups of 3 dairy cows (source and body weight: not specified) were dosed orally for 28 days with azinphos-methyl (batch no.: not specified), mixed with a small amount of grain supplement, half the dose in the morning and half in the evening, at dose levels of 544 and 1088 mg/1000 lb (1.2 and 2.4 mg/kg bw). These doses were equivalent to dietary concentrations of 16.8 and 33.6 ppm. Four undosed animals served as a control group. Whole blood cholinesterase activity was determined in blood samples, taken before the start of dosing and on days 0, 1, 3, 7, 10, 14, 21, 28 and 35. Milk production was measured daily.
Findings and conclusion:
Milk production was significantly decreased at 33.6 ppm but not at 16.8 ppm. Cholinesterase activity in the blood of animals receiving 16.8 ppm was reduced by 20-30 %, but no signs of toxicity were observed in these animals. At 33.6 ppm, cholinesterase activity was depressed by 40-50 %.

B.5.8.5.1.4 DAIRY COWS

Wargo, 1978: The effect of feeding Guthion to dairy cattle. Report no. 66448 of 19 June 1978; Chemagro Corporation, Research and Development Department. Dates of experimental work: not specified.
Guidelines and GLP:
The method employed was the scientific standard at the time the study was performed. When the study was performed, GLP was not compulsory. The study is acceptable.
Material and methods:
Groups of three dairy cows were fed with a diet containing 11, 33 or 77 ppm azinphos-methyl over a period of 28.5 days. One cow was designated as control

animal (0 ppm). The cows were investigated for toxicity signs, body weight, food consumption, milk production, blood cholinesterase (ChE) activity and residues.
Findings and conclusion:
The appearance and behaviour, food consumption, body weight and milk production were unaffected by treatment. Starting at 33 ppm, dose-related inhibition of the ChE activity in whole blood was determined at the end of the treatment period (33 ppm = 50 % inhibition; 77 ppm = 77 % inhibition).

B.5.8.5.2 HORSES

Giri et al., 1974: Oral toxicity of azinphos-methyl in horses. Amer. J. Vet. Res., 35 (8), 1031-1035, 1974.
This publication provides supplementary information.
Material and methods:
Seven groups of three horses were administered daily azinphos-methyl doses at concentrations equivalent to 0, 5, 15, 25, 50, 75 or 100 ppm. The 5 and 15 ppm concentrations were administered over a period of 30 days, whereas the higher levels were only given for seven days. The latter four groups (25-100 ppm) were observed for 10 additional days after the conclusion of treatment. The horses were investigated for toxicity signs as well as plasma and erythrocytic cholinesterase (ChE) activity.
Findings:
No toxicity signs were observed. The 5 and 15 ppm doses induced no detectable inhibition of the ChE activities. The 25 ppm concentration caused slight (5 %) inhibition of the erythrocytic ChE activity. Doses in the 50-100 ppm range led to 53-75 % inhibition of ChE activity in the blood plasma and between 50-61 % in the erythrocytes. The plasma ChE values recovered rapidly, reaching pretreatment activities. The 50 ppm dose group achieved full restitution of erythrocytic ChE activity within 10 days after conclusion of treatment, whereas slight inhibition was still observed at 75 and 100 ppm (7 and 16 %).
Conclusion:
A concentration of 100 ppm azinphos-methyl fed to horses for 7 days caused an approx. 50 % inhibition of plasma and erythrocytic ChE activity, however without manifesting any overt signs of toxicity.

B.5.9 MEDICAL DATA AND INFORMATION

Generally, no cases of health effects were observed in male and female workers subjected to regular medical monitoring and employed in formulating azinphos-methyl using the normal safety precautions. However, in one case, it is highly probable that handling azinphos-methyl led to exacerbation of a pre-existing generalized skin disease characterized by apparently hypersensitive, very dry skin (Faul, 1981; Miksche, 1981).
Rider et al. (1971, 1972, **abstracts only**) found no plasma or erythrocyte cholinesterase inhibition in male volunteers receiving daily doses of 10 to 20 mg azinphos-methyl. Based on a mean body weight of 70 kg, the no-adverse-effect-dose may be calculated to be around 0.3 mg/kg bw.
A total urinary excretion study in male volunteers indicated a skin penetration rate of about 16 % (Feldmann & Maibach, 1974). The absorption rate can increase by a factor of around 4 if the application site is occluded or damaged (Wester & Maibach, 1985). A useful method to determine an exposure of humans (manufactoring personnel, users, bystanders etc.) seems to be the measuring of dimethylthiophosphate (DMTP), an urinary metabolite of azinphos-methyl (Franklin et al., 1986).

B.5.9.1 PLANT PERSONNEL

Simpson, 1965: Exposure to Guthion during formulation. Arch. of Environ. Health, 10, 53-54, 1965.
Subject:
By chance, a plant was visited by scientific officers of the division of occupational health, New South Wales department of public health, Australia. In this plant, azinphos-methyl was formulated under rather primitive conditions. A concentration of 0.5-1.0 mg/m^3 air was measured in the breathing zone of the workers (The tentative limit set by this division of occupational health was 0.3 mg/m^3). The activity of whole blood cholinesterase was at several measure points well below 60 units (modified method of Fleischer et al., AMA Arch. Industr. Health, 1, 510-520, 1956, range 0-120 units, "normal" level 78-110 units, symptoms expected at 20-30 units, recommended removing from contact to organophosphates when the ChE level reached 60 units). Two of the workers showed symptoms and one of them had to be hospitalized. The author attributed the high exposure level and the consequences for health to the not existing safety precautions in this plant.

Miksche, 1981: Information on the effects in man / occupational experience. Bayer AG, Medical Department, Leverkusen, Germany (12 June 1981).
Subject:
Fax concerning the absence of any observations of health impairment in male and female employees, subjected to regular medical examination, working with the Gusathion MS formulation using the usual protective measures.

Faul, 1981: BBA request - effects on humans. Bayer AG, Department DO Medical, Dormagen, Germany (6 February 1981).
Subject:
Letter confirming the likelihood that contact with Gusathion was the cause of generalized dermatosis in an apparently hypersensitive, very dry skin.

B.5.9.2 OCCUPATIONAL EXPOSURE

Mahler, 1991: Health effects attributed to azinphos-methyl exposure in 1991. California Department of Agriculture, pesticide illness surveillance program, worker health and safety branch.

Subject:
About ten case reports received by the California pesticide illness surveillance program in which health effects were attributed to azinphos-methyl exposure, comprehended in 1991.

Anonymous, 1991: Pesticide residues in food - 1991. Joint FAO/WHO meeting on pesticide residues, evaluation 1991, Part II - Toxicology.
Subject:
An incident which occurred in 1987 and involved 32 workers. These workers experienced symptoms including headache, nausea, weakness and vomiting upon entering a field to pick peaches three days after methomyl had been applied to the crop, and about six weeks after an application of azinphos-methyl.

B.5.9.3 VOLUNTEER STUDIES

B.5.9.3.1 Thornton, 1971: Analysis of urine samples from human subjects treated orally with Guthion®. Report no.: 30201 of 20 May 1971; Chemagro corporation, research and development department, USA. Dates of experimental work: December 1970 to January 1971. The study provides supplementary information on the active ingredient.
Objective:
To develope new diagnostic tests it should be found out, whether correlations exist between
a) the amount of azinphos-methyl ingested and the level of anthranilic acid precursors found in urine and
b) the amount of anthranilic acid precursors in urine and the depression of blood cholinesterase activity.
Material and methods:
Five male volunteers received 16 mg azinphos-methyl/day for 30 days. The total 24-hour urine was collected two times before, during and three times after the dosing period. The urine was analyzed for azinphos-methyl residues. From two volunteers, the urine was fluorimetrically analyzed after hydrolysis to convert all azinphos-methyl related compounds to anthranilic acid. The blood cholinesterase activity was measured.
Findings:
A definite increase in the level of azinphos-methyl (and/or metabolites) in the urine was found on the day after the start of administration. A return to pre-treatment levels was recorded on the day following discontinuation of treatment. After hydrolysis of urine samples, a correlation was observed between the intake of azinphos-methyl and the amount of azinphos-methyl residues in urine, which were converted to anthranilic acid. No depression of blood cholinesterase was detected.
Conclusion:
A definite correlation between the azinphos-methyl intake and the residues in urine, which were converted to anthranilic acid, could be observed, but no correlation between the latter and a blood cholinesterase depression.

B.5.9.3.2 Rider, Swader and Puletti, 1971: Anticholinesterase toxicity studies with methyl parathion, Guthion and Phosdrin in human subjects. Fed. Proc. 30, 443, 1971, **abstract** no. 1382. This abstract provides supplementary information.
Material and methods:
Groups of 5 men received daily doses of azinphos-methyl at levels of 10, 12, 14 or 16 mg/day. Two men served as control persons. The plasma and red blood cell (RBC) cholinesterase activity was measured twice weekly during a control period followed by a 30 day treatment period.
Findings and conclusion:
No cholinesterase depression was observed.

B.5.9.3.3 Rider, Swader and Puletti, 1972: Anticholinesterase toxicity studies with Guthion, Phosdrin, Di-syston and Trithion in human subjects. Fed. Proc. 31, 520, 1972, abstract no. 72-1073. This abstract provides supplementary information.

Material and methods:
Groups of 5 men received daily doses of azinphos-methyl at levels of 18 or 20 mg/day. Two men served as control persons. The plasma and red blood cell (RBC) cholinesterase activity was measured twice weekly during a control period followed by a 30 day treatment period.

Findings and conclusion:
No cholinesterase depression was observed.

B.5.9.3.4 Feldmann and Maibach, 1974: Percutaneous penetration of some pesticides and herbicides in man. Toxicol. and Appl. Pharmacol., 28, 126-132, 1974. This publication is acceptable for evaluation purposes.

Objective:
The urinary excretion rate of some pesticides was determined in order to quantify the absorption from the undamaged skin surface.

Material and methods:
Onto the ventral forearm of six men, ^{14}C-azinphos-methyl, dissolved in acetone, was pipetted. The dose was 4 µg/cm^2, corresponding to the amount that would be deposited by a thin film of 0.25 % solution, which can be related to actual exposure conditions. The area was not washed for 24 hours. The total urine was collected for 5 days and the urinary excretion of ^{14}C was quantified. To correct the skin penetration data for incomplete urinary recovery, ^{14}C-azinphos-methyl (in propylene glycol), was intravenously administered and ^{14}C was also quantified in urine.

Findings:
After 5 days, the total urinary excretion after i. v. administration was 69.5 % (SD 6.9) of the dose. The half-life was 30 hours. After topical administration 15.9 % (SD 7.9, corrected by the i. v. data) were excreted via the urine.

Conclusion:
After topical application of azinphos-methyl onto the intact skin under conditions which can be related to actual exposure conditions, the excretion rate via urine is about 16 %.

B.5.9.3.5 Wester and Maibach, 1985: *In vivo* percutaneous absorption and decontamination of pesticides in humans. J. Toxicol. and Environ. Health, 16, 25-37, 1985. This publication (review) is acceptable for evaluation purposes.

Objective:
The principles of percutaneous absorption from a number of pesticides under different conditions are reviewed in this publication.

Findings and conclusion:
The applied dose, the surface area, the anatomic region, the contact time and the species influence the extent of absorption. Other factors which enhance the percutaneous absorption rate are occlusion and application to damaged skin. The latter two factors can increase the percutaneous absorption rate of azinphos-methyl from 15.9 % to 56.1 % and to 60.5 %, respectively.

B.5.9.3.6 Franklin, Muir and Moody, 1986: The use of biological monitoring in the estimation of exposure during the application of pesticides. Toxicology Letters, 33, 127-136, 1986.

Objective:
In order to find out wether the estimation of urinary metabolites is a useful method to assess an exposure with a pesticide, a biological monitoring study was done on orchardists (a). The results were compared with dermal absorption studies on animals (see B.5.8.4) and human volunteers (b).

Material and methods:
a) Three groups of orchardists (12 people in British Columbia, 6 in Ontario and 5 in Nova Scotia), who sprayed their own properties with azinphos-methyl 50WP at the rate of 0.8 to 2.6 kg a.i./ha on one day (1 to 9.5 hours) participated in the study. Patches, which were pinned under the clothing, and thin gloves, worn under the protective gloves, were analyzed for azinphos-methyl. 24 hour-urine samples were collected one day prior spraying, on spray day and on one or two days after spraying and analyzed for dimethylthiophosphate (DMTP). The data found for analysis of patches and of DMTP in urine were correlated.
b) Azinphos-methyl was applied to human volunteers (500, 1000, 2000, 4000, 6000 µg). The absorption rate was monitored by measuring renal excretion of dimethylthiophosphate.

Findings:
a) The DMTP levels in urine collected for 48 hours (spray day and one day after) correlated significantly with the amount of active ingredient sprayed.
b) A consistent correlation between the amount of azinphos-methyl applied percutaneously and the concentration of DMTP excreted was observed. The ratio between the applied dose and the DMTP in urine was about 10 to 1 (similar to animal studies).

Conclusion:
In general, urinary metabolite data seems to provide a more reliable and accurate estimation of exposure than patch data.

B.5.9.4 OBSERVATION ON EXPOSURE OF THE GENERAL POPULATION

Between 1982 and 1988, a small number of incidents which have been definitely, probably or possibly associated with azinphos-methyl either alone or in combination with other pesticides has been reported annually (involving 5-12 persons each year). In addition, an incident occurred in 1987, in which 26 people were affected from spray drift under adverse weather conditions (Anonymous, 1991).

B.5.9.5 DIAGNOSIS OF POISONING

Following absorption in the organism, azinphos-methyl is activated by microsomal liver enzymes to form a P=O compound (the azinphos-methyl oxygen analogue), which represents a powerful cholinesterase inhibitor (Dubois et al., 1957; Murphy & Dubois, 1957; Dahm et al., 1962; Murphy et al., 1968; Hitchcock & Murphy, 1971). In common with other organophosphates, the toxic symptoms induced by azinphos-methyl arise due to an anticholinesterase effect. Diagnosis of acute poisoning may be assisted, therefore, by determination of blood cholinesterase activity.

B.9.5.6 PROPOSED TREATMENT

If accidental contamination leads to poisoning, oxime or oxime/atropine therapy is effective.

B.9.5.7 EXPECTED EFFECTS OF POISONING

The expected signs of acute poisoning with azinphos-methyl, are those common to the organophosphate class, and associated with overstimulation of the cholinergic system due to acetylcholine excess.

B.5.10 SUMMARY OF MAMMALIAN TOXICOLOGY AND PROPOSED ADI, AOEL AND DRINKING WATER LIMIT

B.5.10.1 ABSORPTION, DISTRIBUTION, EXCRETION AND METABOLISM

Following oral administration of azinphos-methyl to rats, the biokinetic studies showed a high degree of absorption (3 hours after dosing the concentration of radioactivity in the blood had reached its highest value) followed by fast elimination from the body. Thus, >94 % of the orally administered dose had been eliminated after two days (62-73 % via urine, 23-34 % via feces). The bile-fistulated animals eliminated 27 % of the applied amount with the bile within one day. A part of this was subject to enterohepatic circulation. After distribution of the radioactivity, the highest levels were found in liver and kidney. 16 days post-administration, extremely low levels were found in all organs.
Benzazimide, a metabolite of azinphos-methyl, has a biokinetic behaviour very similar to the parent molecule. Benzazimide was quickly and completely absorbed in the GIT, followed by fast elimination from the body.
About 75 % of the total dose was identified in metabolism studies. None of the individual azinphos-methyl metabolites constituted more than 5 % of the total dose. Steps in the process of degradation are cleavage of the organophosphorus ester, methylation and oxidation reactions and formation of conjugates.

B.5.10.2 SUMMARY OF MAMMALIAN TOXICOLOGY

Azinphos-methyl is very toxic after single oral administration in both aqueous and non-aqueous vehicles (oral LD50 in rats: 4.4-26 mg/kg bw) and after intraperitoneal injection (ip LD50 in rats: 5.7-11.6 mg/kg bw). The greatest inhibition of plasma and erythrocyte ChE activity following a single oral dose was measured 5 to 24 hours after treatment while the peak brain ChE inhibition was already determined two hours after the treatment. Whereas the mouse (oral LD50: 11-20 mg/kg bw) is as susceptible as the rat, the guinea pig (oral LD50: 80 mg/kg bw) is less susceptible than the rat.
Azinphos-methyl applied dermally is moderately toxic in both aqueous and non-aqueous vehicles (dermal LD50 in the rat: 72.5-250 mg/kg bw) although in one study using a 25 % Cremophor emulsion as vehicle, the LD50 was determined as 2500-5000 mg/kg bw. It is very toxic by inhalation (4h LC50 in the rat: 132-155 µg/l air) when administered as an aerosol.
Clinical symptoms of acute azinphos-methyl intoxication in laboratory animals are qualitatively similar for the oral, dermal, inhalation and intraperitoneal routes of administration. Generally, they are typical of organophosphate poisoning, predominantly comprising palmospasm, ataxia, clonic cramps, prostration, salivation and dyspnea, followed by apathy and piloerection. Clinical symptoms are generally evident shortly after administration (within 5 to 20 minutes of treatment in the lethal dose range), except in the case of dermal exposure where symptoms appear at the day after dosing. Some symptoms of intoxication persist for up to 11 days after exposure.
The substance is not an irritant to either intact or abraded skin of the rabbit, but it elicits mild conjunctival irritation which is reversible within 48 hours. It is not, however, classified as a primary eye irritant. Using the guinea pig maximization test of Magnusson and Kligman, evidence was obtained of skin-sensitizing properties of technical azinphos-methyl. Two additional Bühler patch tests on guinea pigs elicited at high concentrations (6-25 %) also a sensitizing potential. A subsequent challenge at a concentration of 0.6 % in the second study did not elicit a sensitization response.
With regard to the high acute toxicity after oral, dermal and inhalative application as well as the sensitizing potential the following classification and labelling of azinphos-methyl, according to Directive 67/548/EEC, 18th

adaptation, Annex IV (General Classification and Labelling Requirements for Dangerous Substances and Preparations), is proposed:

T+ (Very toxic)
R26 (Very toxic by inhalation)
R28 (Very toxic if swallowed)
R24 (Toxic in contact with skin)
X_i (Irritant)
R43 (May cause sensitization by skin contact).

Short-term studies were performed in rats, dogs and rabbits by oral (dietary), inhalative or dermal administration.
In rats, inhibition of ChE activity was evident at dietary concentrations of 20 ppm and above, and there was some evidence of complete recovery within 30 days after cessation of exposure. Marked cholinergic effects and mortality occured at 50 ppm and above following administration for more than 4 weeks. Limited histopathological examination revealed no specific organ toxicity at dose levels up to 100 ppm. The NOEL in rats was 5 ppm, equivalent to about 0.25 mg/kg bw/d.
In dogs, ChE inhibition was evident at 10 ppm and above. General clinical findings (diarrhoea, impaired general condition, reduced weight gain) and signs of cholinergic stimulation (spasms, tremors) occured at 25 and 100 ppm and above, respectively, and mortality at 400 ppm. Comprehensive histopathological examination of dogs receiving doses up to 125 ppm for 52 weeks revealed no evidence of organ toxicity. The NOEL in dogs was 5 ppm, equivalent to 0.15-0.16 mg/kg bw/d.
Inhalative exposure of rats revealed ChE inhibition and decreased body weight gain in males at air concentraions of 50 µg/l for 1 hour/day over 10 days and 4.72 µg/l for 6 hours/day over 12 weeks. The NOEL for inhalative exposure for 6 hours/day over 12 weeks was 1.24 µg/l.
Dermal application to both abraded and non-abraded skin of rabbits over 3 weeks revealed slight reduced weight gain and ChE inhibition in RBC at 20 mg/kg bw/d. The NOEL for dermal exposure was 2 mg/kg bw/d.
The genotoxic potential of azinphos-methyl was studied *in vitro* in an extensive test battery suitable to assess gene mutations, chromosome aberrations and DNA perturbations using bacteria, yeast cells and mammalian cells, as well as *in vivo* on somatic cells by means of the micronucleus test and in germinal cells by the dominant lethal test. A test for recessive lethal mutations in *Drosophila melanogaster* was also conducted, with negative result. The large majority of the *in vitro* tests, and in particular all the *in vivo* assays, revealed no evidence of a genotoxic potential of azinphos-methyl.
Long-term feeding studies in rats, mice and dogs revealed no specific toxicity to organs and tissues, including the nervous system. When compared with the short-term studies, no additional effects were revealed in either rats or dogs.
No evidence of oncogenic potential of azinphos-methyl was obtained in long-term feeding studies in rats and mice. In one study in Osborne-Mendel rats (NCI, 1978), the incidence of pancreatic tumors (islet-cell adenomas and carcinomas) in the high-dose males (13 %) appeared to be elevated when compared to the pooled controls (2 %). However, since the spontaneous incidence of this lesion varied from 0 % to 22 % in the performing laboratory, the incidence found in the high-dose males cannot be clearly implicated as a chemically induced effect. Similarly, in the same study, a significant higher incidence of thyroid follicular-cell neoplasms in the male low (32 %) and high (33 %) dose groups was found when compared with the matched control (11 %) and pooled control (8 %) groups. However, since the spontaneous incidence of these neoplasms in male Osborne-Mendel rats varied from 0 % to 43 % at the performing laboratory, the incidence found in azinphos-methyl-treated male rats cannot be clearly implicated as a chemically induced effect. Thus, the results of the study suggest but do not provide sufficient evidence for the carcinogenicity of azinphos-methyl in male Osborne-Mendel rats (NCI, 1978). The NOEL in rats, dogs and mice is 5 ppm in the diet, equivalent to dose levels of 0.25-0.31, 0.15-0.26 and 0.79-0.98 mg/kg bw/d, respectively.

Two reproduction toxicity studies (a two-generation-study and an one-generation-study) were performed in rats with doses of 0, 5, 15, 45 ppm. There was evidence of reduced fertility and pup viability at 15 ppm and above and, additionally, at 45 ppm, reduced birth weight and subsequent growth retardation of the offspring. In the one-generation-study an inhibition of ChE activity was demonstrated in the brain of pups at 45 ppm. In the parents brain ChE activity was inhibited at 15 ppm and above in females and at 45 ppm in males. No malformations were observed and histological evaluation of major organs showed no treatment-related changes. In the one-generation-study, the treatment of F0 male animals and subsequent mating with untreated females elicited no effects on reproduction parameters or on the progeny. The NOEL for reproduction toxicity is 5 ppm, equivalent to a dose level of 0.33-0.42 mg/kg bw/d in male rats and 0.48-0.67 mg/kg bw/d in female rats.

In teratogenicity studies, no embryotoxic, fetotoxic or teratogenic effects were observed in rats and rabbits at dose levels up to 5 and 6 mg/kg bw/d, respectively. Doses of 5 and 6 mg/kg bw/d, respectively, elicited overt signs of maternal toxicity (reduced ChE activity, clinical signs related to this finding, reduced weight gain and feed consumption). Treatment of female rats up to the end of the lactation period (peri-/postnatal toxicity study) indicated that dams were more sensitive to azinphos-methyl later in gestation. Treatment-related mortalities and a reduced gestation index of the dams as well as reduced survival and weight gain of the pups were observed at 5 mg/kg bw/d. In mice, there was a slight increase in naturally occuring anomalies at a dose level causing maternal toxicity (5 mg/kg bw/d), but there were no specific (teratogenic) defects.

Administration of azinphos-methyl to hens did not elicit any evidence of delayed neurotoxicity, neither after single nor after continuous oral administration.

Major metabolites of azinphos-methyl have a substantially lower acute oral toxicity to the rat than the parent compound. Similarly, the acute inhalation toxicity is lower for benzazimide than for the parent compound.

An additive effect was observed after simultaneous acute oral administration of azinphos-methyl with methamidophos, azinphos-ethyl or propoxur and a slight super-additive (potentiating) effect with chlorpyrifos.

Oximes such as toxogonin and pralidoxime, alone or in combination with atropine, were found to represent effective antidotes in cases of acute poisoning.

Short-term studies on cattle demonstrated that azinphos-methyl is highly acutely toxic. The highest no-effect-level established in cattle for cholinesterase inhibition was 15 ppm for 30 days, equivalent to a daily dose level of 0.45 mg/kg bw/d. No reduction in milk yield resulted from the administration of 17 ppm to dairy cows for 28 days. In horses the dose of 100 ppm led to inhibition of plasma and erythrocytic cholinesterase activity by about 50 % without signs of toxicity.

Generally, no cases of adverse health effects were observed in male and female workers subjected to regular medical monitoring and employed in formulating azinphos-methyl using the normal safety precautions.

In male volunteers receiving daily doses of 10 to 20 mg azinphos-methyl no plasma or erythrocyte cholinesterase inhibition was found (results only as **abstracts**). Based on a mean body weight of 70 kg, the no-adverse-effect-dose may be calculated to be around 0.3 mg/kg bw.

A total urinary excretion study in male volunteers indicated a skin penetration rate of about 16 %. The absorption rate can increase by a factor of around 4 if the application site is occluded or damaged. A useful method to determine an exposure of humans (manufactoring personnel, users etc.) seems to be the measuring of dimethylthiophosphate (DMTP), an urinary metabolite of azinphos-methyl.

Table B.5.10.2-1: Summary of the most relevant toxicity studies with azinphos-methyl

Study	Dose levels	Result	Reference
Acute oral, rat	1-10 mg/kg bw	LD50: 4.6/4.4 mg/kg bw (m/f) - aqueous vehicle	Mihail, 1978
Acute oral, rat	2-16 mg/kg bw	LD50: 5.6/6.4 mg/kg bw (m/f) - non-aqueous vehicle	Crawford & Anderson, 1974
Acute dermal, rat	100-400 mg/kg bw (m) 63-250 mg/kg bw (f)	LD50: 200-250/155 mg/kg bw (m/f)	Heimann & Lorke, 1982
Acute 4 h inhalation, rat	80-250 mg/l air	LC50: 0.155/0.132 mg/l (m/f)	Shiotsuka, 1987a
Skin irritation, rabbit	0.5 g	No irritation	Zorbas, 1994a
Eye irritation, rabbit	87 mg	Mild irritation, not classified as primary irritant	Zorbas, 1994b
Sensitization, guinea pig	0.1 ml 1 % i/d, 12.5 % topical	Sensitizer	Flucke, 1986
28 d feeding, rat	0, 5, 20, 50 ppm	NOEL = 5 ppm (0.35-0.46 mg/kg bw/d)	Eiben et al, 1983
16 wk feeding, rat	0, 2, 5, 20 ppm	NOEL = 5 ppm (0.25 mg/kg bw/d)	Doull & Rehfuss, 1957
12 wk feeding, dog	5, 10, 20, 50 ppm	NOEL = 5 ppm (0.125 mg/kg bw/d)	Doull & Anido, 1957
3 wk dermal, rabbit @	0, 2, 20 mg/kg bw/d	NOEL = 2 mg/kg bw/d	Flucke & Schilde, 1980
12 wk inhalation, rat @	0, 0.195, 1.24, 4.72 µg/l	NOEL = 1.24 µg/l	Kimmerle, 1976
52 wk feeding, dog	0, 5, 25, 125 ppm	NOEL = 5 ppm (0.15-0.16 mg/kg bw/d)	Allen et al, 1990
104 wk feeding, mouse	0, 5, 20, 40 ppm	NOEL = 5 ppm (0.79-0.98 mg/kg bw/d)	Hayes, 1985
2 yr feeding, rat	0, 5, 15, 5 ppm	NOEL = 5 ppm (0.25-0.31 mg/kg bw/d)	Schmidt, 1987
2 yr feeding, dog	5, 20-50, 50-300 ppm	NOEL = 5 ppm (0.15-0.26 mg/kg bw/d)	Worden et al, 1973
2-generation feeding, rat #, @	0, 5, 15, 5 ppm	NOEL = 5 ppm (0.33-0.48 mg/kg bw/d)	Eiben & Janda, 1987
Teratogenicity, rat	0, 0.5, 1.0, 2.0 mg/kg bw/d	NOEL: (mat) = 1.0 mg/kg bw/d (fet) = 2.0 mg/kg bw/d	Kowalski et al, 1987
Teratogenicity, rabbit	0, 1.0, 2.5, 6.0 mg/kg bw/d	NOEL: (mat) = 1.0 mg/kg bw/d (fet) = 6.0 mg/kg bw/d	Clemens et al, 1988
Neurotoxicity, hen	330 mg/kg bw	No neurotoxicity at overtly toxic dose level	Glaza, 1988
30 d oral human volunteer	10, 12, 14, 16, 18, 20 mg/d	NOEL=20 mg/d (about 0.3 mg/kg bw/d)	Rider et al., 1971, 1972

Basis for the proposed ADI, @ basis for the proposed AOELs

B.5.10.3 ADI

The calculation of the ADI is usually based on the highest dose at which no adverse effect is observed in the most appropriate study in the most sensitive species. However, since the critical toxicological endpoint was not acetylcholinesterase inhibition, the human studies (NOEL: about 0.3 mg/kg bw/d) or the 52-week dog study (NOEL: 0.15-0.16 mg/kg bw/d) were not considered appropriate for estimation of the ADI. For human risk assessment, the adverse effects of azinphos-methyl on reproduction should be considered as critical toxicological endpoint, and thus, the ADI should be based on the NOEL in the 2-generation study in rats (NOEL: 0.48 mg/kg bw/d).

In view of the quality, extent and consistency of the database, it is appropriate to apply the conventional safety/uncertainty factor of 100 and, thus, to derive an **acceptable daily intake (ADI) of 0.005 mg/kg bw.**

The JMPR (1991) estimated an ADI of 0.005 mg/kg bw, based on the NOEL in the 2-generation study in rats using a safety factor of 100.

B.5.10.4 AOEL

According to the principles of Annex VI to directive 91/414 EEC, the proposed AOEL should be established on the basis of the highest dose at which no adverse effect is observed in relevant studies in the most sensitive species. Therefore, the most conservative approach is to use the NOEL from the 2-generation study in rats (5 ppm: 0.48 mg/kg bw/d) to derive the AOEL (which is also the basis for the ADI). The same NOEL (5 ppm) was found in dietary 90-day studies in rats and dogs. Because exposure of operators is likely to occur not persistently throughout their life, but azinphos-methyl exhibited sensitizing properties, an uncertainty factor of 100 is considered to be adequate. On this basis, the calculation results in an

AOEL of 0.005 mg/kg bw/d (oral exposure).

In an subchronic inhalation study (60 x 6 h/d) on rats a NOEL of 1.24 µg/l air (equal to 0.33 mg/kg bw/d) was established. Applying a safety factor of 100 it results in an

AOEL of 0.003 mg/kg bw/d (inhalative exposure).

For direct extrapolation of the dermal exposure, the systemic NOEL of 2 mg/kg bw/d of the subacute (15 x 6 h/d) dermal rabbit study is used. When applying a safety factor of 100, an

AOEL of 0.02 mg/kg bw/d (dermal exposure)

can be derived. Regarding the oral studies, this value reflects a dermal absorption rate of about 25 %. This is in the range of experimental results from a dermal absorption study on rats with a WP formulation and a human volunteer study in which the percutaneous absorption rate has been determined to be 16 % for intact nonoccluded skin.
This derivation of the specific AOELs is in contrast to the applicants proposal to determine these values on the basis of two human volunteer studies (Rider et al., 1971, 1972), submitted as abstract only, without considering any safety factor. This procedure results in an AOEL (oral) of 0.3 mg/kg bw/d which is identical with the NOEL. However, the applicant stated that the available human data were considered not appropriate for establishing the ADI.

B.5.11 ACUTE TOXICITY INCLUDING IRRITANCY AND SKIN SENSITIZATION OF THE PREPARATIONS

Data for two formulations have been submitted representing the common uses of azinphos-methyl: Glusathion M EC 19.5 and Glusathion M WP 25. For the evaluation of Gusathion M WP 25, the tests were performed with Guthion 35 WP (equivalent to Gusathion M WP 35). The content of active ingredient is higher in WP 35 formulation (35 %) than in the WP 25 formulation (25 %) and with respect to the inert ingredients the formulations are very similar. Therefore the submitted studies are acceptable.

Table B.5.11-1: Summary of acute toxicity studies with Gusathion M EC 19.5

Study	Species/sex	Result	Reference
Oral	Rat/m, f	LD_{50}: <18, ca 16 mg/kg bw	Krötlinger, 1995
Dermal	Rat/m, f	LD_{50}: 1,021, 727 mg/kg bw	Krötlinger, 1995
Inhalation	-	-	-
Skin irritation	Rabbit	moderate to severe irritation	Krötlinger, 1995
Eye irritation	Rabbit	severe irritation	Krötlinger, 1995
Sensitization (Buehler test)	Guinea pig	Skin sensitization	Vohr, 1995

Table B.5.11-2: Summary of acute toxicity studies with Gusathion M WP 35

Study	Species/Sex	Result	Reference
Oral	Rat/m, f	LD_{50}: 58, 53 mg/kg bw	Sheets, 1990
Dermal	Rat/m + f	LD_{50}: >2,000 mg/kg bw	Sheets, 1990
Inhalation	Rat/m, f	LC_{50}: 0.596, 0.422 mg/l air	Warren, 1990
Skin irritation	Rabbit	No irritation	Sheets, 1990
Eye irritation	Rabbit	Mild irritation	Sheets, 1990
Sensitization (Buehler test)	Guinea pig	Skin sensitization	Porter, 1987

Gusathion M EC 19.5 has a very high acute oral and moderate acute dermal toxicity. Testing for inhalation toxicity has not been conducted because this is not compulsory for the formulation in question. The formulation showed moderate to severe irritations on the skin and severe irritations on the eyes. The formulation was sensitizing in the Buehler patch test.

Proposal for classification/labelling in accordance with the Directive 78/631/EEC in combination with the latest classification and labelling guidance under Directive 67/548/EEC (ie in the 18th ATP published as Directive 93/21/EEC):

T+ Very toxic
R 21 Harmful in contact with skin
R 28 Very toxic if swallowed
R 38 Irritating to skin
R 41 Risk of serious damage to eyes
R 43 May cause sensitization by skin contact

Gusathion M WP 25 is assessed on the basis of studies with Gusathion M WP 35. It can be said that the formulation has a moderate acute oral and slight dermal toxicity. The LC_{50} was below 1.0 mg/l air. The formulation was not irritating to the skin and mild irritating to the eyes. It was sensitizing in the Buehler patch test.
Proposal for classification/labelling in accordance with the Directive 78/631/EEC in combination with the latest classification and labelling guidance under Directive 67/548/EEC (ie in the 18th ATP published as Directive 93/21/EEC):

T Toxic
R 22 Harmful if swallowed
R 23 Toxic by inhalation
R 43 May cause sensitization by skin contact

B.5.11.1 STUDIES WITH GUSATHION M EC 19.5

B.5.11.1.1 ORAL STUDY IN RATS

Krötlinger, F. (1995): E 1582 19.5 EC 00126/0667 (c.n. Azinphos-methyl); Study for Acute Oral Toxicity in Rats. Bayer report no.: 23910 of April 05, 1995; Bayer AG, Toxicology Department, Wuppertal-Elberfeld, Germany. Dates of experimental work: November 03, 1994 - December 02, 1994.
Guidelines and GLP:
The test study was performed according to OECD Guideline 401 and is in compliance with the demands of Directive 92/69/EEC, Part B, July 31, 1992.
The study is considered acceptable.
Materials and methods:
E 1582 19.5 EC 00126/0667, Azinphos-methyl (Formulation No.: 0807 based on formulation No. 00126/0667; content: 19,3 %) in demineralized water was administered once, orally by intubation (volume: 10 ml/kg bw), at dose levels of 18 and 25 mg/kg bw to male and 12.5, 18 and 25 mg/kg bw to female fasted Wistar rats (Source: Hsd Win:Wu from Winkelmann GmbH, Borchen/Paderborn; body weight range 170-192 g for males and 189-199 g for females; five rats per sex and group). Symptoms and mortality were recorded for 14 days after dosing and the LD_{50} values calculated according to Bliss in the manner described by Rosillo et al., J. Toxicol. Environ. Health 3, 797-809, 1977.
Findings:
Clinical signs: Mortalities occurred within 20 minutes after administration. 3 of 5 and 5 of 5 male rats died following doses of 18 and 25 mg/kg bw respectively and from the female rats 1 of 5, 3 of 5 and 5 of 5 died following doses of 12.5, 18 and 25 mg/kg bw respectively. The signs observed in all animals (palmospasm, increased salivation and swollen buccal region) occured shortly after administration and lastet 1-2 hours. The body weights were not affected by the treatment.
Gross pathology: Animals which died during the post-treatment observation period showed slightly collapsed lung, dark-red liver, pale spleen and moderately red glandular stomach. Rats that were sacrificed at the end of the post-treatment observation period showed slightly red glandular stomach in one animal.
Conclusion:
A LD50 of <18 mg/kg bw was determined for male rats and an approximative LD50 of 16 mg/kg bw was determined for female rats. Accordingly the test substance was of high toxicity to rats following acute oral administration.

B.5.11.1.2 DERMAL STUDY IN RATS

Krötlinger, F. (1995): E 1582 19.5 EC 00126/0667 (c.n. Azinphos-methyl); Study for Acute Dermal Toxicity in Rats. Bayer report no.: 23909 of April 05, 1995; Bayer AG, Toxicology Department, Wuppertal-Elberfeld, Germany. Dates of experimental work: November 23, 1994 - January 26, 1995.
Guidelines and GLP:
The test study was performed according to OECD Guideline 402 and is in compliance with the demands of Directive 92/69/EEC, Part B, July 31, 1992.
The study is considered acceptable.
Materials and methods:
E 1582 19.5 EC 00126/0667, Azinphos-methyl (Formulation No.: 0807 based on formulation No. 00126/0667; content: 19,3 %) as a paste with cellulose powder

(400 g cellulose powder/g test substance) was applied to the intact dorsal skin, shorn on the previous day, of 5 Wistar rats (Source: Hsd Win:Wu from Winkelmann GmbH, Borchen/Paderborn; Germany; body weight range 241-293 g for males and 218-256 g for females) per dose and sex respectively for a period of 24 hours under occlusive dressing. The doses were 220, 800, 1,100 and 2,000 mg/kg bw for males and 220, 530, 800 and 2,000 mg/kg bw for females. Symptoms and mortality were recorded for 14 days after dosing and the LD_{50} values calculated according to Bliss in the manner described by Rosillo et al., J. Toxicol. Environ. Health 3, 797-809, 1977.
Findings:
Clinical signs: At dermal doses of ≥800 mg/kg bw in male and of ≥530 mg/kg bw in female rats clinical signs were observed, like decresased motility and decreased reactivity, labored breathing, palmospasm, piloerection, swollen buccal region, increased salivation, lacrimation and temporary tremor of the head. The signs occurred shortly after administration and lasted in the males until day 4 and in the females until day 5 of the study. Male animals died at doses of ≥800 mg/kg bw and female animals at doses of ≥530 mg/kg bw. A dose of 220 mg/kg bw was tolerated without signs of systemic toxicity and skin reaction by male rats. Local skin reaction were observed in males from day 2-12 and in females from day 2-8. They constisted of partial reddening and formation of scab. There were no treatment-related influences on the body weights.

Table B.5.11.1.2-1: Acute dermal toxicity of Gusathion M EC 19.5 in rats

Dose (mg/kg bw)	Toxicol. results*	Death after	LD_{50}
Males			
220	0/0/5	-	approx. 1,021 mg/kg bw
800	1/5/5	2 days	
1,100	3/5/5	4 hours - 4 days	
2,000	5/5/5	2-5 hours	
Females			
220	0/2/5	-	approx. 727 mg/kg bw
530	1/3/5	3 days	
800	3/5/5	2 - 5 days	
2,000	5/5/5	2-3 hours	

* number of animals: dying/with symptoms/exposed

Gross pathology: Animals which died during the post-treatment observation period showed esophagus change in contents, shavings markedly (once); lung slightly collapsed, dark red (single); liver dark red, pale, distinct lobulation (single); spleen pale; kidneys red medulla, both; stomach change in contents, shavings markedly (once); glandular stomach areas, few, up to 0.2 cm in diameter, dark red; testes displacement abdomen (once); autolysis. In animals sacrified at the end of post-treatment observation period only once both testes diminished in size were seen.
Conclusion:
Gusathion M EC 19.5 was of moderate toxicity to rats following acute dermal administration. Female rats were more sensitive than the males. The dermal LD_{50} was determinated to be approximately 1,021 mg/kg bw in male rats and 727 mg/kg bw in female rats.

B.5.11.1.3 INHALATION

Testing for inhalation toxicity has not been conducted because this is not compulsory for the formulation in question.

B.5.11.1.4 SKIN IRRITATION

Krötlinger, F. (1995): E 1582 19.5 EC 00126/0667; Study for Skin and Eye Irritation/Corrosion in Rabbits. Bayer report no.: 23673 of January 25, 1995; Bayer AG, Toxicology Department, Wuppertal-Elberfeld, Germany. Dates of experimental work: October 04, 1994 - November 15, 1994.

Guidelines and GLP:
The test study was performed according to OECD Guidelines 404 and 405 and is in compliance with the demands of Directive 92/69/EEC, Part B, July 31, 1992.
The study is considered acceptable.

Materials and methods:
E 1582 19.5 EC 00126/0667, Azinphos-methyl (Formulation No.: 0807 based on formulation No. 00126/0667; content: 19,3 %; 500 µl, unchanged) was applied to the intact dorsal skin, shorn on the previous day, of altogether four female albino rabbits (Source: Interfauna U.K. Ltd., Wyton, Huntingdon, England; body weight range 2.8-4.1 kg) for a period of 4 hours under semiocclusive dressing. Due to the expected irritation potency of the test substance in the first step one animal was used. At a later date, the test was completed using three additional animals from which one rabbit died 48 hours after application (reason unknown). Readings were made 1, 24, 48 and 72 hours and 7 and 14 days after application according to Draize.

Findings:
Exposure to the test substance resulted in moderate to severe (score 1-3) erythematous and slight (score 0-2) exsudative skin reaction in all animals. In 2 rabbits, the signs subsided by day 7 and 14, respectively. In the third animal, the signs did not prove to be reversible up to day 14. On day 7 the skin was chapped and on day 7 and 14 the skin showed a white squamous coat.

Table B.5.11.1.4-1: Irritant effects of Gusathion M EC 19.5 on the skin

Animal No.	Draize grade after											
	1 h		24 h		48 h		72 h		7 d		14 d	
	e	oe	e	oe	e	oe	e	oe	e	oe	e	oe
E17	2	1	2	0	2	0	2	0	0	0	-	-
F6	2	1	1	0	dead							
F14	3	2	3	1	3	1	3	1	2 f, g	0 f	1	0
D16	2	1	2	0	2	0	1	0	1	0	0	0

e = erythema and eschar oe = oedema; f = white squamous coat; g = exposed skin area: chapped skin

Conclusion:
Under the given test conditions and considering the described findings, the product has to be classified as irritating to skin.

B.5.11.1.5 EYE IRRITATION

Krötlinger, F. (1995): E 1582 19.5 EC 00126/0667; Study for Skin and Eye Irritation/Corrosion in Rabbits. Bayer report no.: 23673 of January 25, 1995; Bayer AG, Toxicology Department, Wuppertal-Elberfeld, Germany. Dates of experimental work: October 04, 1994 - November 15, 1994.

Guidelines and GLP:
The test study was performed according to OECD Guidelines 404 and 405 and is in compliance with the demands of Directive 92/69/EEC, Part B, July 31, 1992.
The study is considered acceptable.

Materials and methods:
E 1582 19.5 EC 00126/0667, Azinphos-methyl (Formulation No.: 0807 based on formulation No. 00126/0667; content: 19,3 %; 100 µl, unchanged) was placed into

the conjunctival sac of one eye of a female albino rabbit (Source: Interfauna U.K. Ltd., Wyton, Huntingdon, England; 4.1 kg bw). The other eye remained untreated and served as control. 24 hours after instillation of the test substance the treated eye was rinsed with normal saline. Only one animal was used in the test because of the known severe skin irritation. At a later date, this test was repeated with a 1%-formulation of the test substance in deionized water on one rabbit (3.6 kg bw). Eye irritation was scored and recorded as described by Draize, whereas the aqueous humor (opacy) was evaluated according to McDonald and Shadduck (Eye irritation: in Marzulli and Maibach: Dermatotoxicology and Pharmacology, 3rd ed. 1987; Wiley, New York, London).

Findings:
The exposure to the undiluted test substance caused reactions on the mucous membranes, effects on the cornea and discharge. The iris was also transiently affected. Corneal effects, redness and swelling of the mucous membranes did not prove to be reversible within 21 days. The 1 % aqueous formulation was not irritating to the eye.

Table B.5.11.1.5-1: Primary irritation scores; eye: contact time 24 h

Animal no.	Tissue	Signs *	Draize grades Time after exposure and rinsing						
			1 h	24 h	48 h	72 h	7 d	14 d	21 d
undiluted test substance									
D 6	Cornea	o a	2 4	2 3	1 3	1 3	2 u 4	1 u 3	1 u 3
	Fluorescein	i a	- -	2 4 c	2 4 c	1 3 c	2 3 c	1 2 c	1 2 c
	Iris		0	1	1	1	0	0	0
	Conjunctiva	r s	1 2 j	2 1	2 z 1	2 z 1	1 k 1	1 k 2	1 1
	Aqueous humour		0	0	0	0	0	0	0
	Discharge		2	1	2	1	0	0	0
1%-formulation									
G 11	as above		0	0	0	0	0	0	0

* a = area; c = confluent diffuse areas; i = intensity;
j = conjunctivae and nictitating membrane: vesicles;
k = conjunctivae and nictitating membrane: injection of blood vessels;
o = opacity; r = redness; s = swelling; u = vascularisation;
z = conjunctivae and nictitating membrane: red areas.

Conclusion:
The weight of the described findings indicates that the test substance has to be considered as severely irritating to the eye.

B.5.11.1.6 SKIN SENSITIZATION

Vohr, H.-W. (1995): E 1582 19.5 EC 00126/0667; Study for Skin Sensitizing Effect in Guinea Pigs (Buehler Patch Test). Bayer report no.: 23726 of February 10, 1995; Bayer AG, Toxicology Department, Wuppertal-Elberfeld, Germany. Dates of experimental work: October 18, 1994 - November 25, 1994.

Guidelines and GLP:
The test study was performed according to OECD Guidelines 406 and is in compliance with the demands of Directive 92/69/EEC, Part B, July 31, 1992.
The study is considered acceptable.

Materials and methods:
The Buehler epicutaneous patch test was performed with E 1582 19.5 EC 00126/0667, Azinphos-methyl (Formulation No.: 0807 based on formulation No.

00126/0667; content: 19,3 %) using emulsions in sterile physiological saline with the following concentrations (basing on a range-finding test and the results of the 1st challenge):

first to third induction:	50 %	(250 mg test substance/animal)
1st chalenge:	25 %	(125 mg test substance/animal)
	and 12 %	(60 mg test substance/animal)
2nd chalenge:	6 %	(30 mg test substance/animal)
	and 1 %	(5 mg test substance/animal)

The control animals were induced with sterile physiological saline solution. 3 times at days 0, 7 and 14, one group of 20 (test) and 2 groups of 10 (control) female guinea pigs, strain Hsd Win:DH, previously termed Bor:DHPW (source: Winkelmann, Borchen, Germany; body weight range 282-350 g) were induced epidermal with occlusion for 6 hours (administration volume per animal 0.5 ml). The challenges were performed four and five weeks after the first induction in comparison with the control animals.

Findings:

There was no difference between test substance group and control group in appearance, behaviour, and the body weight gain.

After the first challenge, 45 % and 25 % of the test substance animals responded with "slight localised" to "moderate confluent" skin redness to the 25 % and the 12 % test substance formulation, respectively. No skin reactions occurred in the control group. After the 2nd challenge, the 6 % and 1 % test substance formulations led to skin redness in 10 % of the test animals in both cases. There were no skin reactions in the control group.

Table B.5.11.1.6-1: Number of animals exhibiting skin reddening after initiation of challenges

chall-enge	Test substance group							1st and 2nd Control group					
	Test subst. patch				Control patch			Test subst. patch			Control patch		
	after challenge				after chall.			after chall.			after chall.		
	30h	54h	78h	total	30h	54h	78h	30h	54h	78h	30h	54h	78h
1th													
25 %	1	8	8	9	0	0	0	0	0	0	0	0	0
12 %	1	3	5	5	0	0	0	0	0	0	0	0	0
2nd													
6 %	2	0	0	2	0	0	0	0	0	0	0	0	0
1 %	2	0	0	2	0	0	0	0	0	0	0	0	0

Conclusion:

The test substance exhibited a skin-sensitizing potential under the conditions of the Buehler patch test.

B.5.11.2 STUDIES WITH GUSATHION M WP 35

B.5.11.2.1 ORAL STUDY IN RATS

Sheets, L.P. (1990): Acute Oral Toxicity Study with Guthion 35 WP in Rats. Bayer file no.: 5420, Report of December 10, 1990; Mobay Corporation, Corporate Toxicology Department, South Metcalf, Stilwell, KS; USA. Dates of experimental work: July 09, 1990 - July 27, 1990.

Guidelines and GLP:

The test study was performed according to OECD Guideline 401 and is in compliance with the demands of Directive 92/69/EEC, Part B, July 31, 1992. The study is considered acceptable.

Materials and methods:

Gusathion M WP 35 (Batch Nos. 0-14-3651; 0-03-0128, 36,3 % and 35,9 % active ingredient) in deionized water was administered once, orally by intubation

(volume: 5 ml/kg bw), at dose levels of 20, 42 and 80 mg/kg bw to male and 42, 52, 63 and 80 mg/kg bw to female fasted Sprague-Dawley rats (Sas: CD(SD)BR; Source: Sasco Inc., Houston, TX; body weight range 290-360 g for males and 192-229 g for females; five rats per sex and group). Symptoms and mortality were recorded for 14 days after dosing and the LD_{50} values calculated using a modified probit analysis program according to Stephen (Stephen C.E., 1982. EPA, Envir. Res. Lab., Personal communication to Bahner L.).

Findings:

Clinical signs: The incidence of mortality increased with dose for both males and females, with all deaths on day 0. Treatment-related signs of toxicity (red nasal, oral and lacrimal stains, urine and anal stains, clear nasal discharge, muscle fasciculations and tremors) were apparent on days 0-1 and resolved in surviving animals by day 7. Alopecia was seen at various locations on three animals beginning on days 5-8. Body weight gain decreased from days 0-7 in a dose-related manner in surviving males, with recovery evident by day 14. Body weight gain was not effected in surviving females.

Gross pathology: Evidence of salivation and lacrimation, as well as reddened lungs, were considered treatment-related gross lesions in animals found dead. Alopecia was the only gross lesion observed in animals that survived day 14.

Table B.5.11.2.1-1: Acute oral toxicity of Gusathion M WP 35 in rats

Dose (mg/kg bw)	Toxicol. results*	Death after days	LD_{50}
Males			
20	0/0/5	-	58 mg/kg bw
42	1/1/5	0	
90	4/1/5	0	
Females			
42	0/2/5	-	53 mg/kg bw
52	2/3/5	0	
63	5/0/5	0	
80	5/0/5	0	

* number of animals: dying/with symptoms/exposed

Conclusion:

The acute oral LD_{50} for males, with 95 % confidence intervals, was 58 mg/kg bw (27-167 mg/kg bw), with a slope in the dose-mortality curve of 6.2. For females the LD_{50} (determined by nonlinear interpolation) was 53 mg/kg bw. The NOEL was 20 mg/kg bw for males and <42 mg/kg bw for females. The test substance has to be classified accordingly.

B.5.11.2.2 DERMAL STUDY IN RATS

Sheets, L.P. (1990): Acute Dermal Toxicity Study with Guthion 35 WP in Rats. Bayer file no.: 5409, Report of December 05, 1990; Mobay Corporation, Corporate Toxicology Department, South Metcalf, Stilwell, KS; USA. Dates of experimental work: July 10, 1990 - October 09, 1990.

Guidelines and GLP:

The test study was performed according to OECD Guideline 402 and is in compliance with the demands of Directive 92/69/EEC, Part B, July 31, 1992. The study is considered acceptable.

Materials and methods:

Gusathion M WP 35 (Batch Nos. 0-14-3651; 0-03-0128, 36,3 % and 35,9 % active ingredient) moistened with tap water was applied to the intact dorsal skin, shorn on the previous day, of 5 Sprague-Dawley rats (Sas: CD(SD)BR; Source: Sasco Inc., Houston, TX; body weight range 231-260 g for males and 200-219 g for females) per group and sex respectively for a period of 24 hours under occlusive dressing. Collars were applied on all animals for seven days. One group/sex received the dermal limit dose (2,000 mg/kg bw) and the other group/sex served

as the untreated control. Control animals received exactly the same treatment except the test substance. Symptoms and mortality were recorded for 14 days after dosing.

Findings:

Clinical signs: No death resulted from treatment at the dermal limit dose, therefore, additional dose levels were not tested and LD_{50} values were not determined. Treatment-related clinical signs (muscle fasciculations and decreased motor activity) were observed only in one female. All other clinical signs were attributed to the collars. Body weight gain was not affected by treatment.

Gross pathology: No treatment-releated gross lesions were observed.

Conclusion:

The dermal LD_{50} for Gusathion M WP 35 was >2,000 mg/kg bw for males and females. The no-observed-effect level was 2,000 mg/kg bw for males and <2,000 mg/kg bw for females.

B.5.11.2.3 INHALATION STUDY IN RATS

Warren, D.L. (1990): Acute Four-Hour Inhalation Toxicity Study with Guthion 35 WP in Rats. Bayer file no.: 5419, Report of December 10, 1990; Mobay Corporation, Corporate Toxicology Department, South Metcalf, Stilwell, KS, USA. Dates of experimental work: August 02, 1990 - September 06, 1990.

Guidelines and GLP:

The test study was performed according to OECD Guideline 403 and is in compliance with the demands of Directive 92/69/EEC, Part B, July 31, 1992. The study is considered acceptable.

Materials and methods:

The acute toxicity of Gusathion M WP 35 (Batch Nos. 0-14-3651; 0-03-0128, 36,3 % and 35,9 % active ingredient) tested as a dust wes evaluated in Sprague-Dawley rats (Sas: CD(SD)BR; Source: Sasco Inc., Houston, TX; body weight range 192-315 g for males and 176-238 g for females). Six rats per sex and group were exposed for four hours under nose-only exposure conditions to concentrations of 248, 334, 445, 590 and 780 mg/m^3 (gravimetric concentration, resp. 727-1,818 mg/m^3 nominal; mass median aerodynamic diameter, MMAD: 2.3-4.2 µm). Comparable groups (5 or 6) of rats were sham-exposed (to conditioned room air) and served as control. Symptoms and mortality were recorded for 14 days after dosing and the LC_{50} values with 95 % confidence limits were estimated by probit analysis.

Findings:

Clinical signs: No deaths from either sex of animals were observed from controls or groups exposed to 280 mg/m^3 of Guthion 35 WP. Signs related to exposure to the test substance (hypoactivity, muscle fasciculation, red nasal stain, salivation, tremor) were observed in all treated groups. Signs that may be releated to exposure were dyspnea lacrimation, moribundity, and rales. These signs were first observed shortly after exposure (day 0) and a complete recovery from both sexes generally occured by day 3. Decreases in body weight gain were recovered by day 14.

Gross pathology: Gross lesions related to Gusathion M WP 35 exposure were salivation and reddened lungs (observed from animals found dead before day 14).

Table B.5.11.2.3-1: Acute inhalation toxicity of Gusathion M WP 35 in rats

Gravimetric concentration (mg/m^3)	Mortality (number of animals) (dying/exposed)	Death after days	LC_{50}
Males			
control I	0/5	-	596 mg/m^3
248	0/6	-	
445	3/6	0	
590	3/6	0	
control II	0/6	-	
334	1/6	0	
780	4/6	0	
Females			
control I	0/6	-	422 mg/m^3
248	0/6	-	
445	4/6	0	
590	3/6	0	
control II	0/6	-	
334	4/6	0, n=3; 3, n=1	
780	6/6	0	

Conclusion:

While a NOEL was not identified by this study, LC_{50} values were for males 0.596 mg/l (95 % confidence limits: 0.448-1.004 mg/l) and females 0.422 mg/l (no confidence limits calculable). Therefore the test substance was toxic to rats and has to be classified accordingly.

B.5.11.2.4 SKIN IRRITATION

Sheets, L.P. (1990): Primary Dermal Irritation Study with Guthion 35 WP in Rabbits. Bayer file no.: 4706, Report of August 30, 1990; Mobay Corporation, Corporate Toxicology Department, South Metcalf, Stilwell, KS; USA. Dates of experimental work: May 23, 1990 - May 26, 1990.

Guidelines and GLP:

The test study was performed according to OECD Guideline 404 and is in compliance with the demands of Directive 92/69/EEC, Part B, July 31, 1992. The study is considered acceptable.

Materials and methods:

500 mg of Gusathion M WP 35 (Batch Nos. 0-14-3651; 0-03-0128, 36,3 % active ingredient) was applied to a the intact dorsal skin, shorn on the previous day, of 3 male and 3 female New Zealand White Rabbits (Source: Small Atock, Industries, Pea Ridge, Arkansas, USA; approximately 15 weeks of age) for a period of 4 hours under occlusive dressing. Readings were made 1, 24, 48 and 72 hours after application according to Draize.

Findings:

No signs of irritation were seen in any animal and there were no other lesions or toxic signs onserved. The primary irritation index was 0.0.

Conclusion:

Under the given test conditions, the product was not irritating to skin.

B.5.11.2.5 EYE IRRITATION

Sheets, L.P.: Primary Eye Irritation Study with Guthion 35 WP in Rabbits. Bayer file no.: 5298, Report of August 24, 1990; Mobay Corporation, Corporate Toxicology Department, South Metcalf, Stilwell, KS; USA. Dates of experimental work: May 22, 1990 - May 29, 1990.

Guidelines and GLP:
The test study was performed according to OECD Guideline 405 and is in compliance with the demands of Directive 92/69/EEC, Part B, July 31, 1992.
The study is considered acceptable.
Materials and methods:
0.1 ml (approx. 42 mg) of Gusathion M WP 35 (Batch Nos. 0-14-3651; 0-03-0128, 36,3 % active ingredient) was placed into the conjunctival sac of the left eye of 4 male and 2 female New Zealand White Rabbits (Source: Small Atock, Industries, Pea Ridge, Arkansas, USA; approximately 15 weeks of age) respectively. The right eye served as the untreated control. Readings were made 1, 24, 48 and 72 hours and 7 days after application according to Draize.
Findings:
The cornea and iris showed no lesions or signs of irritation in any animal. Only one hour after dosing ocular discharge (grades 1-3) was seen in two animals. Chemosis and conjunctival redness (max. grade 1) were observed in all six rabbits and had resolved in all animals by 48 hours and 7 days respectively.
Conclusion:
Under the test conditions chosen and considering the described findings the product was only very mild irritating to the eye and there is no needs for classifying.

B.5.11.2.6 SKIN SENSITIZATION

Porter, M.C., Craigo, R.E., and Hartnagel Jr., R.E. (1987): Dermal Sensitization of Guthion 35 WP in the Guinea Pig. Bayer file no.: 885, Report of June 29, 1987; Miles Laboratories, Inc., Toxicology Department, Elkhart, IN, USA. Dates of experimental work: not indicated in the report.
Guidelines and GLP:
The test study was performed in compliance with the demands of Directive 92/69/EEC, Part B, July 31, 1992.
The study is considered acceptable.
Materials and methods:
The Buehler epicutaneous patch test was performed with Gusathion M WP 35 (Batch No. 86R0330I from Mobay Corporation, Stillwell, KS,; content: 35 %) in a 50 % ethanol/distilled water vehicle using the highest nonirritating concentration of 5.0 % basing on a pretest. Thirty male Guinea pigs, Hartley strain (source: Harlan Sprague Dawley, Indianapolis, IN, USA) were assigned to Gusathion M WP 35 and control groups of 15 and 5 animals respectively, and 1-chloro-2,4-dinitrobenzene (DNCB - positive control; 0.05 % solution in 50 % ethanol/distilled water) test and control groups, each consisting of 5 animals. Animals in the test groups received 3 topical sensitizing applications of the appropriate formulation on study days 0, 7, and 14, followed by a 2-week rest period and s topical challenge application on day 28. Animals in the 2 control groups received no sensitizing applications, but a single topical application of the appropriate formulation on day 28. All induction and challenge sites were scored for erythema at 24 and 48 hours post-application.
Findings:
The challenge of 15 guinea pigs, previously exposed to Gusathion M WP 35 during the induction period, resulted in slight (10 animals) to moderate (1 animal) erythema at 24 and/or 48 hours in 11 animals. None of the naive control animals exhibited a positive response when exposed to Gusathion M WP 35. Erythema (ranging from slight to severe)was observed for 4 Of 5 DNCB test animals, while no response was seen for DNCB negative controls.

Table B.5.11.2.6-1: Dermal scores for induced and naive guinea pigs following challenge with Gusathion M WP 35 and DNCB

Test group	Incidence*	Severity**
Gusathion M WP 35-induced	11/15 = 0.73	18/30 = 0.60
naive control	0	0
DNCB-induced	4/ 5 = 0.80	17/10 = 1.70
naive control	0	0

* Number of animals showing positive response at 24 or 48 hourrs / animals tested
** Mean of all test grades at 24 and 48 hours, corrected for reading at vehicle site

Conclusion:
The test substance exhibited a skin-sensitizing potential under the conditions of the described Buehler patch test and has to be classified accordingly.

B.5.12 DERMAL ABSORPTION

Schroeder, R.S. (1992): Dermal absorption of azinphos-methyl by rats from a Guthion® 35 % wettable powder formulation using ^{14}C-azinphos-methyl. Bayer file no.: 6387 of march 27, 1992; Miles Inc., Toxicology, Stilwell, Kansas, USA. Dates of experimental work: October 30, 1990 to March 8, 1991.
Guidelines and GLP:
The method employed was based on the draft EPA guideline , dated 7 February 1990. The study is compliant with EPA-FIFRA GLP standards. The study is considered acceptable.
Material and methods:
Three groups of 24 (treated) and 2 groups of 4 (control) male Sprague-Dawley rats (source: SASCO, Inc., St. Louis, MI., USA; body weight range 202-275 g) were treated once with [phenyl-UL-^{14}C]azinphos-methyl (radiochemical purity 99.3 %), as the formulation suspended in water, at doses of 0, 0.04, 0.4 and 4.0 mg/animal (equivalent to 0, 2.67, 26.7 and 267 µg formulation/cm^2) resulting in 0, 0.056, 0.56 and 5.6 mg azinphos-methyl/kg bw and 0, 0.93, 9.3 and 93 µg azinphos-methyl/cm^2. (The selection of the doses based on the sponsors information, that human dermal exposure to acinphos-methyl range from 0.083-5.0 mg/kg bw/day by use dilution rates of Guthion WP 35 from 0.52-18.9 µg/µl. The doses chosen for the descibed study were 0.27, 2.67 and 26.7 µg/µl.) All treatments were applied to 15 cm^2 of shaved, occluded dorsal skin. Groups of rats/dose group were monitored at 1, 4, 10, 24, 72 and 168 hours after application. The application sites of animals on test for more than 10 hours were wiped with a detergent-wetted pad after 10 hours to model a worker cleaning up after a full day's field work. A blood sample was withdrawn from each animal at termination for the determination of plasma and erythrocyte cholinesterase activities.
Statistical methods: analysis of variance, Bonferroni's t-test and Dunnett's test using SAS software.
Findings:
The majority of azinphos-methyl was absorbed by the skin during the first hour after application of the WP-suspension. Approximately 2/3 of the dose absorbed after 10 hours was excreted in the urine during a 168 hour period, the majority of the remaining radioactivity being located in the treated skin sample, suggesting that it may be immobilized there. No more than 2 % of the absorbed dose is excreted in a 24 hour urine sample obtained later than 48 hours after exposure. The extent of absorption of the highest dose level was 21 %, but this did not result in a depression of plasma or erythrocyte cholinesterase of more than 20 % at 168 hours (ie the suggested threshold limit for significant exposure; given Reference: Gage, J.C. (1967), The Significance of Blood Cholinesterase Activity Measurements, Residue Reviews, 18, 159-173).

Table B.5.12-1: Extent* of absorption of [phenyl-UL-^{14}C]azinphos-methyl after 10 hours exposure

Nominal dose (µg formulation/cm^2)	Absorption (% of applied dose)	mg (-equivalents)
2.67	54	0.018
26.7	32	0.115
267	21	0.860

* mg in skin, carcass, blood, urine, feces and cage washings

Conclusion:
As appropriate for risk evaluations, an absorption rate of 20 % (ie the average of absorption at all time points for the highest dose used) is proposed there.
Assessment:
On the basis of the subchronic animal studies with azinphos-methyl a dermal absorption rate of about 25 % can be assumed. The dermal absorption study on rats with Guthion WP 35 using ^{14}C-azinphos-methyl showed absorption rates of 20-50 % depending on the dose and the exposure time. However, an absorption rate of 100 % for rats and rabbits was reported (in Franklin et al., 1986; see B.5.9.3.6. The applicant will be asked to submit this publication.).
In dermal studies with azinphos-methyl on human volunteers (see B.5.9.3.4; B.5.9.3.5), the absorption through the skin was found to be 16 % for normal nonoccluded skin, but about 60 % if the skin was occluded or the skin was damaged. The high dermal bioavailability has to be taken into account in the risk assessment for operator, worker and bystander.

B.5.13 TOXICOLOGICAL DATA ON NON ACTIVE SUBSTANCES

Data for all coformulants are given as Safety Data Sheets. The major coformulants of Gusathion M EC 19.5 are the solvent (about 70 %) and an emulsifier (>10 %) which are classified as harmful (Xn) and irritating (Xi) respectively. These properties are covered by the studies with the preparation. Gusathion M WP 25 (resp. Gusathion M WP 35) containes coformulants without need of classification, only, or in a concentration of <1 %. For this preparation, there were also studies available.

B.5.14 EXPOSURE DATA

B.5.14.1 Operator exposure
According to the applicants statement, Gusathion M EC 19.5 and Gusathion M WP 25 are applied on crops using field crop sprayers, portable or hand held sprayers, and broadcast air assisted fruit tree sprayers. On this basis, operator exposure estimates were calculated using the German model:
Uniform Principles for Safeguarding the Health of Applicators of Plant Protection Products (Uniform Principles for Operator Protection); Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft, Berlin-Dahlem, no. 277, 1992 ("German model").
Calculation of operator exposure:
The following assumptions have been made:
Treated area per day:
20 ha/d for field crops/tractor mounted (FCTM)
8 ha/d for high crops/tractor mounted (HCTM)
1 ha/d for high crops/hand held (HCHH)
Application rates for both formulations, recommended by the applicant:
1000 g ai/ha for field crops/tractor mounted
3000 g ai/ha for high crops/tractor mounted
3000 g ai/ha for high crops/hand held

The calculation of the estimated operator exposure was carried out for three different scenarios regarding personal protective equipment (PPE):

I. no PPE: No personal protective equipment is used when handling the undiluted product and during application.

II. with PPE, recommended by the applicant:
- gloves during mixing/loading and application; reduction coefficient: 0.01
- standard protective garment (plant protection) and sturdy footwear during mixing/loading and application; reduction coefficient: 0.05

III. with PPE, recommended by the applicant (see II.) and additional PPE in field crops:
- half-mask with combination filter A1P2 during mixing/loading and application; reduction coefficient: 0.02 for inhalation, 0.8 for dermal exposure (head region) in high crops
- hood and visor during application; reduction coefficient: 0.05 for dermal exposure (head region)

Gusathion M WP 25 is marketed in watersoluble bags (PVAL-containers, polyvinylalcohol), which are passed unopened into the spay tank, where they dissolve. This kind of handling precludes contamination during mixing and loading and the corresponding values can be set to zero exposures for both formulations.

Table B.5.14.1-1: Gusathion M EC 19.5 - estimated operator exposure
I.: no PPE

		FCTM	HCTM	HCHH
dermal exposure			mg/person/d	
	mixing/loading	48.00	57.60	615.0
	application	40.80	276.00	121.2
	total	88.80	333.60	736.2
inhalation exposure			mg/person/d	
	mixing/loading	0.012	0.0144	0.15
	application	0.02	0.432	0.9
	total	0.032	0.4464	1.05

Table B.5.14.1-2: Gusathion M EC 19.5 - estimated operator exposure
II.: PPE recommended by applicant

		FCTM	HCTM	HCHH
dermal exposure			mg/person/d	
	mixing/loading	0.48	0.576	6.15
	application	2.876	40.488	18.468
	total	3.356	41.064	24.618
inhalation exposure			mg/person/d	
	mixing/loading	0.012	0.0144	0.15
	application	0.02	0.432	0.9
	total	0.032	0.4464	1.05

Table B.5.14.1-3: Gusathion M EC 19.5 - estimated operator exposure
III.: PPE recommended by applicant + additional PPE

		FCTM	HCTM	HCHH
dermal exposure			mg/person/d	
	mixing/loading	0.48	0.576	6.150
	application	2.636	13.128	4.788
	total	3.116	13.703	10.938
inhalation exposure			mg/person/d	
	mixing/loading	0.00024	0.000288	0.003
	application	0.0004	0.00864	0.018
	total	0.00064	0.008928	0.021

Table B.5.14.1-4: Gusathion M WP 25 - estimated operator exposure
I.: no PPE

		FCTM	HCTM	HCHH
dermal exposure			mg/person/d	
	mixing/loading	0.0	0.0	0.0
	application	40.80	276.00	121.2
	total	40.80	276.00	121.2
inhalation exposure			mg/person/d	
	mixing/loading	0.0	0.0	0.0
	application	0.02	0.432	0.9
	total	0.02	0.432	0.9

Table B.5.14.1-5: Gusathion M WP 25 - estimated operator exposure
II.: PPE recommended by applicant

		FCTM	HCTM	HCHH
dermal exposure			mg/person/d	
	mixing/loading	0.0	0.0	0.0
	application	2.876	40.488	18.468
	total	2.876	40.488	18.468
inhalation exposure			mg/person/d	
	mixing/loading	0.0	0.0	0.0
	application	0.02	0.432	0.9
	total	0.02	0.432	0.9

Table B.5.14.1-6: Gusathion M WP 25 - estimated operator exposure
III.: PPE recommended by applicant + additional PPE

		FCTM	HCTM	HCHH
dermal exposure			mg/person/d	
	mixing/loading	0.0	0.0	0.0
	application	2.64	13.128	4.788
	total	2.64	13.128	4.788
inhalation exposure			mg/person/d	
	mixing/loading	0.0	0.0	0.0
	application	0.0004	0.00864	0.18
	total	0.0004	0.00864	0.018

Determination of the tolerable exposure:
The proposed acceptable operator exposure level should be established on the basis of the highest dose at which no adverse effect is observed in relevant studies in the most sensitive species. In a subchronic inhalation study (60 x 6 h/d) on rats a NOEL of 0.00124 mg/l air equal to 0.3348 mg/kg bw/d (assuming a respiration volume of 45 l/kg bw/h during 6 h per day: factor 270) was established. A systemic NOEL of 2 mg/kg bw/day was observed in a subacute (15 x 6 h/d) dermal rabbit study. This should be the basis for deriving the AOEL for

inhalation and dermal exposure (see B.5.10.4). Assuming a body weight of 70 kg and using an extended safety factor of 100 because of the sensitizing properties of azinphos-methyl, the tolerable inhalation exposure (I_{tol}) and the tolerable dermal exposure (D_{tol}) are calculated to be:

D_{tol} = 2 mg/kg bw/d x 70 kg : 100 = 1,4 mg/person/day (0.02 mg/kg bw/day)

I_{tol} = (0.00124 x 270) mg/kg bw/d x 70 kg : 100 = 0,234 mg/person/day (0.003 mg/kg bw/day)

Comparison of estimated and tolerable exposure:
Using the following equations, the total degree of exposure (E) is calculated for both types of formulation, for the three application methods and for the three scenarios regarding operator protection respectively; values of E = <1 indicate that no risk for the applicator is to be assumed.

For Gusathion M EC 19.5:

(D : D_{tol}) + (I : I_{tol}) = E

FCTM I. no PPE: (88.8 : 1.4) + (0.032 : 0.234) = **63.56**
II. PPE recommended by applicant:
(gloves, standard protective garment and sturdy footwear)
(3.356: 1.4) + (0.032 : 0.234) = **2.53**
III. PPE recommended by applicant + additional PPE:
(PPE as given in II. + half-mask with combination filter)
(3.116: 1.4) + (0.00064 : 0.234) = **2.53**

HCTM I. no PPE: (333.6 : 1.4) + (0.4464 : 0.234) = **240.20**
II. PPE recommended by applicant:
(gloves, standard protective garment and sturdy footwear)
(41.064: 1.4) + (0.4464 : 0.234) = **31.24**
III. PPE recommended by applicant + additional PPE:
(PPE as given in II. + hood and visor)
(13.704: 1.4) + (0.00893 : 0.234) = **9.84**

HCHH I. no PPE: (736.2 : 1.4) + (1.05 : 0.234) = **530.32**
II. PPE recommended by applicant:
(gloves, standard protective garment and sturdy footwear)
(24.618: 1.4) + (1.05 : 0.234) = **22.04**
III. PPE recommended by applicant + additional PPE:
(PPE as given in II. + hood and visor)
(10.938: 1.4) + (0.021 : 0.234) = **7.92**

For Gusathion M WP 25:

(D : D_{tol}) + (I : I_{tol}) = E

FCTM I. no PPE: (40.8 : 1.4) + (0.02 : 0.234) = 29.24
II. PPE recommended by applicant:
(gloves, standard protective garment and sturdy footwear)
(2.876: 1.4) + (0.02 : 0.234) = 2.12
III. PPE recommended by applicant + additional PPE:
(PPE as given in II. + half-mask with combination filter)
(2.64 : 1.4) + (0.0004 : 0.234) = 1.88

HCTM I. no PPE: (276.0 : 1.4) + (0.432 : 0.234) = 199.00
II. PPE recommended by applicant:
(gloves, standard protective garment and sturdy footwear)
(40.488: 1.4) + (0.432 : 0.234) = 30.76
III. PPE recommended by applicant + additional PPE:
(PPE as given in II. + hood and visor)
(13.128: 1.4) + (0.00864 : 0.234) = 9.32

HCBH I. no PPE: (121.2 : 1.4) + (0.9 : 0.234) = 90.40
II. PPE recommended by applicant:
(gloves, standard protective garment and sturdy footwear)
(18.468: 1.4) + (0.9 : 0.234) = 17.04
III. PPE recommended by applicant + additional PPE
(PPE as given in II. + hood and visor)
(4.788: 1.4) + (0.018 : 0.234) = 3.48

In all cases, the degree of exposure was calculated to be >1. This means that for both Gusathion formulations the margins of safety between the estimated and the tolatable operator exposure are not acceptable with regard to systemic toxicity of azinphos-methyl even when high protection measures are considered. To facilitate comparison with other assessment procedures the total systemic exposure (ie absorbed dose) - on the basis of estimates made using the German model - as percentage of the AOEL (oral) can be calculated (see tab. B.5.14.1-7 and B.5.14.1-8). Using the AOEL value of 0.005 mg/kg bw, assuming 16 % skin absorption and applying a value for body weight of 60 kg, it can be seen that there are great differences between the formulations and the effects of the various PPE. However, the relation to the AOEL is unacceptable in all cases.

Table B.5.14.1-7 Comparison of estimated systemic exposure/resorbed dosis with the AOEL (oral) for Gusathion M EC 19.5

application	absorbed dose (mg/person/d)		total absorbed dose		%
	dermal	inhalation	mg/person/d	mg/kg bw/d	of AOEL
FCTM					
I. no PPE	14.21	0.03	14.24	0.24	4,800
II. recomm. PPE*	0.54	0.03	0.57	0.01	200
III. add. PPE**	0.50	0.00064	0.50	0.01	200
HCTM					
I. no PPE	53.38	0.45	53.83	0.90	18,000
II. recomm. PPE*	6.57	0.45	7.02	0.12	2,400
III. add. PPE***	2.19	0.008928	2.20	0.04	800
HCBH					
I. no PPE	117.79	1.05	118.84	1.98	39,600
II. recomm. PPE*	3.94	1.05	4.99	0.08	1,600
III. add. PPE***	1.75	0.021	1.77	0.03	600

* = gloves, standard protective garment and sturdy footwear
** = PPE as given in II.(*) + half-mask with combination filter
*** = PPE as given in II.(*) + hood and visor

Table B.5.14.1-8 Comparison of estimated systemic exposure/resorbed dosis with the AOEL (oral) for Gusathion M WP 25

application	absorbed dose (mg/person/d)		total absorbed dose		%
	dermal	inhalation	mg/person/d	mg/kg bw/d	of AOEL
FCTM					
I. no PPE	6.53	0.02	6.55	0.109	2,180
II. recomm. PPE*	0.46	0.02	0.48	0.008	160
III. add. PPE***	0.4224	0.0004	0.4228	0.007	140
HCTM					
I. no PPE	44.16	0.43	44.59	0.743	14,860
II. recomm. PPE*	6.48	0.43	6.91	0.115	2,300
III. add. PPE***	2.1005	0.0086	2.1090	0.035	700
HCBH					
I. no PPE	19.39	0.90	20.29	0.338	6,760
II. recomm. PPE*	2.95	0.90	3.85	0.064	1,280
III. add. PPE***	0.7661	0.0180	0.7841	0.013	260

* = gloves, standard protective garment and sturdy footwear
** = PPE as given in II.(*) + half-mask with combination filter
*** = PPE as given in II.(*) + hood and visor

It can be concluded that without wearing PPE the total systemic exposure for the estimated uses of Gusathion M EC 19.5 range between 4800 % and 39600 % of the AOEL, oral. By wearing the PPE recommended by applicant these values are between 200 % and 2400 % of the AOEL, and by additional wearing of half-mask with combination filter in field crops and hood and visor in high crops the values are between 200 % and 800 % of the AOEL.
For Gusathion M WP 25, the estimated exposure levels are a little bit lower, between 2180 % and 14860 % of the AOEL, oral (without PPE), between 160 % and 2300 % of the AOEL (with PPE recommended by applicant), and between 140 % and 700 % of the AOEL (with increased PPE), nevertheless in all cases unacceptable high.
In addition it has to be taken into account that the calculation is based on a dermal resorption rate of 16 % (human volunteer study, intact unoccluded skin). In a worst case scenario (occlusion; damaged skin) the rate can increase to about 60 %.

Therefore the recommended uses are not acceptable.
(In contrast, the applicant came to the conclusion that sufficient margins of safety exist for both Gusathion formulations when the appropriate protective equipment is used. This assessment was based on very high AOEL's derived from a human volunteer study without applying any safety factor.)
For the estimation of the operator exposure given above the applicant's statement about application rates (1 kg ai/ha for field crops and 3 kg ai/ha for high crops) were used. But it should be mentioned that there are listed application rates between 0.196 kg ai/ha (blackberry and boysenberry) and 15 kg ai/ha (ornamentals and forest) in the applicant's documents for approved uses of formulations for spray application in the EU. Thus, for special applications, the estimated operator exposure and likewise the degree of exposure can be decreased by factors up to about 1/5 or has to be multiplied by factors up to about 5.

B.5.14.2 BYSTANDER EXPOSURE

Because of the high toxicity of azinphos-methyl, the sensitizing properties of the substance, and the resulting data from the different operator exposure calculations every bystander exposure should be avoided or extremely minimized. It must be taken into account that bystanders are not wearing personal protective equipment.

B.5.14.3 WORKER EXPOSURE

Gusathion M formulations are normally used at times, when entering the crop shortly after spraying is not necessary. The half-life of the active ingredient in air is less than 3 hours. By the dermal route the high rate of percutaneous absorption has to be taken into account.
For the recommended uses (without and with PPE), the estimated operator exposure rates in relation to the AOEL's were calculated to be unacceptable. On this basis, the same problems for workers can be anticipated. Therefore adequate PPE and particular re-entry times for workers are considered to be necessary.

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EG:AIIA-5.1	1988. Gronberg, R.R., Lemke, V.J. and Lasley, M.B. Metabolism of azinphos-methyl in lactating goats. 5.1.2 /04 ! Bayer MR95649. TOX95-50613.	N	N	BAY	
EG:AIIA-5.1	1988. Kao, L.R.M. Disposition and metabolism of azinphos-methyl in rats. 5.1.1 /03 ! Bayer MR98327 !. TOX95-50609.	Y	N	BAY	
EG:AIIA-5.1	1976. Patzschke, K., Wegner, L.A. and Weber, E. 14C-azinphos-methyl (14C-Gusathion M): Biokinetic investigations on rats. 5.1.1 /01 ! Bayer PF1054. TOX95-50607.	N	N	BAY	
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	Landwirtschaftliche Forschung, 30, 1, 1977, 56-68. 5.1.2 /02 ! Bayer IM437 ! 66563. TOX95-50611.				
EG:AIIA-5.1	1980. Weber, H., Patzschke, K. and Wegner, L.A. [Phenyl-UL-14C]benzazimide: Biokinetic study on rats. 5.1.1 /02 ! Bayer PH9005 ! 69015. TOX95-50608.	N	N	BAY	
EG:AIIA-5.1; EG:AIIA-5.8.2	1973. Benke, G.M., Cheever, K.L. and Murphy, S.D. Comparative toxicity, anticholinesterase action and metabolism of methyl parathion, Parathion and Guthion in sunfish and mice. Tox. Appl. Pharmacol., 25, 1973, 473-474. 5.2.7 /03. TOX95-50635.	N	Y		
EG:AIIA-5.2	1993. Krötlinger, F. E 1582 (c.n.: azinphos-methyl) - Study for acute intraperitoneal toxicity to rats. 5.2.7 /04 ! Bayer 22647 ! T 9044245. TOX95-50636.	Y	N	BAY	
EG:AIIA-5.2.1	1957. DuBois, K.P., Thursh, D.R. and Murphy, S.D. Studies on the toxicity and pharmacologic actions of the dimethoxy ester of benzotriazine dithiophosphoric acid (DBD, Guthion). Journal of Pharmacology and Experimental Therapeutics, 119, 2, 1957, 208-218. 5.2.1 /01 ! Bayer LITR158257A ! 29454. TOX95-50614.	N	Y		
EG:AIIA-5.2.1	1979. Flucke, W.	N	N	BAY	

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EG:AIIA-5.2.1	1987. Heimann, K.G. Determination of acute toxicity (LD50). 5.2.1 /15 ! Bayer R1582231187. TOX95-50622.	N	N	BAY	
EG:AIIA-5.2.1; EG:AIIA-5.8.1	1974. Crawford, C.R. and Anderson, R.H. The acute oral toxicity of Guthion technical, benzazimide and methyl benzazimide to rats. 5.8.5 /01 ! Bayer USAR1582230774 ! 41190 TOX95-50689.	N	N	BAY	
EG:AIIA-5.2.1; EG:AIIA-5.8.2	1970. Crawford, C.R. and Doull, J. Antagonism of the lethal effects of Dipterex and Guthion with atropine and related drugs. (Abstract). Fed. Proc., 29, 1970, 349. 5.2.1 /02. TOX95-50615.	N	Y		
EG:AIIA-5.2.1; EG:AIIA-5.2.2	1982. Heimann, K.G. R 1582 (azinphos-methyl, the active ingredient of Guthion) - Study of the acute oral and dermal toxicity to rats. 5.2.1 /14 ! Bayer R1582300682. TOX95-50621.	N	N	BAY	
EG:AIIA-5.2.1; EG:AIIA-5.8.1	1974. Lamb, D.W. and Anderson, R.H. The acute oral toxicity of Guthion, benzazimide and methyl benzazimide to	N	N	BAY	

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EG:AIIA-5.2.1; EG:AIIA-5.2.2	1976. Pasquet, J., Mazuret, A., Fournel, J. and Koenig F.H. Acute oral and percutaneous toxicity of phosalone in the rat, in comparison with azinphos-methyl and parathion. Toxicology and Applied Pharmacology, 37, 1976, 85-92. 5.2.1 /06. TOX95-50616.	N	Y		
EG:AIIA-5.2.1; EG:AIIA-5.8	1979. Sterri, S.H., Rognerud, B., Fiskum, St.E. and Lyngaas, S. Effect of toxogonin and P2S on the toxicity of carbamates and organophosphorus compounds. Acta Pharmacol. et Toxicol., 45, 1979, 9-15. 5.2.1 /12. TOX95-50619.	N	Y		
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EG:AIIA-5.2.4	1994. Zorbas, M.A. Primary dermal irritation study with technical grade Guthion in rabbits. 5.2.4 /02 ! Bayer 7354. TOX95-50628.	Y	N	BAY	
EG:AIIA-5.2.4; EG:AIIA-5.2.5	1981. Thyssen, J. R 1582 - (Azinphos-methyl, the active ingredient of Guthion) - Study of the irritant effect on the skin and mucous membranes (eye). 5.2.4 /01 ! Bayer R1582191081. TOX95-50627.	N	N	BAY	
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EG:AIIA-5.2.6	1986. Flucke, W. E 1582 (c.n. azinphos-methyl) - Study for skin sensitizing effect on guinea pigs (Magnusson and Kligman's maximization test). 5.2.6 /01 ! Bayer 15003 ! T 8021276.	Y	N	BAY	

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	TOX95-50630.				
EG:AIIA-5.2.6	1987. Heimann, K.G., E 1582 technical (c.n.: azinphos-methyl) - Study of skin sensitization effect on guinea pigs (Bue hler-Patch-Test). 5.2.6 /03 ! Bayer 16188 ! T 0022376. TOX95-50632.	Y	N	BAY	
EG:AIIA-5.2.6	1989. Mihail, F. Azinphos-methyl - Assessment of skin sensitizing effect. 5.2.6 /04. TOX95-50633.	N	N	BAY	
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EG:AIIA-5.3.2	1957.	N	N	BAY	

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EG:AIIA-5.3.2	1956. Doull, J. and Rehfuss, P.A. The effect of diets containing Guthion (Bayer 17147) on rats (final report). 5.3.2 /01 ! Bayer USAR1582030556 ! 1077. TOX95-50638.	N	N	BAY	
EG:AIIA-5.3.2	1967. Lö¨ser, E ad, Lorke, D. Cholinesterase activity in dogs following administration of Gusathion in food. 5.3.2 /04 ! Bayer 292. TOX95-50641.	N	N	BAY	
EG:AIIA-5.3.3	1969. DuBois, K.P. and Flynn, M. Effects of repeated inhalation exposure of rats to Guthion. 5.3.3 /01 ! Bayer USAR1582021269 ! 26254 TOX95-50642.	N	N	BAY	
EG:AIIA-5.3.3	1980. Flucke, W. and Schilde, B. Gusathion-M active ingredient (R 1582) - Subacute cutaneous study of toxicity to rabbits. 5.3.3 /03 ! Bayer 8959. TOX95-50644.	N	N	BAY	
EG:AIIA-5.3.3	1976. Kimmerle, G. Subchronic inhalation toxicity of azinphos-methyl in rats. Arch. Toxicol., 35, 1976, 83-89.	N	Y		

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	5.3.3 /02 ! Bayer LITR158276A. TOX95-50643.				
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EG:AIIA-5.4.1	1978. Herbold, B. R 1582 (Gusathion M active ingredient) - Salmonella/microsome test to evaluate for point mutation. 5.4.1 /02 ! Bayer 7965. TOX95-50647.	N	N	BAY	
EG:AIIA-5.4.1	1984. Herbold, B. R 1582 (c.n. azinphos-methyl) - Pol test on e. coli to evaluate for potential DNA damage. 5.4.1 /07 ! Bayer 12478. TOX95-50651.	N	N	BAY	
EG:AIIA-5.4.1	1986. Herbold, B. E 1582 (c.n. azinphos-methyl) - Cytogenetic study with human lymphocyte cultures in vitro to evaluate for harmful effect on chromosomes.	Y	N	BAY	

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	5.4.1 /08 ! Bayer 15145. TOX95-50652.				
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EG:AIIA-5.4.1	1983. Hoorn, A.J.W. Mutagenicity evaluation of R 1582 (c.n. azinphos-methyl) in the reverse mutation induction assay with saccharomyces cerevisiae strains S138 and S211. 5.4.1 /05 ! Bayer R2503. TOX95-50649.	Y	N	BAY	
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EG:AIIA-5.4.1; EG:AIIA-5.4.2; EG:AIIA-5.4.3	1982. Waters, M.D., Sandhu, S.S., Simmon, V.F., Mortelmans, K.E., Mitchell, A.D., Jorgenson, T.A., Jones, D.C.L. et al. Study of pesticide genotoxicity. In: Fleck, R.A. and Hollaender, A. (eds.) "Genetic toxicology, an agricultural perspective" Plenum Press, New York and London, 1982, 1982, 275-326. 5.4 /02 ! Bayer LITR158282B. TOX95-50646.	N	Y		

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EG:AIIA-5.4.2	1995. Herbold, B. R 1582 - Micronucleus test on the mouse. 5.4.2 /03 ! REPORT NO.: 24015. TOX95-50874.	Y	N	BAY	
EG:AIIA-5.4.3	1979. Herbold, B. R 1582 - Dominant lethal study on male mouse to test for mutagenic effects. 5.4.3 /01 ! Bayer 8425. TOX95-50656.	N	N	BAY	
EG:AIIA-5.5	1978. Anonym Bioassay of azinphos-methyl for possible carcinogenicity. DHEW Publication No. (NCI) 78-1319. National Institutes of Health Bethesda, Maryland 20014. National Cancer Institute carcinogenesis technical report series No. 69., 1978, 1-126. 5.5.2 /02 ! 69072. TOX95-50659.	N	Y		
EG:AIIA-5.5	1985. Hayes, R.M. Oncogenicity study of azinphos-methyl (Guthion) in mice. 5.5.3 /02 ! Bayer MOB612 ! 80-271-02. TOX95-50662.	Y	N	BAY	
EG:AIIA-5.5	1980. Kimmerle, G. Comments on the bioassay of azinphos-methyl for possible carcinogenicity (National Cancer Institute carcinogenesis technical	N	N	BAY	

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	report series No. 69, 1978). TOX95-50660.				
EG:AIIA-5.5	1987. Schmidt, W.M. R 1582 (c.n.: azinphos-methyl) - Study of chronic toxicity and carcinogenicity to Wistar rats (administration in the feed for up to 2 years) in three sections. 5.5.2 /03 ! Bayer 16290 ! T 2015169. TOX95-50661.	Y	N	BAY	
EG:AIIA-5.5	1973. Worden, A.N., Wheldon, G.H., Noel, P.R.B. and Mawdesley-Thomas, L.E. Toxicity of Gusathion for the rat and dog. Toxicology and Applied Pharmacology, 24, 1973, 405-412. 5.5.1 /01. TOX95-50657.	N	Y		
EG:AIIA-5.6	1990. Holzum, B. E 1582 (R 1582) (c.n. azinphos-methyl) - Investigation of inhibition of cholinesterase activity in plasma, erythrocytes and brain in a 1-generation study. 5.6.1 /02 ! Bayer 19594 ! T0027362. TOX95-50664.	Y	N	BAY	
EG:AIIA-5.6.1	1987. Eiben, R. and Janda, B. R 1582 (c.n.: azinphos-methyl, the active ingredient of Guthion) - Two generation study on rats. 5.6.1 /01 ! Bayer R3956 ! T 6006415. TOX95-50663.	Y	N	BAY	
EG:AIIA-5.6.2	1988. Clemens, G.R., Bare, J.J. and Hartnagel Jr., R.E. A teratology study in the rabbit with azinphos-methyl (Guthion technical). 5.6.2 /06 ! Bayer MOB1030 ! MTD0070. TOX95-50670.	Y	N	BAY	

Annex point(s) (91/414/EEC);	year. author(s). title. source. report number registration number.	GLP GEP Y/N	publ. Y/N	owner	data prot
EG:AIIA-5.6.2	1987. Kowalski, R.L., Clemens, G.R., Bare, J.J. and Hartnagel Jr., R.E. A teratology study with azinphos-methyl (Guthion technical) in the rat (addendum to 5.6.2 /04, Bayer 973; EG:AIIA-5.6.2, TOX 95-50668). 5.6.2 /05 ! Bayer MOB1074. TOX95-50669.	Y	N	BAY	
EG:AIIA-5.6.2	1987. Kowalski, R.L., Clemens, G.R., Bare, J.J. and Hartnagel Jr., R.E. A teratology study with azinphos-methyl (Guthion technical) in the rat. 5.6.2 /04 ! Bayer 973 ! MTD0043. TOX95-50668.	Y	N	BAY	
EG:AIIA-5.6.2	1975. Machemer, L. R 1582 (active ingredient of Gusathion) - Studies for embryotoxic and teratogenic effects on rabbits following oral administration. 5.6.2 /01 ! Bayer 5455. TOX95-50665.	N	N	BAY	
EG:AIIA-5.6.2	1980. Short, R.D., Minor, J.L., Lee, C.-C., Chernoff, N. and Baron, R.L. Developmental toxicity of Guthion in rats and mice. Arch. Toxicol., 43, 1980, 177-186. 5.6.2 /03. TOX95-50667.	N	Y		
EG:AIIA-5.6.2	1978. Short, R.D., Minor, J.L., Unger, T.M. and Lee, C.-C. Teratology of Guthion. 5.6.2 /02 ! 68096. TOX95-50666.	N	N	BAY	
EG:AIIA-5.7	1988. Glaza, St.M. Guthion technical - Acute delayed neurotoxicity study in the domestic	Y	N	BAY	

Annex point(s) (91/414/EEC);	year. author(s). title. source. report number registration number.	GLP GEP Y/N	publ. Y/N	owner	data prot
	fowl. 5.7 /05 ! Bayer MOB1067 ! HLA 6232-101. TOX95-50675.				
EG:AIIA-5.7	1965. Grundmann, E. Histological findings (addendum to 5.7 /02, Bayer R1582200565; EG:AIIA-5.7, TOX 95-50672). 5.7 /02A ! Bayer R1582200565. TOX95-50673.	N	N	BAY	
EG:AIIA-5.7	1974. Kimmerle, G and Lö¨ser, E. Delayed neurotoxicity of organophosphorus compounds and copper concentration in the serum of hens. EQS Environmental Quality and Safety. Ed. by Coulston, F. and Korte, F.; Georg Thieme Verlag Stuttgart, Academic Press New York, London. 3. 1974. 173-178. 5.7 /04. TOX95-50674.	N	Y		
EG:AIIA-5.7	1964. Kimmerle, G. Neurotoxic study with Gusathion active ingredient. 5.7 /01. TOX95-50671.	N	N	BAY	
EG:AIIA-5.7	1965. Kimmerle, G. Neurotoxicity study with Gusathion active ingredient. 5.7 /02 ! Bayer R1582200565. TOX95-50672.	N	N	BAY	
EG:AIIA-5.8.1	1970. Edery, H., Soroker, D. and Kuhnberg, W. Antidotal action of new oximes in experimental organophosphate intoxication. Israel J. Med. Sci., 6, 2, 1970, 209-218. 5.8.1 /04. TOX95-50678.	N	Y		

Annex point(s) (91/414/EEC);	year. author(s). title. source. report number registration number.	GLP GEP Y/N	publ. Y/N	owner	data prot
EG:AIIA-5.8.1	1987. Eigenberg, D.A. Primary dermal irritation of benzazimide in albino rabbits. 5.8.5 /03 ! Bayer USAR1582200587 ! 854. TOX95-50691.	Y	N	BAY	
EG:AIIA-5.8.1	1969. Lorke, D. and Kimmerle, G. The action of reactivators in phosphoric acid ester poisoning. Naunyn-Schmiedebergs Arch. Pharmak. Exp. Path., 263, 1, 1969, 237. 5.8.1 /02. TOX95-50677.	N	Y		
EG:AIIA-5.8.1	1961. Sanderson, D.M. Treatment of poisoning by anticholinesterase insecticides in the rat. J. Pharm. Pharmacol., 13, 1961, 435-442. 5.8.1 /01. TOX95-50676.	N	Y		
EG:AIIA-5.8.1	1988. Sheets, L.P. Acute dermal toxicity of technical grade benzazimide in rabbits. 5.8.5 /05 ! Bayer USAR1582281088 ! 1077. TOX95-50693.	Y	N	BAY	
EG:AIIA-5.8.1	1987. Shiotsuka, R.N. Acute inhalation toxicity study with benzazimide technical in rats. 5.8.5 /04 ! Bayer USAR1582290787 ! 911. TOX95-50692.	Y	N	BAY	
EG:AIIA-5.8.2	1963. Anderson, C.A. The effect of feeding Guthion on the blood cholinesterase activity and milk production of dairy cows. 5.8.4 /01 ! Bayer 16950. TOX95-50684.	N	N	BAY	
EG:AIIA-5.8.2	1973.	N	N	BAY	

Annex point(s) (91/414/EEC);	year. author(s). title. source. report number registration number.	GLP GEP Y/N	publ. Y/N	owner	data prot
	Crawford, C.R. and Anderson, R.H. The effect of daily oral administration of Guthion to cattle at doses of 5 and 15 ppm for 30 days. 5.8.4 /03 ! Bayer 35408. TOX95-50686.				
EG:AIIA-5.8.2	1974. Giri, S.N., Peoples St.A., Llaguno G.K. and Mull, R.L. Oral toxicity of azinphos-methyl in horses. Amer. J. Vet. Res., 35, 8, 1974, 1031-1035. 5.8.4 /04. TOX95-50687.	N	Y		
EG:AIIA-5.8.2	1971. Kamienski, F.X. and Murphy, S.D. Biphasic effects of methylenedioxyphenyl synergists on the action of hexobarbital and organophosphate insecticides in mice. Toxicology and Applied Pharmacology, 18, 1971, 883-894. 5.2.7 /02. TOX95-50634.	N	Y		
EG:AIIA-5.8.2	1976. Thyssen, J. Studies to determine the toxic effects of the simultaneous application of azinphos-methyl or azinphos-ethyl and methamidophos. 5.8.2 /01 ! Bayer 6354. TOX95-50679.	N	N	BAY	
EG:AIIA-5.8.2	1977. Thyssen, J. Study for combination toxicity of azinphos-methyl and propoxur. 5.8.2 /02 ! Bayer 7174. TOX95-50680.	N	N	BAY	
EG:AIIA-5.8.2	1977. Thyssen, J. Study for the combination toxicity of azinphos-methyl and azinphos-ethyl.	N	N	BAY	

Annex point(s) (91/414/EEC);	year. author(s). title. source. report number registration number.	GLP GEP Y/N	publ. Y/N	owner	data prot
	5.8.2 /03 ! Bayer 7178. TOX95-50681.				
EG:AIIA-5.8.2	1977. Thyssen, J. Study for combination toxicity of Chlopyrifos, Cytrolane, Cyolane, Tamaron, Gusathion-ethyl and Gusathion-methyl active ingredient. 5.8.2 /04 ! Bayer 7179. TOX95-50682.	N	N	BAY	
EG:AIIA-5.8.2	1978. Wargo, J.P. The effect of feeding Guthion to dairy cattle. 5.8.4 /05 ! Bayer 66448. TOX95-50688.	N	N	BAY	
EG:AIIA-5.8.2	1968. White, R.G., Nelson, D.L. and Allen, A.D. The toxicity of Guthion to cattle. 5.8.4 /02 ! Bayer 23065. TOX95-50685.	N	N	BAY	
EG:AIIA-5.8.2; EG:AIIA-5.9.1	1986. Franklin, C.A., Muir, N.I. and Moody, R.P. The use of biological monitoring in the estimation of exposure during the application of pesticides. Toxicology Letters, 33, 1986, 127-136. 5.9.1 /07. TOX95-50700.	N	Y		
EG:AIIA-5.8.2; EG:AIIIA-7.3	1992. Schroeder, R.S. Dermal absorption of azinphos-methyl by rats from a Guthion 35% wettable powder formulation using 14C-azinphos-methyl. 5.8.3 /01 ! Bayer 6387 ! 90-722-GE. TOX95-50683.	Y	N	BAY	
EG:AIIA-5.9	1962. Dahm, P.A., Kopecky, B.E. and Walker, C.B. Activation of organophosphorus	N	Y		

Annex point(s) (91/414/EEC);	year. author(s). title. source. report number registration number.	GLP GEP Y/N	publ. Y/N	owner	data prot
	insecticides by rat liver microsomes. Toxicology and Applied Pharmacology, 4, 1962, 683-696. 5.9.3 /03. TOX95-50705.				
EG:AIIA-5.9	1971. Hitchcock, M. and Murphy, S.D. Activation of parathion and Guthion by mammalian, avian, and piscine liver homogenates and cell fractions. Toxicology and Applied Pharmacology, 19, 1971, 37-45. 5.9.3 /05. TOX95-50707.	N	Y		
EG:AIIA-5.9	1957. Murphy, S.D. and DuBois, K.P. Enzymatic conversion of the dimethoxy ester of benzotriazine dithiophosphoric acid to an anticholinesterase agent. J. Pharmacol. Exp. Ther., 119, 1957, 572-583. 5.9.3 /02. TOX95-50704.	N	Y		
EG:AIIA-5.9	1968. Murphy, S.D., Lauwerys, R.R. and Cheever, K.L. Comparative anticholinesterase action of organophosphorus insecticides in vertebrates. Toxicol. Appl. Pharmacol., 12, 1968, 22-35. 5.9.3 /04. TOX95-50706.	N	Y		
EG:AIIA-5.9.1	1974. Feldmann, R.J. and Maibach, H.I. Percutaneous penetration of some pesticides and herbicides in man. Toxicology and Applied Pharmacology, 28, 1974, 126-132. 5.9.1 /05 ! 86514. TOX95-50698.	N	Y		
EG:AIIA-5.9.1	1971. Rider, J.A., Swader, J.I. and Puletti,	N	Y		

Annex point(s) (91/414/EEC);	year. author(s). title. source. report number registration number.	GLP GEP Y/N	publ. Y/N	owner	data prot
	E.J. Anticholinesterase toxicity studies with methyl parathion, Guthion and phosdrin in human subjects. Fed. Proc., abstract no. 1382, 30, 1971, 443. 5.9.1 /02. TOX95-50695.				
EG:AIIA-5.9.1	1965. Simpson, G.R. Exposure to Guthion during formulation. Arch. Environ. Health, 10, 1965, 53-54. 5.9.1 /01. TOX95-50694.	N	Y		
EG:AIIA-5.9.1	1985. Wester, R.C. and Maibach, H.I. In vivo percutaneous absorption and decontamination of pesticides in humans. J. Toxicol. Environm. Health, 16, 1985, 25-37. 5.9.1 /06. TOX95-50699.	N	Y		
EG:AIIA-5.9.2	1981. Faul, J. BBA request: Effects on humans. 5.9.2 /01. TOX95-50701.	N	N	BAY	
EG:AIIA-5.9.2	1991. Mahler, L. Health effects attributed to azinphos-methyl exposure. Pesticide Illness Surveillance Program, Worker Health and Safety Branch, California Department of Agriculture, 1991. 5.9.2 /04. TOX95-50703.	N	Y		
EG:AIIA-5.9.2	1981. Miksche, L. Information on effects on man / occupational experience. 5.9.2 /02. TOX95-50702.	N	N	BAY	

Annex point(s) (91/414/EEC);	year. author(s). title. source. report number registration number.	GLP GEP Y/N	publ. Y/N	owner	data prot
EG:AIIA-5.9.4	1972. Rider, J.A., Swader, J.I. and Puletti, E.J. Anticholinesterase toxicity studies with Guthion, Phosdrin, Di-syston and Trithion in human subjects. Fed. Proc., abstract no. 72-1073, 31, 1972, 20. 5.9.1 /04. TOX95-50697.	N	Y		
EG:AIIA-5.9.4	1971. Thornton, J.S. Analysis of urine samples from human subjects treated orally with Guthion. 5.9.1 /03 ! Bayer 30201. TOX95-50696.	N	N	BAY	
EG:AIIA-5.9; EG:AIIA-5.10	1991. Anonym Azinphos-methyl. Pesticide residues in food - 1991. Joint FAO/WHO meeting on pesticide residues. Evaluations 1991 Part II - Toxicology., 1991. 5.10.2.1 /01. TOX95-50708.	N	Y		
EG:AIIA-5.10	1989. Anonym Maximale Arbeitsplatzkonzentration und biologische Arbeitsstofftoleranzwerte 1989. Mitteilung XXV der Senatskommission zur Prü fung gesundheitsschädlicher Arbeitsstoffe. 5.10.2.3 /01. TOX95-50709.	N	Y		
EG:AIIIA-7.1.1	1995. Krö¨tlinger, F. E 1582 19.5 EC 00126/0667 (c.n.: azinphos-methyl): Study for acute oral toxicity in rats. 7.1.1 /01 ! Bayer 23910 ! T 1058053. TOX95-50711.	Y	N	BAY	

Annex point(s) (91/414/EEC);	year. author(s). title. source. report number registration number.	GLP GEP Y/N	publ. Y/N	owner	data prot
EG:AIIIA-7.1.1	1990. Sheets, L.P. Acute oral toxicity study with Guthion 35 WP in rats. 7.1.1 /01 ! Bayer 5420. TOX95-50715.	Y	N	BAY	
EG:AIIIA-7.1.2	1995. Krö¨tlinger, F. E 1582 19.5 EC 00126/0667 (c.n.: azinphos-methyl): Study for acute dermal toxicity in rats. 7.1.2 /01 ! Bayer 23909 ! T 2058054. TOX95-50712.	Y	N	BAY	
EG:AIIIA-7.1.2	1990. Sheets, L.P. Acute dermal toxicity study with Guthion 35 WP in rats. 7.1.2 /01 ! Bayer 5409. TOX95-50716.	Y	N	BAY	
EG:AIIIA-7.1.3	1990. Warren, D.L. Acute four-hour inhalation toxicity study with Guthion 35 WP in rats. 7.1.3 /01 ! Bayer 5419. TOX95-50717.	Y	N	BAY	
EG:AIIIA-7.1.4	1990. Sheets, L.P. Primary dermal irritation study with Guthion 35 WP in rabbits. 7.1.4 /01 ! Bayer 4706. TOX95-50718.	Y	N	BAY	
EG:AIIIA-7.1.4; EG:AIIIA-7.1.5	1995. Krö¨tlinger, F. E 1582 19.5 EC 00126/0667 (c.n.: azinphos-methyl): Study for skin and eye irritation/corrosion in rabbits. 7.1.4 /01 ! Bayer 23673 ! T9058321. TOX95-50713.	Y	N	BAY	
EG:AIIIA-7.1.5	1990. Sheets, L.P. Primary eye irritation study with Guthion 35 WP in rabbits.	Y	N	BAY	

Annex point(s) (91/414/EEC);	year. author(s). title. source. report number registration number.	GLP GEP Y/N	publ. Y/N	owner	data prot
	7.1.5 /01 ! Bayer 5298. TOX95-50719.				
EG:AIIIA-7.1.6	1987. Porter, M.C., Craigo, R.E. and Hartnagel Jr., R.E. Dermal sensitization evaluation of Guthion 35 WP in the guinea pig. 7.1.6 /01 ! Bayer 885 ! MTD0014. TOX95-50720.	Y	N	BAY	
EG:AIIIA-7.1.6	1995. Vohr, H.-W. E 1582 19,5 EC 00126/0667: Study for skin sensitizing effect in guinea pigs (Bue hler Patch Test). 7.1.6 /01 ! Bayer 23726 ! T 5058327. TOX95-50714.	Y	N	BAY	

Annex B

Azinphos-methyl

B-7: Environmental fate and behaviour

B.7 Environmental fate and behaviour

B.7.1 Route and rate of degradation in soil (Annex IIA 7.1.1; Annex IIIA 9.1.1)

B.7.1.1 Aerobic and anaerobic studies

(a) Ref.: Gronberg et al., 1979 (revised 1995), BOD 95-50038

Material and Method:
The metabolism of [phenyl-UL-^{14}C]azinphos-methyl (27.7 mCi/mmole specific radioactivity; 97.2 % radiochemical purity) admixed to a 50 WP formulation blank, was investigated under aerobic, anaerobic and sterile conditions. A sandy loam (soil characteristics see Table B.7.1-1; soil no. 1) was treated with 2 mg/kg azinphos-methyl (corresponding to 3 kg as/ha) and maintained at 22 °C and 63 % of field moisture content in Erlenmeyer flasks connected with two tubes containing Chromosorb 102, a NaOH trap and a dry ice-acetone trap in series. After ageing 30 d under aerobic conditions, soil was in part sub-merged with 1 cm water to generate anaerobic conditions. Other samples were sterilised by heating and thereafter incubated at room temperature. Duplicate samples were processed from the aerobic soil system after 0, 1, 3, 7, 14, 30, 60 and 120 d incubation with single samples taken at 186, 242, 304 and 365 d. Duplicate samples were processed from the anaerobic soil flasks 60 and 90 d post application. Duplicate sterile samples were processed 7, 14, 30, 60 and 120 d post application. After refluxing in methanol/chloroform (1:1) followed by methanol/conc. HCl (150:1), analyses were made by LSC and TLC. The Chromosorb 102 trap and cold trap were extracted with methanol. Unextracted radioactivity was determined by combustion and LSC.

Aerob → 30 → aerob / anaerob; steril

365 d aerob
90 d anaerob
120 d steril

Results:
- Microbial populations before application:
  Fungi, 1 · 10^5 organisms/g; Actinomycetes, 3 · 10^6 organisms/g; Bacteria, 1 · 10^6 organisms/g

- Aerobic soil:
  - Mass balance for radioactivity: 0 – 120 d: 85 – 100 %
    186 – 365 d: 79 – 91 %
  - Degradation rate of azinphos-methyl:

	DT50	DT90
1.5th order kinetics (optimum fit) :	25 d	132 d
1st order kinetics :	63 d	209 d

    statistical model according to Timme et al. (1986, BOD 96-50001)
  - At each interval through 120 d post application, the majority of the extractable radioactivity was identified as azinphos-methyl. At later intervals, azinphos-methyl accounted for < 5 % AR.
  - 4.1 % AR was found as $^{14}CO_2$ after 365 d.
  - No residues (< 0.01 %) were found in the Chromosorb and cold traps.
  - Identified metabolites:
    M01: azinphos-methyl oxygen analogue, maximum: 5.3 % AR at 186 d
    M05: mercaptomethyl benzazimide, maximum: 4.2 % AR at 120 d
    M06: hydroxymethyl benzazimide and/or

    M08: benzazimide, maximum: 5.9 % AR at 120 d
    (could not be separated by TLC)
    M15: dimethyl benzazimide sulfide, maximum: 3.1 % AR at 186 d
  - The sum of unknown compounds never exceeds 10 % AR.
  - nonextractable residues: increasing to 72.7 % after 365 d

ANAEROB

- Anaerobic soil:
  - Mass balance for radioactivity: 95.4 – 102.9 %
  - Degradation rate of azinphos-methyl: DT50 = 66.5 d (1st order kinetics), DT90 was not evaluable with the statistical model employed (Timme et al., 1986, BOD 96-50001)
    Azinphos-methyl was the primary residue with 23.7 % AR after 60 d under anaerobic conditions.
  - 1.7 and 1.4 % AR were found as $^{14}CO_2$ after 30 and 60 d anaerobic incubation.
  - No residues (< 0.01 %) were found in the Chromosorb and cold traps.
  - Identified metabolites:
    M01: azinphos-methyl oxygen analogue, maximum: 2.8 % AR at 0 d anaerobic conditions
    M05: mercaptomethyl benzazimide, maximum: 5.0 % AR after 30 d anaerobic conditions
    M06: hydroxymethyl benzazimide and/or
    M08: benzazimide, maximum: 3.3 % AR after 30-60 d anaerobic conditions
    (could not be separated by TLC)
    M15: dimethyl benzazimide sulfide, maximum: 0.8 % AR after 60 d anaerobic conditions
  - Single unknown compounds never exceed 10 % AR.
  - nonextractable residues: increasing to 50.3 % after 60 d anaerobic conditions

STERIL

- Sterile soil:
  - Mass balance for radioactivity: 92.7 – 98.4 %
  - Degradation rate of azinphos-methyl: DT50 = 780 d (square root 1.5 order kinetics), DT90 was not evaluable with the statistical model employed (Timme et al., 1986, BOD 96-50001)
    Azinphos-methyl was the primary residue with 68.4 % AR after 120 d.
  - $^{14}CO_2$ could not be determined; no residues (< 0.01 %) were found in the Chromosorb and cold traps.
  - Identified metabolites:
    M01: azinphos-methyl oxygen analogue, maximum: 2.1 % AR at 30 d
    M05: mercaptomethyl benzazimide, maximum: 2.5 % AR at 60 d
    M06: hydroxymethyl benzazimide and/or
    M08: benzazimide, maximum: 1.7 % AR at 120 d
    (could not be separated by TLC)
    M15: dimethyl benzazimide sulfide, maximum: 0.8 % AR after 14 d
  - Single unknown compounds never exceed 10 % AR.
  - nonextractable residues: increasing to 11.8 % after 120 d

Comment:
Acceptable with some reservations because of the following deficiencies: high pH and soil humidity; no information if samples were incubated in the dark; incubation of 50 WP formulation

Conclusion:
Azinphos-methyl was the major residue and degraded for the most part by microbial action. Over the course of the experiment, most of the applied radioactivity was accounted for in the decreasing organosoluble and the increasing bound residue fraction except for a small amount of approximately 4 % of $^{14}CO_2$ (day 365, aerobic incubation).
The same degradation products were detected in the aerobic and the anaerobic part of the experiment. The major metabolic pathway in the degradation of azinphos-methyl involved hydrolysis of the phosphorus ester, with or without a previous oxidation to the oxygen analogue, resulting in various benzazimides which underwent further reactions (oxidation, dimerization) and ultimate cleavage of the heterocyclic and benzene ring structure and mineralisation to carbon dioxide.

(b) Ref.: Wagner et al., 1982 (revised 1995), BOD 95-50039
Material and Method:
The degradation of [phenyl-UL-^{14}C]azinphos-methyl (approx. 25 mCi/mmole specific radioactivity; > 97 % radiochemical purity) and of [carbonyl-^{14}C]azinphos-methyl (approx. 2.7 mCi/mmole specific radioactivity; > 97 % radiochemical purity) was investigated in a reactivated loamy sand (standard soil 1, soil characteristics see Table B.7.1-1; soil no. 2) according to BBA Merkblatt 36. The application rate was 0.59 mg/kg and 4.49 mg/kg, respectively. The metabolism was investigated in a natural soil (field plot Laacherhof, soil no. 3) after treatment with 11.9 mg [carbonyl-^{14}C]azinphos-methyl (approx. 2.7 mCi/mmole specific radioactivity; > 97 % radiochemical purity) per kg wet soil (corresponding to approx. 18 kg as/ha).
Samples were maintained dark under aerobic conditions at 22 °C, at 40% of the maximum water capacity (standard soil 1; pre-incubated over 14 d) or 11.2 % moisture (soil Laacherhof). The $^{14}CO_2$ formed was absorbed in 3 NaOH traps connected in series. Volatile, basic compounds were absorbed in a trap filled with 50 % sulfuric acid. $^{14}CO_2$ was collected at about weekly to 14 d intervalls. Metabolites were determined after 37, 67, 100, 136, 164, and 197 d. The studies were terminated after 222 d (metabolism), 552 d (degradation; carbonyl-labelled) and 365 d (degradation; phenyl-labelled). After successive extraction of the soil samples with chloroform, acetone, and water, analyses were made by LSC, TLC, NMR and MS. Unextracted radioactivity was determined by combustion and LSC.

Results:
- Mass balance for radioactivity: 97.5 - 106.3 %
  (metabolism study, 37 ... 197 d post application)
- Degradation rate of azinphos-methyl:

	DT50	DT90
1st order kinetics (optimum fit) :	49 d	162 d

  in soil Laacherhof,
  statistical model according to Timme et al. (1986, BOD 96-50001)
  At each interval post application, the majority of the extractable radioactivity was identified as azinphos-methyl.
- Formation of $^{14}CO_2$:
  carbonyl-lab. as, soil Laacherhof (No. 3): 18.6 % AR after 222 d

  carbonyl-lab. as, standard soil 1 (No. 2): 9 % AR after 552 d
  phenyl-lab. as, standard soil 1 (No. 2): 10 % AR after 365 d
- No residues were found in the sulfuric acid trap.
- Sixteen metabolites and two unidentified components were found; the concentration of which remained below 10 % throughout the incubation period
- Identified metabolites (soil Laacherhof):
  M05: mercaptomethyl benzazimide, maximum: 0.9 % AR at 67 d
  M06: hydroxymethyl benzazimide maximum: 0.4 % AR
  M08: benzazimide, maximum: 0.2 % AR
  M15: dimethyl benzazimide sulfide, maximum: 1.3 % AR at 67 d
  M16: dimethylbenzazimide disulfide, maximum: 6.7 % AR at 67 d
- The transmethylation products methylsulfonylmethylbenzazimide (M11), methylsulfinylmethylbenzazimide (M10), and methylthio-methylbenzazimide (M09) were broken down to benzamide (M14), and salicylic acid (M26).
- nonextractable residues: increasing to 62.0 % after 197 d

Comment:
Acceptable with some reservations because of the following deficiencies: metabolism study with highly exaggerated dose rate; no information about microbial biomass; incomplete soil characteristics; number of replicates is not reported.
Mass balance and distribution of metabolites are reported first after 37 d. But, sampling intervals obviously meet the requirement to be concentrated where the rates of decline of as and the formation and decline of major degradation products are expected to be greatest.

Conclusion:
Azinphos-methyl was the major residue.
Over the course of the experiment, most of the applied radioactivity was accounted for in the decreasing organosoluble and the increasing bound residue fraction.
Degradation of azinphos-methyl derived from hydrolysis, methylation, oxidation, hydroxylation, dimerisation, heterocyclic and benzene ring cleavage. Cleavage of the heterocyclic and benzene ring structure resulted in ultimate mineralisation to carbon dioxide.
The proposed degradation pathway is provided in Figure B.7.1-1.

(c) Ref.: Wagner, 1974 (revised 1988), BOD 95-50045
Material and Method:
The rate of degradation was investigated in 2 soils in laboratory according to BBA Merkblatt No. 36. The formulated test substance (formulation Gusathion M) was dissolved in water and 0.5 mg as/100 g soil were applied (corresponding to 7.5 kg/ha). Standard soil 1 (soil no. 9, Table B.7.1-1) and standard soil 2 (soil no. 10) were moist incubated at 25 °C in the dark. Samples were collected after 0, 1, 2, 3, 5, 7, 9, and 12 d post application. Analyses were made by GC with nitrogen detector. The lower limit of determination was 0.01 ppm in both soils; the recovery rate was 95 % and 99.5 % in soil 1 and 2, respectively.

Results:

Dissipation	Soil 1	Soil 2	Best fit[1]

DT50	4.0 d	6.3 d	first order
DT66	-	9.9 d	first order
DT80	9.4 d	-	first order

1 - according to Timme et al. (1986, BOD 96-50001)

Comments:
The study was conducted according to the test methods existing at this time. It can be used only for orientation because of the following deviations from the requirements of Annex II:
- highly exaggerated application rate
- investigation of formulated test substance
- incomplete soil characteristics, no information about microbial activity
- The duration of the study was to short for the estimation of degradation kinetics and DT90 values.
- incomplete description of analytical method

(d) Ref.: Fritz, 1988, BOD 95-50057
cf. Chapter B.7.2.2 (Mobility in soil)
Material and Method:
The leaching behaviour and metabolism of a mixture of [phenyl-UL-^{14}C] azinphosmethyl (> 99 % radiochemical purity, 3.2 MBq/mg specific radioactivity) and non-radioactive as (99.1 % purity) in Standard soil 2.1 (sand) and a silt soil ("Höfchen") was studied according to BBA guideline IV, 4-2.
Soil characteristics:

Soil type	Organic carbon (%)	pH $CaCl_2$	Sand (%)	Silt (%)	Clay (%)	CEC (meq/ 100g)	Biomass at start (mg C/kg dried soil)
sand	0.75	5.6	87.8	8.7	3.5	4	131
silt soil	1.8	5.3	2.3	88.6	9.1	10.5	1283

Amounts of 0.17 mg and 0.18 mg as were applied to 100 g of dry soil (corresponding to 1 kg as/ha) and aged for 0, 30, 62 and 92 d in the dark at 22 °C and at about 40 % of the maximum water capacity. After ageing, there were 2 replications for soil analysis. The aged soil samples were subsequently extracted with water, acetone and chloroform and this extracts were radioassayed and analysed by TLC. The remaining soil was radioassayed after combustion. Possible volatiles were trapped in paraffine oil coated quartz wool and soda lime and radioassayed after extraction and liberation, respectively.

Results:
- Recovery after aerobic ageing

Soil	Sand (2.1)				Silt soil (Höfchen)			
Incubation period	1 d	30 d	62 d	90 d	1 d	30 d	62 d	92 d
CO_2 (%)	< 0.1	6	9	10	< 0.1	2	3	5
non extracted (%)	6	57	64	69	9	56	57	70

extracted (%)	102	33	32	29	83	34	32	28
consisting of e.g.:								
azinphos-methyl	90	7	5	5	71	9	7	6
methylsulfonyl-methylbenzazimide	n.d.	8	11	10	n.d.	9	12	11
methyl benzazimide sulfonic acid	0.6	5	5	5	0.4	2	2	2
recovery (%)	108	96	104	108	92	92	93	102

n.d.: not detected

The other metabolites identified did not amount > 6 % AR at any sampling time. Other volatile compounds could not be detected > 0.1 % AR.

- Degradation rate of azinphos-methyl:

	DT50	DT90	kinetics (optimum fit)
Sand	7.8 d	55.4 d	root function first order
Silt soil	10.6 d	79.9 d	root function first order

Conclusion:
The proposed metabolic pathway given in Figure B.7.1-1 is confirmed by the results of this study. Deviating from the results of Gronberg et al. (1979, revised 1995) and Wagner et al. (1982, revised 1995), the metabolite methylsulfonylmethyl benzazimide exceeded 10 % AR after 62-92 d post application.

Comment:
The study is accepted.

B.7.1.2 Photolysis on soil

(a) Ref.: Liang and Lichtenstein, 1976, BOD 95-50040
Material and Method:
The degradation of [carbonyl-^{14}C]azinphos-methyl (2.7 µCi/mg specific radioactivity) on glass surfaces and soil after exposure to sunlight was compared to the results obtained after irradiation with UV light. Thin layers of 1-2 mm soil (sand, soil no. 4, Table B.7.1-1, and silt loam, soil no. 5) and 2-4 mm soil muck (soil no. 6) contained 100 µg of as (corresponding 37.3 g as/ha). Soils were maintained moist and were exposed for 8 h to sunlight (outdoors; cloudless June or August days between 9 a.m. and 5 p.m.; 30-42°C), UV light (257.3 nm, two 15-W Westinghouse 15T8 germicidal lamps, 30 cm from the test substrate, 25-31°C), or kept in the dark. In addition, the effects of formulations (746 g/ha EC formulation (22 % as), 10.67 kg/ha granules (7 % as), and 2 mg/268 cm² analytical grade) on its photodegradation on loam soil was investigated. Duplicate samples were extracted twice with (1:1) methanol-acetone followed by a single extraction with (1:1:1) methanol-acetone-benzene. Analyses were made by LSC, GLC, TLC, and bioassay with mosquito larvae. Unextracted radioactivity was determined by combustion and LSC.

Results and Conclusion:

- Radioactivity balance:
With glass surfaces, a total of only 51 % (UV light) or 65 % (sunlight) of the applied radiocarbon was recovered, probably due to volatilisation losses. This loss was to some extent reduced with the sand (radiobalance 59.6% under UV light and 82.6% under sunlight) and practically no losses occurred with the loam and muck soils where totals of 95.0 to 99.1 % of the applied radiocarbon were recovered.

- Degradation of azinphos-methyl:
In the absence of light almost no degradation occurred, since practically all (> 97 %) of the AR was still recovered from the benzene extraction phases which were associated with unaltered azinphos-methyl. In general, it appeared that UV light had the most pronounced effect on degradation of azinphos-methyl. With sunlight, the amounts of benzene-soluble radiocarbon (associated with unaltered as) were smallest on glass surfaces (46.7 % AR) and increased from the sand (75.3 %) to the loam (83.5 %) and the muck (91.1 %).
Soils reduced the effect of irradiation on the photodecomposition of as and this reduction increased with increasing organic matter of these soils.

- Water-soluble non-insecticidal degradation products were produced by irradiation, primarily on glass surfaces but also in the sandy soils where 20.1 % and 6.5 % AR was water-soluble after irradiation with UV light and sunlight, respectively. With soils of higher organic matter contents, the amounts of water-soluble radioactivity were reduced and were smallest in the muck soil (2.5 % or 2.6% under sunlight or UV light, respectively).

- The non-extractable radioactivity in the irradiated samples ranged from 0.8 % (sandy soil, sunlight) to 12.6 % of applied (muck soil, UV light). Since in the dark no bound residues were noticed in the soils, irradiation may have affected the binding of as, or what seemed more likely, irradiation enhanced the appearance of degradation products which in turn were "bound" to the soil.

- In the formulation test minor variations were found between results obtained after application of the emulsions (irradiation by sunlight) and those obtained with analytical grade azinphos-methyl. With analytical grade and emulsions, 78 % and 79 % of the applied as, were recovered from glass surfaces, 16 % and 17 % from water, and 99 % and 96 % from loam soil. With granules, however, the insecticide was better protected against photolysis since nearly all of the azinphos-methyl applied was recovered from glass surfaces and soils after exposure to sunlight.

Comment:
The study report did not refer to any guideline. Only accepted as additional information.
Deficiencies: high temperatures, wavelength of 254 nm, not specified sunlight, missing qualitative and quantitative distribution of as and degradation products, no degradation rate.

(b) Ref.: Wilkes et al., 1979, BOD 95-50041
Material and Method:

Soil plates with sandy loam (soil no. 1, Table B.7.1-1) containing 0.15 µg/cm² [phenyl-UL-^{14}C]azinphos-methyl (27.7 mCi/mmole, 98.2 % radiochemical purity) were exposed in a merry-go-round photoreactor equipped with a 200 W Hanovia Mercury Lamp No. 6515-32. Borosilicate glass was used to exclude irradiation of < 280 nm. Duplicate samples were used as dark controls and 0-time samples, respectively; single samples were withdrawn until 240 h of continous exposure. Samples were refluxed with (1:1) methanol-chloroform. Analyses were made by LSC and two-dimensional TLC. Total and unextracted radioactivity was determined by combustion and LSC.

Results:
- Mass balance for radioactivity: 97.1 - 103.7 %, averaged 101.4 % for exposed samples. No volatilization loss occured during the exposure period.
- The non-extractable radioactivity increased from 0.8 % at initiation of the experiment to 25.6 % after 240 h compared to 2.9 % in the dark control.
- relative contribution of as and its degradates after photolysis for 240 h:

Sample	Parent	M01	M05	M06+ M08	M07	M13	M15	Un-known	Ori-gin
		[% of the applied radioactivity]							
Exposed	48.2	3.4	0.9	4.1	1.2	0.7	1.3	1.1	7.5
Dark control	92.0	0.4	0.7	0.4	<0.1	0.3	0.4	0.1	1.6

M01: azinphos-methyl oxygen analogue
M05: mercapto-methyl benzazimide
M06: hydroxy-methyl benzazimide
M07: methyl benzazimide
M08: benzazimide
M13: anthranilamide
M15: dimethylbenzazimide sulfide
(M06 and M08 could not be separated by TLC)
No metabolite accounts for more than 5 % AR at any time during the study.
- Degradation rate of azinphos-methyl: graphically extrapolated half-life of 220 h

Comment:
The study report did not refer to any guideline. The results can be used only for orientation since a high intensity mercury lamp was used and wavelengths in the range of 280 - 290 nm were not excluded. The temperature was not reported.

(c) Ref.: Morgan, 1987 (revised 1990), BOD 95-50042
Material and Method:
The decomposition of [phenyl-UL-^{14}C]azinphos-methyl (46.9 mCi/mmole specific radioactivity, > 98 % radiochemical purity) under sunlight was studied according to EPA guideline 161-3. Guthion was added to 1 mm thin-layers of sterilised sandy loam (soil no. 7, Table B.7.1-1). The samples contained 33.8 µg a.i./cm² (corresponding to 3.38 kg/ha). The soil samples were exposed to natural sunlight (March - April 1987, Kansas City, USA, 17.8 Watt/cm²/d energy) for 31 days. Soil covered with foil were taken as dark controls. The average temperatures over the entire course were 17.7 °C for the exposed samples (daytime

maximum at 34.2 °C) and 17.0 °C for the dark samples (daytime maximum at 27.4 °C). At 0, 4, 8, 16, and 31 d intervals, duplicate dark and exposed samples were collected. Volatiles were collected in XAD and NaOH traps. Soil samples were extracted four times with (150:100:1) water-acetonitrile-acetic acid. Analyses were made by LSC, HPLC with radioactivity detection and GC-MS. Unextracted radioactivity was determined by combustion and LSC.

Results:
- Mass balance for radioactivity: 94.9 - 104.6 %, averaged 102.1.
- < 1% AR was accumulated in the NaOH traps and no volatiles were entrapped in the XAD-4 resin.
- The non-extractable radioactivity ranged from 0.3 % (day 0) to a maximum of 3.8 % AR (day-31 exposed).
- Degradation rate of azinphos-methyl:
  The amount of parent declined slowly to 80 % AR on day 31 (dark control: 90 % azinphos-methyl after the same interval).
  The graphically extrapolated half-life was estimated 99 d.
  Half-life of degradation due to only photolysis (irradiated degradation minus dark control degradation): 231 d.
- There were six additional, non-identified peaks displayed by the HPLC system which contained from 0.1 to 3.7 % AR (day-31 exposed).

Comment:
Since the test was conducted under natural sunlight, reproducibility is not given and no comparability with standardized experiments using artificial light is possible. A highly exaggerated application rate was used. The results can be used only for orientation and may be possibly utilized only for regions near to latitude 40 °N (Kansas City!).

(d) Ref.: Gronberg, 1989, BOD 95-50043
Material and Method:
Abbreviated study to meet the Arizona soil requirements, with the same procedures as described by Morgan (1987 (revised 1990), BOD 95-50042).
In contrast, a sterile sandy loam with a pH of 7.1 was used (soil no. 8, Table B.7.1-1). The study was conducted during August and September, 1989. The average sunlight energy was 18.2 Watts/cm²/d. The sampling was after 0, 15, and 30 d post application.

Results:
After 15 and 30 days of exposure to sunlight, azinphos-methyl residues represented 83 and 71 % AR, respectively, while 90 and 79 % were recovered from the dark controls. The net photolytic contribution to the degradation of azinphos-methyl was 8 % of applied after 30 days, corresponding to a net photolysis half-life of 232 d.

Comment:
Since the test was conducted under natural sunlight, reproducibility is not given and no comparability with standardized experiments using artificial light is possible. A highly exaggerated application rate was used. The results can be used only for orientation and may be possibly utilized only for regions near to latitude 40 °N (Kansas

City!).

Conclusion:
The net photolytic contribution to the degradation of azinphos-methyl in both studies with different soils (Morgan, 1987 (revised 1990), BOD 95-50042, and Gronberg, 1989, BOD 95-50043) was the same or approximately 9 % after 30 - 31 d.

B.7.1.3 Field studies

(a) Ref.: Yaron et al., 1974, BOD 95-50047
Material and Method:
Persistence and leaching of 10 kg as/ha azinphos-methyl were studied in a sandy loam (Negev, Israel, soil no. 11, Table B.7.1-1) in irrigated (401.5 mm and 553.8 mm irrigation) potato fields.
The maximum temperature during the growing period varied from 20.6 °C in March to 30.5 °C in June. No additional rainfall occurred.
Azinphos-methyl was applied twice to the field: prior to potato germination (23 March) and in the middle of the vegetation period (8 June). Samples (0-3, 3-6, 6-9, 9-15, 15-30 cm) were taken after each irrigation. Additional samples to a depth of 120 cm were collected at the beginning, in the middle, at the end, and after the irrigation season. After extraction with a mixture of 1:2 chloroform-methanol and water (9:1), analyses were made by GLC.

Results and Conclusion:
Azinphos-methyl practically disappeared within 30 d after each of two applications. No marked differences were observed between the moist soil and the wet (plus an additional 30% water) soil. The rates of degradation were reported to be similar to a soil laboratory study performed at 40°C which yielded a half-life of 5 days.
It was assumed that the persistence in the field is affected mainly by decomposition and volatilization.
Traces of as were detected in a soil depth of 12-30 cm, but were not found below 30 cm.
No residues were found in the potato tubers or leaves.

Comment:
The study does not meet the requirements of Annex II. Deficiencies are e.g.: high soil pH; incomplete characteristics of climate and soil; concentrations, recovery rates and limit of determination are not reported; high application rate.
Results can be used only as additional information on dissipation under higher temperature.

(b) Ref.: Morris, 1979, BOD 95-50048 and
Morris, 1979, BOD 95-50049
Material and Method:
Field dissipation of azinphos-methyl formulated as Guthion WP 50 and Guthion EC 240 was investigated at three sites in the USA (Indiana, Texas, Florida). Different soils (sand (soil no. 12, Table B.7.1-1), sandy loam (soil no. 13), sandy clay loam (soil no. 14), and silt loam (soil no. 15) were treated with 4.5 kg as/ha each.

Location	Soil/	Application	Precipitation	Soil temperature

	No.	(date)	(mm; sum)	(°C;10 cm depth)
Florida	sand/12	23/11/77	278 (23/11/77-23/1/78)	13-20 (23/11/77-23/1/78)
Florida	silt loam/15			
Indiana	sandy loam/13	24/05/78	174	17-21
Texas	sandy clay loam/14	4/11/77	79.5 (4/11/77-3/1/78)	19-22 (4/11/77-3/1/78)

For detailed weather conditions see Tab. B.7.1-3.

Samples of 0-15 cm and 15-30 cm soil depths were taken at 0, 30, 58-60, 119-120, 181, and 273 d and analysed separately.

Analytical method: Ref.: Wargo and Gronberg, 1979, BOD 95-50044:
According to the method 67084 (U.S. EPA MRID# 41135501) azinphos-methyl and azinphos-methyl oxygen analogue are extracted from soil with a methanol/dichloromethane mixture under reflux. After several clean-up procedures (liquid-liquid partitions), azinphos-methyl and azinphos-methyl oxygen analogue are separated on a silica gel column. Azinphos-methyl is determined by gas chromatography with a flame photometric detector. The fraction containing the azinphos-methyl oxygen analogue is cleaned-up on a Florisil column and determined by reversed-phase HPLC at a wavelength of 280 nm.
Recoveries from individual samples of different soils fortified at 0.05 to 0.10 mg/kg ranged from 72 to 92% for azinphos-methyl and from 51 to 112% for azinphos-methyl oxygen analogue. The limits of determination of both compounds were 0.01 mg/kg soil.

Results:
- There were no distinct differences between the two formulation used. The DT50 and DT90 values calculated according to Timme et al. (1986; BOD 96-50001) ranged from 1.5-9.5 d (average at 4 d) and 17-57 d (average at 32 d), respectively - see Table B.7.1-4.
- The degradation kinetics follows generally a function of square root first order.
- The azinphos concentrations decreased rapidly; latest quantitative detects were in the top layer (0-15 cm) after 120 d with 0.01-0.03 mg/kg (Texas) and in the 15-30 cm layer after 60 d with 0.01 mg/kg (Texas).
- The azinphos-methyl oxygen analogue could never be detected.

Comment:
The studies were conducted according to the test methods existing at this time and can be accepted with some reservations because of the following deficiencies: high application rate; the initial concentrations (0.22-0.82 mg/kg) were to low (approximately 2 mg/kg were expected in top layer!).
Because of the high soil temperatures, it may be possible to utilize some of these results for South European conditions.

(c) Ref.: Grace and Cain, 1990, BOD 95-50050
Material and Method:
The degradation and leaching behaviour of azinphos-methyl

(formulated as Guthion 50 WP) was studied according to EPA Guideline section 161-1 in two irrigated field plots in California, USA, after application of 3.4 kg as/ha to alfalfa (planted on 26 April 1989). The sites are located at Fresno (soil no. 16, Table B.7.1-1) and at Chualar (soil no. 17); soil characteristics refer to the 0-45/60 cm horizon. The soil from both sites demonstrated microbial activity (^{14}C-glucose degradation test).
At each site, one plot received a single application (for degradation kinetics); the other plot was treated twice to determine the leaching potential.
Core samples were driven to a depth of 1.2 m (0-15 cm on day 0) and segmented into 15 cm soil horizons. Sampling occured on days 0, 3, 7, 14, 28-29, 60, 88-90.

Location	Soil/ No.	Application (date)	Precipitation (mm) rain/irrigation	Soil temperature (°C;? cm depth)
Fresno	sandy loam/loamy sand/16	1. 30/5/89 2. 13/6/89	254 (30/5-29/7) 413 (30/5-11/9)	25-35 (max. temp.)
Chualar	sand - sandy loam/17	1. 24/7/89 2. 31/7/89	423 (24/7-23/10) 436 (24/7-29/9)	18-21

For detailed weather conditions see Tab. B.7.1-3.

Analytical method: Ref.: Wargo and Gronberg, 1979, BOD 95-50044
Grace, 1990, BOD 95-50045:
Method 67084 (cf. (b)) was somewhat modified by Grace (1990). The further clean-up procedure after the silica gel column is no longer performed and both compounds are determined by gas chromatography with a flame photometric detector.
Recoveries from soil fortified at 0.01 to 1.0 mg/kg ranged from 86 to 120 % for azinphos-methyl and from 70 to 100 % for azinphos-methyl oxygen analogue. The limits of determination of both compounds were 0.01 mg/kg soil.

Storage stability:
Studies on stability of as and its oxygen analogue in the presence of soil under freezer storage conditions were performed by Grace (1990, BOD 95-50045). The study was conducted using 0-15 cm soil horizon composites of two field plots (sandy loam; Grace and Cain, 1990). Average values of residues were calculated and used to determine percent decomposition versus time 0 measured values. Samples were maintained frozen at temperatures between -15°C and -35°C for periods of 0, 3, 4, 6, and 8 month.

Results:
- Storage stability:

	Percent Degradation			
	Azinphos-methyl		Oxygen Analogue	
	Chualar	Fresno	Chualar	Fresno
Day 122	1	0	17	34
Day 181	0	0	0	4
Day 244	51	31	19	0

No corrections for concurrent analytical procedural recoveries were made.
The stability of [phenyl-UL-^{14}C]azinphos-methyl (27.7 mCi/mmole specific radioactivity) in silt loam (soil no. 18, Table B.7.1-1) under frozen storage was already investigated by Close (1976, BOD 95-50051). Soil was fortified with 10 mg/kg and stored at -10°C. After 93 d, the recovery was 100% of initial concentration with 97% as parent compound.

Most all of the samples for the report of Grace and Cain (1990) were extracted in </= 151 d from the time of sampling where stability is given. Only 3 samples were analysed later (190 d, 227d, 238 d after sampling).

- Degradation of azinphos-methyl: after single application	Chualar	Fresno
first-order dissipation rate constant	0.06338	0.12998
correlation coefficient	0.9850	0.9811
half-life	10.9 d	5.3 d

cf. Table B.7.1-4.

- The azinphos concentrations decreased rapidly in both soils, even after repeated application.
The latest quantitative detects after single application were in the top layer (0-15 cm) after 28 d with 0.01-0.04 mg/kg. In a single case a residue of 0.03 mg/kg was found in the 15-30.5 cm soil horizon after 28 d.

- Of the 115 samples containing measurable residues of as, only one of these were found to contain residues of the oxygen analogue >/= 0.01 mg/kg, this being < 10 % of the as residue present in that same sample.

- No measurable residues of either azinphos-methyl or its oxygen analogue were found below the 0-15 cm soil horizon. In a single case a residue of 0.09 mg/kg was found in the 15-30.5 cm soil horizon at Chualar site after 28 d.

Comment:
The study can be accepted. The use rates correspond to the maximum use rates listed on label 6521 (8/8/88) of Guthion 50 WP.
Because of the high soil temperatures, it may be possible to utilize some of these results for South European conditions.

Table B.7.1-1: Soil characteristics

Soil number	Soil classification	Organic matter (%)	pH	Sand (%)	Silt (%)	Clay (%)	WHC (%)	CEC meq/100g
1	sandy loam	1.4	7.9	73.0	17.0	10.0	-	4.6

2	loamy sand	4.6	6.1	(85.1)[1]	(9.1)[2]	5.8	27.5	10 mval
3		1.4	6.0	-	(34.9)[3]	19.8	(11.2)[4]	-
4	sand	0.6	5.6	94	3	3	-	-
5	silt loam	4.7	6.0	5	71	24	-	-
6	muck soil	57	4.5	22	17	4	-	-
7	sandy loam	2.4	5.1 $CaCl_2$	66	32	2	-	17
8	sandy loam	0.3	7.1 $CaCl_2$	53	37	10	-	6
9		4.4	6.8	-	(10.1)[3]		(11.5)[4]	-
10		1.0	5.3	-	(19.5)[3]		(9.6)[4]	-
11	sandy loam	0.6	8.4				(13)[5] (14-17)[4]	13.4
12	sand	0.8	5.9	92	1	7	(6.3-14.7)[4]	1.1
13	sandy loam	2.3-2.5	5.3-5.4	66-68	23	9-11	(6.8-11.8)[4]	14.4-14.9
14	sandy clay loam	2.4-2.6	7.5-7.6	60	17	23	(4.3-14.6)[4]	19.7-21.4
15	silt loam	49	7.3	32	57	11	(4.2-11.2)[4]	21
16	sandy loam[6] loamy sand	0.2-0.75	7.6-8.7	-	-	-	(1.3-12.8)[4]	4.7-16.5
17	sand-sandy loam[6]	0.2-1.92	6.9-8.0	-	-	-	(5.2-17.6)[4]	14-22
18	silt loam	2.3	6.4 $CaCl_2$	3	75	22	-	-
19	sand	1.3	5.6	87	9	4	-	4
20	silt soil	3.1	5.3	2	89	9	-	11

[1] - particle size > 0.2 - 0.02 mm
[2] - particle size 0.02 - 0.002 mm
[3] - suspended particles </= 0.02 mm
[4] - moisture
[5] - field capacity (w/w)
[6] - refer to the 0-45/60 cm horizon

B.7.1.4 Soil residue studies

Soil residue studies were not submitted. Several rotational crop studies were performed (see Chapters B.6.1.2 and B.6.9). Radioactive residues were found in each crop, but unchanged as or its oxygen analogue was never detected in any sample. It can be concluded that rotational crops planted at least 30 d after the last soil treatment with azinphos-methyl will contain no unchanged as or azinphos-methyl oxygen analogue.

B.7.1.5 Summary

The results on route and rate of degradation of azinphos-methyl are summarized in Tab. B.7.1-2 and B.7.1-4.

Table B.7.1-2: Summary of laboratory studies on degradation of azinphos-methyl in soils

Type of study	Soil number Tab. B.7.1-1	Concentration mg as/kg	Incubation °C/ %water	Light Source	Statistical Evaluation DT50 (days)	DT90 (days)	Order	Ref.
degradation	9	5	25/11.5	dark	4	9[1]	1st	(1)
	10	5	25/ 9.6	dark	6	20[2]	1st	
metabolism	3	11.9[3]	22/ 40%WHC[4]	dark	49	162	1st	(2)
metabolism	1	2	22/63[5]	dark?	63	209	1st	(3)
					25	132	1.5th optimum	fit
photolysis	1	0.15 µg/cm²	-	Hg-lamp > 280 nm	9.2	n.r.	n.r.	(4)
photolysis	7	33.8 µg/cm²	17.7/- 34°C max.	sun-light	99	n.r.	n.r.	(5)
					232	(net photolysis)		
photolysis	8	32.6 µg/cm²	21°C min 42°C max.	sun-light	66	n.r.	1st	(6)
					241	(net photolysis)		
aged leaching	19	1.7	22/ 40%WHC[4]	dark	8	55	RF1st[6]	(7)
	20	1.8			11	80	RF1st	

n.r.: not reported
[1]: DT80
[2]: calculated: k = ln2/DT50, DT90 = ln10/k
[3]: mg as/kg wet soil
[4]: WHC: maximum water holding capacity
[5]: field moisture capacity
[6]: root function first order
Ref. (Reference):

(1) Wagner, 1974 (revised 1988), BOD 95-50045
(2) Wagner et al., 1982 (revised 1995), BOD 95-50039
(3) Gronberg et al., 1979 (revised 1995), BOD 95-50038
(4) Wilkes et al., 1979, BOD 95-50041
(5) Morgan, 1987 (revised 1990), BOD 95-50042
(6) Gronberg, 1989, BOD 95-50043
(7) Fritz, 1988, BOD 95-50057

Table B.7.1-4: Summary of field trials to investigate the degradation of azinphos-methyl in soil

Location	Appl. Rate kg as/ha /Formul.	Number of Appl.	Soil Type/number/ (Table B.7.1-1) layer (cm)	Statistical Evaluation DT50 (days)	DT90 (days)	Order	Ref.
California/USA	3.4/ WP50	1	sandy loam/16/ surface layer	5	18[1]	1st	(1)
California/USA	3.4/ WP50	1	sandy loam/17/ surface layer	11	36[1]	1st	(1)
Negev/ Israel	10	1	sandy loam/11/ 0-30	5	n.r.	n.r.	(2)
Indiana USA	4.5/ WP50	1	sandy loam/13/ 0-15	2	23	RF1st[2]	(3)
Texas USA	4.5/ WP50	1	sandy clay loam/ 14/0-15	5	57	RF1st[2]	(3)
Florida USA	4.5/ WP50	1	sand/12/0-15	2	25	RF1st[2]	(3)
Indiana USA	4.5/ EC240	1	sandy loam/13/ 0-15	2	17	RF1st[2]	(4)
Texas USA	4.5/ EC240	1	sandy clay loam/ 14/0-15	4	42	RF1st[2]	(4)
Florida USA	4.5/ EC240	1	silt loam/15/ 0-15	10	32	1st[2]	(4)

n.r.: not reported
RF: Root function 1. or 1.5. order
[1]: ln 2/ DT50 = k, ln 10/ k = DT90
[2]: calculation according to Timme et al. (1986, BOD 96-50001)
Ref. (Reference):
(1) Grace and Cain, 1990, BOD 95-50050
(2) Yaron et al., 1974, BOD 95-50047
(3) Morris, 1979, BOD 95-50048
(4) Morris, 1979, BOD 95-50049

Even after exaggerated application rates, azinphos-methyl was continuously degraded in four sandy loamy soils under laboratory

conditions at 22-25 °C with DT50 values < 50 d (4 ...49 d, optimum fit) and corresponding DT90 values < 170 d. The degradation kinetics was of first, 1.5th order and root function first order.
The net photolytic contribution to the degradation of azinphos-methyl was approximately 9 % after 30 - 31 d.

The major degradates found were methylsulfonylmethyl benzazimide (M11: 11.5 % after 62 d of incubation), azinphos-methyl oxygen analogue (M01: 5.3 % after 186 d) and dimethylbenzazimide disulfide (M16: 6.7 % after 67 days). These metabolites decreased after reaching their respective maxima. Methylsulfonylmethyl benzazimide exceeded 10 % AR in the course of only one (aged leaching study) of total 3 studies.
The mineralization to carbon dioxide was quite different in these studies with 17 % AR within 197 d, 4-10 % AR within 92 d, and only 4 % AR within 365 d. No other volatile residues could be detected. Parallel to mineralization significant amounts of nonextractable residues (55-70 % AR after 90-100 d, 62 % AR after 197 d and 73 % after 365 d) were formed.

Sterilisation of the soil reduced formation of bound residues, indicating the influence of micro-organisms. After 120 days of aerobic incubation 57 % of the radioactivity applied were not extractable, whereas only 12 % were determined after incubation in sterile soil.
Anaerobic conditions did not result in any other metabolites.

The major metabolic pathway (Fig. B.7.1-1) in the degradation of azinphos-methyl involved hydrolysis of the phosphorus ester, with or without a previous oxidation to the oxygen analogue, resulting in various benzazimides which underwent further reactions (oxidation, dimerization) and ultimate cleavage of the heterocyclic and benzene ring structure and mineralisation to carbon dioxide. In addition to the metabolites which occurred after incubation in the dark, anthranilamide (M13) was detected after photolysis on soil.

Under field conditions of USA (California, Florida, Texas, Indiana) and Israel, azinphos-methyl is rapidly degraded in soil with DT90 values < 57 d (17 ...57 d), even after exaggerated application rates (up to 4.5 kg/ha). There were no distinct differences between the two formulations (WP or the EC formulation) tested.

From the results on rotational crop studies it can be concluded that rotational crops planted at least 30 d after the last soil treatment with azinphos-methyl will contain no unchanged as or azinphos-methyl oxygen analogue.

B.7.1.6 Assessment

Azinphos-methyl is fairly degradable in soil. The degradation results mainly in carbon dioxide and in significant amounts of residues that are firmly fixed in the soil matrix. With exception of the degradation product methylsulfonylmethyl benzazimide with 10-12 % AR after 60-90 d of incubation in the course of only one of total 3 studies, no other degradate exceeded an amount of 10 %.

Though the DT50 values in laboratory are always < 60 d at 22-25 °C, an additional study on aerobic metabolism and degradation is required for the final evaluation because of the significant variations of DT50 values, mineralisation rates and amounts of non-extractable residues, and because of different quantitation of metabolites, reported in the studies submitted.
A study on degradation at 10 °C was not submitted because no use of azinphos-methyl is furthermore intended in Northern Europe (Denmark, Sweden, Finland).
Field studies are not required. However, field dissipation studies were performed under climatic conditions which may be possibly be compared to South European conditions. The results cannot be accepted for Middle and Northern Europe.
The relevance of photodegradation on soil for the dissipation of azinphos-methyl under field conditions appears to be small. Therefore, a valid study need not be submitted yet.
Soil residue studies are not required since it can be justified that soil residues do not leave unacceptable residues in rotational crops.

B.7.2 Adsorption, desorption and mobility in soil (Annex IIA 7.1.2; 7.1.3; Annex IIIA 9.1.2)

B.7.2.1 Adsorption, desorption

(a) Ref.: Flint et al., 1970, BOD 95-50052

Material and Method:
5 ml of 2.67, 3.55, and 4.44 µg/ml aqueous solution of technical grade azinphos-methyl were equilibrated with 1 g of each soil (described above) by shaking for 2 h. The aqueous phase were analysed.

Results:
The adsorption coefficients are shown in Table B.7.2-1.

Conclusion:
The adsorption coefficients are in the order expected with the sandy soil being the lowest (due to the larger particle size giving rise to less surface area for adsorption). The high organic soil shows more than twice the affinity of the silt loam (K_d!) illustrating the adsorptive effect of organic matter in soils.
Azinphos-methyl can be classified as having a low mobility in soil.

Comment:
The study on adsorption does not meet the requirements of Annex II. Deficiencies are e.g.: technical grade as; incomplete soil characteristics; other soil properties; no comment if equilibrium is given; no results on desorption.
The results can be used as additional information.

Table B.7.2-1: Adsorption and desorption of azinphos-methyl on different soils

Soil	Sand	Silt	Clay	org.C	pH	Adsorption	Desorption	Ref.

Soil	Sand (%)	Silt (%)	Clay (%)	org. C (%)	pH	[ml/g] K_d	[ml/g] K_{oc}	[ml/g] K_d	[ml/g] K_{oc}	
sand	88	7	5	0.53	4.3	6.79	1282	9.13	1722	(1)
sandy loam	56	30	14	0.58	6.6	4.02	693	6.75	1164	
silt loam	17	66	17	1.53	5.9	12.68	829	16.87	1103	
clay loam	21	50	29	1.16	6.4	8.39	723	11.67	1006	
sandy loam	56	33	11	0.81	6.4	3.33	452	n.r.	n.r.	(2)
silt loam	17	63	21	1.04	5.5	11.04	1165	n.r.	n.r.	
silt loam	24	57	19	2.67	5.4	28.50	1177	n.r.	n.r.	
sandy loam	74	14	13	1.62	6.6	7.60	516	8.95-15.14	552-935	(3)
silt loam	18	57	25	2.90	7.9	16.75	537	24.10-33.04	831-1139	
silty clay	0	41	59	0.29	6.0	9.85	3743	11.44-13.52	3945-4662	

n.r.: not reported
Ref.:
(1): Ziegler and Hallenbeck, 1987, BOD 95-50054
(2): Flint et al., 1970, BOD 95-50052
(3): Lenz, 1979, BOD 95-50053

(b) Ref.: Lenz, 1979, BOD 95-50053

Material and Method:
Adsorption/desorption properties of [phenyl-UL-^{14}C]azinphos-methyl (> 95 % radiochemical purity; 27.7 mCi/mmole specific radioactivity) were studied on three soil types (sandy loam, silt loam, silty clay, cf. Table B.7.2-1). For adsorption, 10 ml of 13.0, 6.5, 1.26, and 0.13 mg/kg aqueous solution were equilibrated with 2 g of each soil in a shaker-bath at 30 °C. Samples were taken from the 13 mg/kg solutions after 1.5, 3, 4.5 and 21.5 h of equilibration and from the other solutions after 24 h of equilibration. Desorption was conducted on the previously adsorbed samples by replacing the 8 ml of pesticide solution previously removed, with 8 ml of distilled water. The samples were desorbed for 24 h under the same conditions as in adsorption. The procedure was repeated 3 additional times. Each sample was centrifuged and the supernatant radioassayed for 2 h.

Results:
Adsorption of as from aqueous solutions on various soil types occured rapidly. Equilibrium was reached within 1.5 h. Azinphos-metyl was moderately adsorbed onto all three soil types at all 4 concentrations. The Freundlich constants and K_{oc} values are shown in Table B.7.2-1. The amount adsorbed ranged from 52-87 %. Desorbed amounts (4 desorption equilibrations) ranged from 32-40 % for silt loam, 47-68 % for sandy loam, and 56-67 % for silty clay.

Conclusion:
Based on K_{oc} values between 468-3396 the mobility of azinphos-methyl was classified as medium to very low according to the classification scheme suggested by McCall et al. (1980: In: Test Protocols for Environmental Fate and Movement of Toxicants, AOAC Symposium Proceedings of the 94th Annual Meeting in Washington DC, October 21-22, pp. 89-109, Arlington, VA).

Comment:
The results can be accepted with some reservations because of the high temperature, the usage of water instead of $CaCl_2$ solution, and deviating soil properties.

(c) Ref.: Ziegler and Hallenbeck, 1987, BOD 95-50054

Material and Method:
The adsorption and desorption of a mixture of [carbonyl-^{14}C] azinphos-methyl (98.6 % radiochemical purity; 46.9 mCi/mmole specific radioactivity) and non-radioactive as (99.9 % purity) was investigated according to U.S. EPA Guideline 163-1. Four soils (sand, sandy loam, silt loam, and clay loam, cf. Table B.7.2-1) were equilibrated with a water(0.01 M Ca^{2+} solution)/soil ratio of 5:1 in an environmental tumbler at 24 °C. Solution contained 0.6, 3.1, 4.6, or 6.1 mg as/l. A preliminary study with sampling after 2, 4, 8, 16, 24, and 48 h was conducted to find out the equilibration period. For desorption, the supernatant was replaced by 5 ml 0.01 M Ca^{2+} solution and mixed under the same conditions as in adsorption. After mixing, each sample was centrifuged and the supernatant radioassayed in triplicate. Some aqueous phases were extracted with benzene and the extracts were analysed by TLC. Some of the soil samples were vacuum-dried and analysed after combustion by LSC.

Results:
Preliminary studies indicated that maximum adsorption occured with a water/soil ratio of 5:1. Equilibrium occurred after 24 h in each soil type. The average recovery was 99 % for sand, 101 % for sandy loam, 102 % for silt loam and 97 % for clay loam.
Adsorption and desorption coefficients are provided in Table B.7.2-1.
No degradation of azinphos-methyl was observed in any of the soil types.

Conclusion:
Based on K_{oc} values between 693-1282 (adsorption) and 1006-1722 (desorption) the mobility of azinphos-methyl in soil was classified as low according to the classification scheme suggested by McCall et al. (1980: In: Test Protocols for Environmental Fate and Movement of Toxicants, AOAC Symposium Proceedings of the 94th Annual Meeting in Washington DC, October 21-22, pp. 89-109, Arlington, VA).

Comment:
The results are accepted.

B.7.2.2 Mobility in soil

(a) Ref.: Thornton et al., 1976, BOD 95-50055
Material and Method:
In a soil thin layer mobility study the Rf-values of azinphos-methyl in different soils (cf. Table B.7.2-2) were determined. Thin-layer plates of the soils were treated at approximately 0.014 µCi ^{14}C-azinphos-methyl per plate and developed with distilled water. The soil plates were placed in contact with x-ray film in the dark for 5 days. Recorded mobility values are the average of at least 3 separate analyses.

Results:
Table B.7.2-2: Soil thin layer mobility of azinphos-methyl

Soil type	Organic matter (%)	pH	Sand (%)	Silt (%)	Clay (%)	Rf value (frontal)
agricultural sand	0.8	5.9	92	1	7	0.18
sandy loam	2.8	6.6	74	14	13	0.22
sandy clay loam	0.6	5.5	56	21	23	0.11
silt loam	5.1	7.9	18	57	25	0.18
silty clay	2.1	6.7	4	53	43	0.14
silty clay	0.5	6.0	0	41	59	0.24

Conclusion:
Results from this study indicate that under the test conditions, azinphos-methyl can be classified to be a compound of low mobility in all the soil types tested. (Metribuzin was chosen as the internal standard because of its intermediate to mobile characteristics on all soils.)

Comment:
The study can be accepted with some reservations because of e.g. incomplete characteristics of test substance, missing information on application rate, and development with distilled water instead of $CaCl_2$ solution.

(b) Ref.: Flint et al., 1970, BOD 95-50052
Material and Method:
Technical grade azinphos-methyl was applied to soil columns (nylon tubing, 45 cm length, 1.6 cm inner diameter) at a rate of 10 mg/kg soil (corresponding to 7.46 kg/ha). The columns were filled with 15 g of each soil (described above) mixed with 6 g of Celite filter-aid to permit a practical eluant flow. The saturation was realized by upward passage of destilled water. One void volume of destilled water was passed into each column to leach as into the soil. The column packing was allowed to air dry and then divided into 1 cm segments which were analysed for as. Aqueous samples and slurries of soil were extracted with chloroform; the extracts were purified, concentrated and analysed by GLC. Recoveries were 90 % or greater in most cases.

Results:
Azinphos-methyl leached only into the second segment (1-2 cm) of both silt loam soils. In the sandy loam the theoretical rainfall required (15700 mm) to leach the as 30 cm into the soil was approximately 66 % lower when compared to soils higher in organic matter and fine particles (silt loam: 49300 mm; high organic silt loam: 47200 mm).

Conclusion:
Azinphos-methyl displayed only a slight tendency to leach into soils.

Comment:
The study does not meet the requirements of Annex II. Deficiencies are e.g.: technical grade as; high application rate; deviating methodology; other soil properties
The results can be used as additional information.

(c) Ref.: Atwell and Close, 1976, BOD 95-50056
Material and Method:
The leaching characteristics of aged [phenyl-UL-^{14}C]azinphos-methyl (radiochemically pure by TLC, 27.7 mCi/mmole) in a silt loam (cf. results) was studied. The soil samples containing 1 mg azinphos-methyl per kg dry soil (corresponding to 0.75 kg/ha) were incubated moist for 28 d. After ageing, 4 g of dried, incubated soil was packed on top of the column (30.5 cm height, 1.5 cm inner diameter) for a total of 30 cm. Water was applied to simulate rainfall (</= 12.7 mm/days) each of 45 d. Total quantity added was 100 ml.
The leachate was collected and radioassayed and additionally extracted with a acetone/chloroform mixture and analysed by TLC. After leaching, the soil was removed from the column in six 5 cm segments and radioassayed after combustion. Before leaching, an aged soil sample was refluxed with methanol and this extract was radioassayed and analysed by TLC. For humic/fulvic acid fractionation, the remaining soil was extracted with NaOH and radioassayed after combustion. The extracts were radioassayed.

Result:

Soil type	Organic matter (%)	pH	Sand (%)	Silt (%)	Clay (%)	Recovered radio-activity in leachate (% AR)
silt loam	2.3	6.4	3	75	22	4.4

Further results:
- Recovery after 28 d of aerobic aging:

extractable:	38.0 %	unextractable:	62.0 %
consisting of:		consisting of:	
azinphos-methyl	19.0 %	humic acid	50.1 %
benzazimide	5.3 %	fulvic acid	10.5 %
unidentified	13.7 %	humin	1.4 %

- Recovery after leaching:

Of the amount of 4.4 % AR in leachate, 12.1 % was found to be extractable, but could not be identified (TLC origin).
Soil column: 0- 5 cm 90.0 % AR (majority in 0-2 cm)
5-15 cm 4.0 % AR
15-30 cm 1.6 % AR

Conclusion:
Azinphos-methyl showed very low leaching behaviour in this soil type.

Comment:
The study does not meet the requirements of Annex II (SETAC-Procedures). Deficiencies are e.g.: deviating methodology; other soil properties and column dimension; elution with water instead of $CaCl_2$ solution.
The results can be used as additional information.

(d) Ref.: Pritz, 1988, BOD 95-50057
Material and Method:
The leaching behaviour of a mixture of [phenyl-UL-^{14}C]azinphos-methyl (> 99 % radiochemical purity, 3.2 MBq/mg specific radioactivity) and non-radioactive as (99.1 % purity) in Standard soil 2.1 (sand) and a silt soil ("Höfchen") was studied according to BBA guideline IV, 4-2.
Soil characteristics:

Soil type	Organic carbon (%)	pH $CaCl_2$	Sand (%)	Silt (%)	Clay (%)	CEC (meq/ 100g)	Biomass at start (mg C/kg dried soil)
sand	0.75	5.6	87.8	8.7	3.5	4	131
silt soil	1.8	5.3	2.3	88.6	9.1	10.5	1283

Amounts of 0.17 mg and 0.18 mg as were applied to 100 g of dry soil (corresponding to 1 kg as/ha) and aged for 0, 30, 62 and 92 d in the dark at 22 °C and at about 40 % of the maximum water capacity. After ageing, the incubated soil was placed on top of the column (30 cm height, 5 cm inner diameter) and irrigated with 200 mm water within 48 h. There were 2 replications for soil analysis and leaching tests each. The leachate was collected and radioassayed or extracted with chloroform and ethylacetate and analysed by TLC and HPLC. After leaching, the soil columns were frozen and cut into 3 segments of even size and radioassayed after combustion.

Results:

Table B.7.2-2: Leaching behaviour of [phenyl-UL-^{14}C]azinphos-methyl in two soils (mean of two replicates) (values are given in % of applied radioactivity after ageing)

Soil	Standard soil 2.1 (slightly humous sand)				"Höfchen" (silt)			
Incubation	0	30	62	92	0	30	62	92

period (d)	0	30	62	92	0	30	62	92
Soil								
Upper layer	66	89	86	82	70	90	89	88
Middle layer	13	6	6	6	14	6	2	2
Lower layer	10	3	2	1	10	2	2	1
* Leachate								
Fraction I	0.1	0.4	0.3	2.5	<0.1	<0.1	<0.1	<0.1
Fraction II	10.4	6.8	6.8	4.3	4.4	1.5	0.9	0.9
Azinphos-methyl	0.3	n.d.	n.d.	n.d.*	0.2	n.d.	n.d.	n.d.
M02	5.1	n.d.	n.d.	n.d.*	1.1	n.d.	n.d.	n.d.
M06/M08	0.3	n.d.	n.d.	n.d.*	0.2	n.d.	n.d.	n.d.
M25	1.5	3.5	3.2	5.1*	2.7	1.4	0.7	0.9
CO_2	-	3.3	6.2	9.9	-	1.8	3.5	3.6
Other volatiles	-	<0.1	<0.1	<0.1	-	<0.1	<0.1	<0.1
Total [%]	99	108	107	105	98	101	97	96

M02: Desmethyl azinphos-methyl
M06/M08: Hydroxymethyl benzazimide / benzazimide (no chromatographic separation)
M25: Methylbenzazimide sulfonic acid
n.d.:not detected
* analysis result of fraction I + II

Conclusion:
The mobility of as and its degradation products can be classified as being low after ageing in soil.

Comment:
The study is accepted.

B.7.2.3 Field leaching studies

(a) Ref.: Yaron et al., 1974, BOD 95-50047
cf. Chapter B.7.1.3

To obtain information on the degradation and leaching behaviour of azinphos-methyl in an irrigated potato field (sandy loam, soil no. 11, Table B.7.1-1), 9 irrigations were made at 10-day intervals throughout the growing season. No additional rainfall occurred. The application rate was 10 kg azinphos-methyl/ha.

Traces of azinphos-methyl (concentrations not reported) were detected in a soil depth of 12-30 cm, but no azinphos-methyl was found below 30 cm.

(b) Ref.: Grace and Cain, 1990, BOD 95-50050
cf. Chapter B.7.1.3

The dissipation of azinphos-methyl (formulated as Guthion 50 WP) after two applications of 3.4 kg as/ha to alfalfa was investigated.

No measurable residues of either azinphos-methyl or azinphos-methyl oxygen analogue (M01) were found below the 0-15 cm soil horizon (LOD 0.01 mg/kg). In a single case (one individual sample of one interval of one of the test plots), a residue of 0.09 mg/kg was found in the 15-30.5 cm soil horizon.

B.7.2.4 Summary

Based on
- the K_{oc} values ranged from 450 to 3700 in quite different soil types,
- the results on soil thin layer mobility with Rf values in the range of 0.11-0.24 in six soil types, and
- the results on column leaching with and without previous ageing,

azinphos-methyl can be classified as having a low mobility in soil. This is confirmed by field trials in Israel and USA.
In total, 4.4 - 10.5 % of applied radioactivity after leaching without ageing and 1 - 7.2 % of applied radioactivity after aged leaching (30 - 92 d incubation) were found in the leachates under laboratory conditions. Azinphos-methyl amounted to 0.2 - 0.3 % after leaching and was not detected in any of the leachates after ageing. After leaching, the degradation product desmethyl azinphos-methyl amounted to 1.1 - 5.1 %. Methyl benzazimide sulfonic acid was the only metabolite being detectable in the leachate after previous ageing at a maximum concentration of about 5 %.
In the field, the major amount was retained in the top layer.

B.7.2.5 Assessment

Azinphos-methyl as well as its metabolites are shown to have a low mobility in soil.
The adsorption/desorption and aged leaching behaviour of azinphos-methyl has been sufficiently investigated. Since the metabolite methylsulfonyl-methyl benzazimide exceeded 10 % of applied slightly only in one of three metabolism studies, further effort concerning adsorption/desorption should not be neccessary. Furthermore, this degradate could not be detected in leachates.
Lysimeter studies are not required.

B.7.3 Predicted environmental concentrations in soil (PEC_s) (Annex IIIA 9.1.3)

The initial maximum PEC_s (PEC_i) were calculated by the applicant (Annex III, point 9.1.3, Bayer AG, September 1995) based on the assumption of a soil depth of 5 cm, a bulk density of 1.5 g/cm³ and a coverage with arable crops or grass of 50 %. Furthermore, it is assumed that in orchards, grapes and olives, 50 % of the amount applied will reach the grass under the target plants.

Tab. B.7.3-1: Initial PEC_s for azinphos-methyl in soil after single application (from Bayer AG)

Crop	Soil reaching the soil %	Application rate (as) kg/ha	Application rate (as) mg/m²	Portion of as reaching the soil kg/ha	Portion of as reaching the soil mg/m²	PECi (mg as/kg dry soil) related to 5 cm soil depth
Gusathion M EC 19.5						
Arable crops	50	0.5	50	0.25	25	0.33
Orchards and olives	25	1.0	100	0.25	25	0.33
Grapes	25	0.8	80	0.2	20	0.27
Gusathion M WP 25						
Arable crops	50	0.7	70	0.35	35	0.47
Orchards	25	1.5	150	0.375	37.5	0.50
Olives	25	1.25	125	0.31	31	0.42
Grapes	25	1.0	100	0.25	25	0.33

Assuming first order kinetics for the decline of the concentration, longer term predicted environmental concentrations were determined as the time weighted average concentration for the respective time interval according to the formula (Annex III, point 9, Bayer AG, September 1995)

$$PEC_{twa} = PEC_i \cdot DT50 \cdot (1 - e^{(-t \cdot \ln(2)/DT50)}) \cdot (t \cdot \ln(2))^{-1}$$

where PEC_{twa} = time weighted average concentration,
PEC_i = initial concentration after last treatment,
DT50 = half-life of disappearance and
t = considered time period.

For these calculations (Table B.7.3-2) a averaged DT50 value of 5 d was used.

Table B.7.3-2: Time course of the PEC in soil after single application (DT50 5 d)

Time (d)	actual concentration (% of initial)	time weigthed average (% of initial)
0	100.00	100.00
1	87.06	92.84
4	57.43	76.32
7	37.89	63.63
28	2.06	25.09
50	0.10	14.33
100	0.00	7.17
180	0.00	3.98
270	0.00	2.66
360	0.00	1.99

PEC_s for as after multiple application were estimated on the basis of the following scenarios and assumptions:
- DT50 value of 5 d for as
- 2 applications of 0.5 kg as/ha as Gusathion M EC19.5 in potatoes (21 d application interval)
- 2 applications of 1.5 kg as/ha as Gusathion M WP25 in fruit (14 d application interval)

The results are given in Table B.7.3-3.

Table B.7.3-3: Initial, short- and long-term PEC_s for azinphos-methyl in soil (actual concentration and time weighted averages (TWA)) over 2 applications (DT50 5 d)

	Gusathion M EC19.5		Gusathion M WP25	
Application rate (kg as/ha)	0.5		1.5	
Interval (d)	21		14	
Time (d)	Concentration actual (mg/kg dry weight)	TWA	actual (mg/kg dry weight)	TWA
0	0.330	0.330	0.500	0.500
14			0.572	0.222
15			0.498	0.243
16			0.433	0.257
18			0.328	0.270
21	0.348	0.108	0.217	0.270
22	0.303	0.118		
23	0.264	0.125		
25	0.200	0.133		
28	0.132	0.136		
42			0.012	0.170
49	0.007	0.096		
64			0.001	0.113
71	0.000	0.067		
114			0.000	0.063
121	0.000	0.039		
180	0.000	0.026	0.000	0.040
270	0.000	0.018	0.000	0.027
360	0.000	0.013	0.000	0.020

Comments:
- The DT50 of 5 d for as is not the worst case.
- The maximum number of applications in registered uses is higher than 2.
- Other recommended uses (worst case situations because of high application rates) should be also taken into consideration.

These PEC_s in Table B.7.3-1, B.7.3-2 and B.7.3-3 are not sufficient. Therefore some further estimations were conducted.
For these worst case calculations, a DT50 value of 11 d from field dissipation studies in USA and an averaged DT50 value of 23 d from laboratory studies were used, assuming that these values can

represent Southern and Middle Europe, respectively. According to the results on the rate of degradation (Table B.7.1-2 and B.7.1-4), a kinetics of root function first order was chosen as worst case:

Actual concentration: $PEC = PEC_i \cdot e^{(-k \cdot t1/2)}$

Time weighted average:

$$PEC_{twa} = PEC_i \cdot 2/(t \cdot k^2) \cdot [(-k \cdot t^{1/2}) - 1] \cdot e^{(-k \cdot t1/2)} + 1]$$

with $k = \ln 2/(DT50)^{1/2}$

The results of these calculations are given in Tab. B.7.3-4.

Table B.7.3-4: Initial, short- and long-term PEC_s after single and multiple application of azinphos-methyl for selected uses **(actual concentration and time weighted averages(TWA))** (coverage: 50 %, 0-5 cm soil depth, bulk density: 1.5 g/cm³)

Crop	Citrus		Apple		Fruit		Apple	
Country	Spain		Italy		Belgium		Ireland	
Application rate (kg as/ha)	3.0		1.5		0.8		0.66	
Number of applic.	1		5		1		5	
Intervall (d)	-		7		-		10	
DT50 (d)	11		11		23		23	
Time (d)	PEC_s (mg/kg)							
	actual	TWA	actual	TWA	actual	TWA	actual	TWA
Initial:								
0 1.appl.	2.0	2.0	1.00	1.00	0.53	0.53	0.44	0.44
2.appl.	-	-	1.58	0.70	-	-	0.72	0.33
3.appl.	-	-	2.03	0.95	-	-	0.95	0.45
4.appl.	-	-	2.42	1.18	-	-	1.15	0.56
5.appl.	-	-	2.75	1.38	-	-	1.32	0.67
after last application:								
Short-term:								
1	1.62	1.74	2.51	1.42	0.46	0.48	1.25	0.68
2	1.49	1.65	2.40	1.46	0.43	0.46	1.22	0.70
4	1.32	1.52	2.23	1.51	0.40	0.44	1.16	0.72
long-term:								
7	1.15	1.40	2.04	1.56	0.36	0.41	1.10	0.75
28	0.66	0.99	1.32	1.59	0.25	0.32	0.83	0.81
50	0.46	0.80	0.95	1.45	0.19	0.28	0.67	0.79
100	0.25	0.57	0.54	1.17	0.12	0.22	0.45	0.70
365	0.04	0.23	0.10	0.57	0.03	0.11	0.15	0.43

It should be mentioned that these PEC_s were determined for EC and WP formulations, respectively.

Registered uses in Northern Europe (Finland, Sweden) are not considered because of missing DT50 values.

The PEC_s in Table B.7.3-4 clearly demonstrate that an elimination of azinphos-methyl of about 90 % (actual concentration) is given within 365 d even under worst case conditions and after 5 applications.

B.7.4 Fate and behaviour in water (Annex IIA 7.2.1; Annex IIIA 9.2.1, 9.2.3)

B.7.4.1 Hydrolytic degradation

(a) Ref.: Heuer et al., 1974, WAS 95-50025

Material and Method:
Dissipation of pure and [methylene-^{14}C]azinphos-methyl (specific radioactivity and radiochemical purity not reported) was studied in aqueous buffers (non-specified) at pH 8.6, 9.6 and 10.7. The samples were maintained at 6, 25 and 40 °C. Azinphos-methyl was extracted from water with chloroform and determined by GLC in the organic phase. Residues in the aqueous phase were tested by LSC .

Results and conclusion:
In aqueous solutions, the azinphos-methyl degradation clearly increased with the pH and temperature and followed first order kinetics. The half-lives are given in Table B.7.4-3.

Comment:
The study does not meet the requirements of Annex II and can be used only for additional information.

(b) Ref.: Wilkes et al., 1979, WAS 95-50026

Material and Method:
Dissipation of [phenyl-UL-^{14}C]azinphos-methyl (27.8 mCi/mmole specific radioactivity; 96.8 % radiochemical purity) was studied in aqueous phosphate buffers at pH 4, 7 and 9 (measured pH 4.22, 6.95 and 9.25) under sterile conditions; fortification was at 1 mg/l and 10 mg/l. The samples were maintained in the dark at 30 °C and 40 °C. Samples from duplicate vials were analysed after 1, 2, 4, 7, 14, 21 and 30 d post-treatment before and after partitioning with ethyl acetate by LSC and two-dimensional TLC.
Benzazimide and hydroxymethyl benzazimide could not be separated by TLC.

Results:
- Radioactivity balance: see Table B.7.4-1
  Minimum: 86.0 %, Maximum: 112.6, overall mean: 98.5 %
- Distribution of main metabolites: see Table B.7.4-1
  Other metabolites never exceeded 10 % AR. There were no volatile degradation products.
- At pH 4 and 7, the majority of the unidentified ^{14}C-material remained in the aqueous layer after extraction. This aqueous phase material was fractionated by chromatography into a number of labelled compounds, none of which represented > 10 % AR. At pH 9,

  < 10 % AR remained in the aqueous phase.
- The half-lives of azinphos-methyl are given in Table B.7.4-3.
  The degradation followed first order kinetics at all pH values, all temperatures, and all fortification levels.

Conclusion:
- Azinphos-methyl degraded faster at higher pH and higher temperature values. It was slightly more stable at the higher concentration.
- Benzazimide and/or hydroxymethyl benzazimide (6.9 - 35.1 %), mercaptomethyl benzazimide (0.4 - 0.7 %), and dimethylbenzazimide sulfide (0.3 - 12.3 %) were identified in each pH level. Anthranilic acid was found at pH 7 (3.5 %) and to a greater extent at pH 9 (31.1 %).
- These metabolites exceeded 10 % AR only under conditions non-relevant for environment (high temperature and/or high or low pH).

Comment:
The study report did not refer to any guideline.
Acceptable with some reservations because differences were noted between the totals given in the sum of radioactivity measured at distinctive TLC spots and the radioassay results of the pooled organic fractions. These variations were explained to be due to the missing background radioactivities of the TLC plates.

(c) Ref.: Wilmes, 1982, revised 1984, WAS 95-50027

Material and Method:
Calculation of half-lives of as at 22 °C from the experimental values (Wilkes et al., 1979, WAS 95-50026) obtained at the starting concentration of 1 mg/l.

Results: see Table B.7.4-3

(d) Ref.: Flint et al., 1970, BOD 95-50052

Material and Method:
In laboratory, saturated solutions of technical grade azinphos-methyl in M/15 phosphate buffers of ph 5, 7 and 9 were incubated at 30 and 50 °C in the dark. Aliquots were taken periodically and analysed for as remaining.
The field study was conducted in a large plastic vessel with approximately 107 cm in diameter. The pool was filled with approximately 5 cm silt and 25 cm water from a nearby pond. A spray concentrate of azinphos-methyl was gently mixed into the system to achieve a final concentration of 100 mg/kg. The pH was 7 and the average temperature over the course of the experiment was 29 °C. The pool was maintained outdoors loosely covered with clear plastic sheet. Aliquots were taken periodically and analysed for as remaining. Aqueous samples were extracted with chloroform; the extracts were purified, concentrated and analysed by GLC. Recoveries were 90 % or greater in most cases.

Results: see Table B.7.4-3

Conclusion:
Under field conditions with abundant air, sunlight and biological

activity as well as presence of silt, the resulting half-life of 1.2 d was nearly eight-fold less than was found under similiar conditions in the laboratory (pH 7 and 30 °C: 10.0 d). The authors concluded that azinphos-methyl was of low stability in aqueous systems approximating field conditions.

Comment:
The studies on water stability do not meet the requirements of Annex II. Deficiencies are e.g.: technical grade as; no information on sampling dates, sterility and degradation products.
The results can be used as additional information.

B.7.4.2 Photochemical degradation

(a) Ref.: Wilkes et al., 1979, revised 1981, WAS 95-50028

Material and Method:
Photodegradation of [phenyl-UL-^{14}C]azinphos-methyl (27.8 mCi/mmole specific radioactivity; 97.2 % radiochemical purity) was studied in aqueous phosphate buffers at pH 4 (measured pH 4.35); fortification was at 10 mg/l. The samples were irradiated at approximately 30 °C in a Ace Photochemical Reaction Assembly Model 6515 equipped with a 200 W Hanovia Mercury Lamp No. 6515-32 in a borosilicate glass immersion well to exclude irradiation of 280 nm or less. Duplicate samples were collected until 48.25 h of continous exposure (19 sampling times). The samples were analysed before and after partitioning with ethyl acetate by LSC and two-dimensional TLC. Volatile photoproducts were collected in Chromosorb 102 traps; CO_2 was trapped in aqueous NaOH solution and radio-assayed, both. Benzazimide and hydroxymethyl benzazimide could not be separated by TLC.

Results:
- Radioactivity balance: 87.3 ... 113.3 % with an average of 102.4 %. The variation of the values from 100 % can be attributed to experimental errors. No significant volatilization losses occured.
- The amount of ^{14}C-residues extracted decreased with time and the amount remaining in the aqueous phase increased with time to 8.4 % after 48 h exposure.
- Half-life of as was 9.4 h at 30 °C and wavelengths > 280 nm The photolytical reaction followed second-order kinetics. The second-order rate constant was calculated to be 0.001 $(h \cdot \%)^{-1}$
- Main photolysis products are given in Table B.7.4-1. Other metabolites never exceeded 10 % AR.
- The unidentified photodegradation products consisted of polar origin material (5.2 %) and one unknown (1.5 %).
- All metabolites (identified and unidentified) were photodegradation products since after 48 h the dark control samples still contained 94 % of the initial radioactivity as azinphos-methyl.

Conclusion:
This study showed that the degradation of azinphos-methyl in water seems to be markedly accelerated in the presence of light. After 48 h of exposure (completion of the test), the azinphos-methyl contents had decreased to 18.7 % AR. Benzazimide (M08) and/

or hydroxymethyl benzazimide (M06) formed the major photodegradation products with around 40 % AR. Other photodegradation products were anthranilic acid (M12: 9.8 %) and methylbenzazimide (M07: 1.5 %).

Comment:
The study report did not refer to any guideline. The results can be used only for orientation since wavelengths in the range of 280 - 290 nm were not excluded and because of irradiation with a mercury lamp and the high temperature.
Furthermore, Morgan (1987, WAS95-50029) stated that the conditions were non-sterile.
Differences were noted between the totals given in the sum of radioactivity measured at distinctive TLC spots and the radioassay results of the pooled organic fractions. These variations were explained to be due to the missing background radioactivities of the TLC plates.
The metabolites M06/M08 exceeded 10 % AR under conditions non-relevant for environment (high temperature, low pH and irradiation with a mercury lamp with wavelengths of 280-290 nm).

(b) Ref.: Morgan, 1987, WAS 95-50029

Material and Method:
Photodegradation of [phenyl-UL-^{14}C]azinphos-methyl (46.9 mCi/mmole specific radioactivity; 98.9 % radiochemical purity) was studied according EPA guideline 161-2 in sterile 0.01 M acetate buffer at pH 4 (measured pH 4.34 ... 4.42), containing 1 % acetonitrile from stock solution; fortification was at 10 mg/l. The samples were exposed to natural sunlight of Kansas City, Missouri, USA, during the period January through March, 1987. Maxima of sunlight intensity were in the range of approximately 5000 - 80000 $\mu W/cm^2$ after 12, 37, 62, and 85 h. The average light energy per hour was 0.4033 $W \cdot min/(cm^2 \cdot h)$. The temperature of exposed fused quartz cells was 25 °C (17.3 - 29.0 °C). The sampling from both the exposed and dark cells was at approximately 0, 4, 5, 8, 32, 56, and 87 h post treatment. The samples were analysed by LSC, HPLC with radioactivity detection, GC-MS and TLC.

Results:
- Radioactivity balance:
  dark control: 97.5 - 103.3 % with an average of 100.0 %
  exposed cells: 99.2 - 105.0 % with an average of 102.6 %
- The photolytical reaction followed first-order kinetics.
  The timed photochemical half-life (i.e. light energy half-life devided by average light energy per hour) at 25 °C was 76.7 h (3.2 d).
  The dark cell showed no loss of as over the course of the study.
- Identified photolysis products are given in Table B.7.4-1.
- Hydroxymethyl benzazimide was shown to be absent from this study.
- The fraction of unknowns consisted of five distinct peaks none of which exceeded 2 % AR. There were no volatile degradates.
- No degradates were found in the dark control. After 87 h of incubation, the amount of azinphos-methyl averaged 100%.

Conclusion:
Aqueous solutions of azinphos-methyl were rapidly degraded by

sunlight. After 87 h of exposure, the azinphos-methyl contents decreased to 41.6 % AR. The major photolytic products were benzazimide (M08: 39.1 %) and anthranilic acid (M12: 7.2 %). Benzazimid can be considered as photolytically stable under sterile conditions at pH 4.

Comment:
The method does not meet the requirements specified in Annex II. Since the test was conducted under natural sunlight, reproducibility is not given and no comparability with standardized experiments using artificial light is possible. Results can be used only for orientation and may be possibly utilized only for regions near to latitude 40 °N (Kansas City!).

(c) Ref.: Hellpointner, 1994, WAS 95-50030
cf. Chapter B.2.1.9.3
Conclusion:
The results indicate that direct photodegradation in water essentially contributes to the overall elimination of azinphos-methyl in the environment. The half-life concerning direct photolysis is expected in the range between 1 and 4 d, for the months of main use.

Comment: Accepted regarding quantum yield and environmental half-life of as. Not accepted regarding aqueous photolysis because of irradiation with a mercury lamp and missing information on the fate of degradation products.

Table B.7.4-1: Distribution of main metabolites in water after application of [phenyl-UL-^{14}C]azinphos-methyl **at selected sampling times** (% of applied radioactivity in organic extracts)

Report	Type of study	Test conditions	Days	as	M05	M06/M08	M11	M12	M15	Unknow	Total (*)
						(%)					
Wilkes et al. (1979)	Hydrolysis 10mg/l (**)	pH 4 30°C	14	74.6	0.4	5.5	-	<0.1	1.1	3.4	96.4
			30	60.4	0.5	7.3	-	<0.1	1.9	3.8	99.0
		pH 7 30°C	14	61.9	0.4	4.6	-	0.8	4.2	3.2	101
			30	50.7	0.5	5.7	-	2.3	5.3	2.8	97.6
		pH 9 30°C	14	3.6	0.7	21.8	-	15.5	9.5	17.4	93.6
			30	2.3	0.2	33.1	-	30.1	2.4	3.9	95.7
		pH 4 40°C	14	61.4	0.3	7.8	-	<0.1	1.9	5.5	96.3
			30	36.4	0.5	10.8	-	<0.1	3.5	8.1	94.7
		pH 7 40°C	14	46.4	0.4	6.6	-	1.7	7.2	6.6	99.0
			30	18.0	0.7	6.9	-	3.5	12.3	9.1	96.8
		pH 9 40°C	14	0.5	0.3	37.1	-	20.1	0.7	12.7	95.9
			30	2.0	0.4	35.1	-	31.1	0.3	5.1	94.0
Wilkes et al. (1979 rev. 1981)	Photolysis	Hg-lamp pH 4 30°C	0.1	80.4	-	9.8	-	1.5	-	1.9	101
			1	29.5	-	37.3	-	8.3	-	6.4	101
			2	18.7	-	38.7	-	9.8	-	6.7	102
						M06 M08					
Morgan (1987)	Photolysis	Natural sunlight	0	100	-	- 0.0	-	0.0	-	0.0	100
			0.2	85.0	-	- 14.0	-	2.7	-	0.0	102
			1.3	52.9	-	- 33.1	-	6.2	-	7.8	100
			2.3	50.7	-	- 35.5	-	7.2	-	6.6	100
			3.6	41.6	-	- 39.1	-	7.2	-	12.1	100

M05 mercaptomethylbenzazimide
M06 hydroxymethylbenzazimide
M08 benzazimide
M11 methylsulfonylmethylbenzazimide
M12 anthranilic acid
M15 dimethylbenzazimide sulfide
(*): Total radioactivity (organic extracts + aqueous phase)
(**): Values were not normalised to 100 %.

B.7.4.3 Ready biodegradability

Not submitted and not required for the classification of as.

B.7.4.4 Water/sediment study

(a) Ref.: Flint et al., 1970, BOD 95-50052
cf. Chapter B.7.4.1

(b) Ref.: Fritz, 1988, WAS 95-50031
Material and Method:
The degradation of [phenyl-UL-^{14}C]azinphos-methyl (> 99 % radiochemical purity; 3.2 MBq/mg specific radioactivity) was investigated in two water/sediment systems in the laboratory according to Dutch recommendations. The sediments and the water were collected from a drainage ditch (Ijzendoorn, NL) of a fruit orchard and from a gravel pit (Lienden, NL) apart from agriculturally used areas. The sediments were characterised as follows:

Parameter	Ijzendoorn (silt loam)	Lienden (loamy sand)
Particle size distribution (%)		
Sand	20.4	73.8
Silt	60.6	14.6
Clay	18.9	11.6
Organic carbon (%)	2.5	0.8
Total N (%)	0.3	0.5
Total P (mg/kg)	1200	230
Calciumcarbonat (mg/kg)	15000	11500
pH (KCl)	7.1	7.4

The concentration was 1 mg as/l which corresponds to 1 kg as/ha

field application rate (assuming 10 cm depth of the water body).
The test solution was applied to the rippling water surface.
The samples (50 g dry weight of each sediment (0.8 - 1.5 cm height); 500 ml batch) were pre-incubated within 10 d and thereafter incubated in the dark at 20°C ± 2°C under continous stirring of water phase. 20°C 91 days
Duplicate samples were taken 3, 34, 62, and 91 d post application.
Possible organic volatiles and CO_2 were trapped in paraffine oil coated quartz wool and soda lime, respectively, and radioassayed after extraction and liberation, respectively.
The water phases were successively extracted with chloroform, ethyl acetate and diethylether; the aqueous phase and the organic phases were radioassayed. Water, acetone and chloroform extracts from the moist sediments were radioasssayed. Additionally, the extracted sediment was refluxed with acetone/HCl solution. All extracts were analysed by TLC and partly HPLC. The remaining sediment was radioassayed after combustion.
Two untreated batches were examined at each processing date for microbial numbers, pH value, redox potential and oxygen content.

Results:
- Analytical data of the aqueous phases in the course of the study:

	Ijzendoorn	Lienden
pH	7.9 - 8.9	7.9 - 8,5
Redoxpotential (mV)		
decreasing from	+ 161-180 (3 d)	+ 161-170 (3 d)
to	+ 105-133 (91 d)	+ 115-141 (91 d)
oxygen (%)	77 - 94	76 - 89

- The microbial numbers were not reported. But, it was mentioned that the determination of the microbial numbers indicated that the system was biologically active during the entire period of the test.
- Distribution of radioactivity:

Table B.7.4-2: Behaviour of [phenyl-UL-^{14}C]azinphos-methyl (1 mg/l) in two water/sediment systems: radioactivity balance in % of the applied radioactivity (mean values of two separate samples)

	Ijzendoorn				Lienden			
Days after Application	3	34	62	91	3	34	62	91
Total	99.2	97.5	95.7	95.2	98.2	91.6	91.1	91.1
liberated $^{14}CO_2$	0.1	2.9	4.4	5.8	0.2	3.4	6.9	9.7
Aqueous Phase	41.8	7.0	7.1	5.9	67.5	21.8	19.9	18.0
extract consisting of e.g.:								
parent	30.5	n.d.	n.d.	n.d.	55.4	n.d.	n.d.	n.d.
M02	5.6	0.5	n.d.	n.d.	5.1	6.7	n.d.	n.d.
M04	0.3	n.d.	n.d.	n.d.	0.5	n.d.	n.d.	n.d.
M06/M08	0.7	0.2	0.1	0.1	0.8	2.1	0.7	0.1
M07	n.d.	0.3	0.2	0.3	n.d.	0.1	0.3	0.1

M10	n.d.	1.0	1.0	1.5	n.d.	2.5	1.5	0.4
M11	n.d.	0.1	0.1	0.3	n.d.	0.3	0.3	0.1
M25	0.4	2.3	3.5	2.3	0.6	4.9	8.5	11.4
unknown	0.9	0.9	0.8	0.3	1.5	1.7	1.8	0.3
dissolved $^{14}CO_2$	0.7	0.5	0.8	0.6	0.8	1.0	4.0	3.0
Sediment	57.3	87.6	84.2	83.5	30.5	66.4	64.3	63.4
- non-extractable	15.2	76.1	74.1	74.0	9.7	55.6	57.3	57.5
- extractable	42.1	11.5	10.1	9.5	20.8	10.8	7.0	5.9
extract consisting of e.g.:								
parent	38.2	2.2	0.7	0.6	18.4	3.8	0.4	0.3
M02	1.4	0.1	0.1	0.1	0.8	0.4	0.1	n.d.
M06/M08	0.2	1.2	0.4	0.3	0.2	1.5	0.3	0.2
M10	n.d.	0.5	0.9	1.4	n.d.	0.6	0.7	0.5
M11	n.d.	0.5	0.2	0.3	n.d.	0.5	0.2	0.2
M25	0.1	0.2	0.4	0.2	0.1	0.2	0.2	0.6
unknown	0.7	2.4	1.0	0.2	0.6	0.8	0.7	0.6

M02 desmethyl azinphos-methyl
M04 desmethyl isoazinphos-methyl
M06 hydroxymethylbenzazimide
M07 methylbenzazimide
M08 benzazimide
M10 methylsulfinylmethylbenzazimide
M11 methylsulfonylmethylbenzazimide
M25 methylbenzazimide sulfonic acid

- Other volatile radioactive metabolites only occurred in very small amounts < 0.1 % AR.
- Preliminary estimation of the rate of degradation:

System	Ijzendoorn DT50	Ijzendoorn DT90	Ijzendoorn kinetics	Lienden DT50	Lienden DT90	Lienden kinetics
Water	< 3 d			circa 4 d		
Total system[1]	1.3 d	14.5 d	RF1st	10.1 d	33.5 d	1st

RF1st: root function first order
[1] statistical model according to Timme et al. (1986, BOD 96-50001) assuming that 100 % at day 0 (optimum fit)

Conclusion:
Over the course of the experiment, the amounts of radioactivity in the surface water fell to 6 - 18 % AR whereas the sediment fractions increased to 63 - 84 % AR. More radioactivity was translocated into the sediment which contained larger amounts of adsorbents (organic substance and clay and silt particles). The amount of non-extractable radioactivity in the sediment samples rose to 57 - 76 % AR.
In both systems the parent compound was rapidly degraded: Azinphos-methyl was no longer detected in the surface waters after 34 d. In the sediments the concentration of the parent compound fell from 18 - 38 % on day 3 to below 1 % on day 91.
Azinphos-methyl was ultimately mineralised to $^{14}CO_2$.

The main metabolites in the water phase comprised desmethyl azinphos-methyl (maximum: 6.7 % AR in Lienden surface water on day 34) which was temporarily observed until day 34 and methyl benzazimide sulfonic acid (M25) (maximum: 11.4 % AR in Lienden surface water on day 91) which was present over the entire incubation period. All other metabolites and the unassigned radioactivity remained below about 3 % AR. Neither the parent compound nor the metabolites tended to accumulate in the water or sediment.
The proposed metabolic pathway is shown in Figure B.7.4-1.

Comment:
The study was conducted according to the test methods existing at this time and does not meet the requirements. The results permit only a preliminary assessment because of the following deficiencies:
- With the information given, it cannot be excluded that the sediment of the orchard drainage ditch (Ijzendoorn, NL) was contaminated with pesticide residues.
- Only 4 sampling intervals. The fate and distribution of residues during the first two days after application are not described. A statistically significant analysis of degradation kinetics is not possible, especially for water phase.
- Microbial activity was not reported.
- Application rate was not the highest possible.

B.7.4.5 Degradation in the saturated zone

Studies on the degradation in the saturated zone were not submitted. It was argued that azinphos-methyl and its metabolites are not expected to leach into deeper soil layers.

B.7.4.6 Summary

The results on route and rate of degradation of azinphos-methyl in water are summarized in Tables B.7.4-1, B.7.4-2 and B.7.4-3.

Table B.7.4-3: Hydrolytical half-lives of azinphos-methyl depending on temperature, pH, and concentration of the test substance

Report	Type of study	Experimental conditions: Temperature (°C)	Concentration (mg/l)	Lightning	Sterile	Half-lives [d]: pH4	pH5	at pH7	pH9	pH11
Wilkes et al. (1979)	Hydrolysis	30	1	no	yes	39	-	23	2	-
		30	10	no	yes	42	-	25	2.5	-
		40	1	no	yes	18	-	11	1	-
		40	10	no	yes	21	-	12	1	-
Wilmes (1982, revised 1984	Hydrolysis	22	1	no	yes	87	-	50	4	-
Flint et al.(1970)	Hydrolysis	30	14[1]	no		-	17	10	0.5	-
laboratory study		50	14[1]	no		-	2	1	0.1	-
field study[2]		29	100	yes	no	-	-	1.2	-	-

Report	Type of study	Temperature (°C)	Concentration (mg/l)	Lightning	Sterile	Half-lives [d]: pH8.6	pH9.6	
Heuer et al. (1974)	Hydrolysis	6	n.r.	n.r.	n.r.	-	-	36	5	4
		25	n.r.	n.r.	n.r.	-	-	28	2	2
		40	n.r.	n.r.	n.r.	-	-	7	0.7	0.4
Wilkes et al. (1976, revised 1981)	Photolysis	30	10	yes	n.r.	9.4h				
Morgan (1987)	Photolysis	17-29	10	yes	yes	3.2				
Hell-pointner (1994)	Photolysis	ambient	n.r.	yes	no	-	-	1.4[3]	-	-
			n.r.	yes	no	-	-	0.9[4]	-	-
			n.r.	yes	no	-	-	3.5[5]	-	-
	modelling		5	yes	no	-	-	1-3[6]	-	-
Fritz (1988)	Biological degradation	20	1	no	no	-	-	< 4[7]	-	-

1: Azinphos-methyl (technical grade) was dissolved in each buffer to form a saturated solution (solubility of azinphos-methyl in water: ca. 28 mg/l). Solutions were filtered and diluted with equal amounts of fresh buffer.
2: A large plastic vessel was filled with silt and water from a nearby pond. The pool was maintained outdoors and exposed to natural sunlight.
3, 4, 5: according to GC-solar program: 0-5 cm surface water, 10th degree of longitude, 50th degree of latitude, seasons:[3] spring,[4] summer,[5] fall; pH: not specified
6: according to the model of Frank and Klöppfer: 0-1 cm stagnant surface water, under geographic and climatic conditions as prevailing in Germany; main month of use (April to September); pH: not specified
7: pH-value of the water: 7.9 - 8.9
n.r.: not reported

It can be concluded that azinphos-methyl is degradable in water even in buffered, sterile solutions in darkness. The hydrolytic half-lives ranged from 4 (pH 9) to 87 days (pH 4) and decreased with increasing temperature and pH. In addition, the hydrolytical breakdown was accelerated by photolytical processes. The environmental half-lives concerning direct photolysis are expected in the range between 1 and 4 d, for the month of main use.

Although the degradation of azinphos-methyl in water seems to be a consequence of chemical processes, the microbial activity of aquatic systems is a important factor. Compared to abiotic and microbial degradation, volatility will probably play a minor role in the elimination of azinphos-methyl from water.
Based on an aerobic water/sediment study (conducted in darkness), DT50 values of approx. < 3 - 4 d in the water phase and 1.3 - 10.1 d in the complete system with corresponding DT90 values of 14.5 - 33.5 d were provisionally estimated from two different systems. Parallel to mineralization significant amounts of nonextractable residues (up to 76 % after 90 d) were formed.
Under natural conditions and in the presence of light, the degradation of azinphos-methyl seems to be significantly faster and resulted in a half-life of 1.2 days.

The main degradation products under sterile conditions at pH 7 were hydroxymethylbenzazimide (M06) and/or benzazimide (M08) and dimethylbenzazimide sulfide (M15). These metabolites and anthranilic acid exceeded 10 % AR only under conditions non-relevant for environment (high temperature and/or pH 4 or pH 9).
The major photolytic product benzazimide can be considered as photolytically stable under sterile conditions at pH 4.

In a water/sediment system the main metabolites were desmethyl azinphos-methyl (M02) and methylbenzazimide sulfonic acid (M25). Desmethyl azinphos-methyl (M02) could only be detected during the first 34 d after application. The major hydrolytic and photolytic products hydroxymethylbenzazimide (M06) and/or benzazimide (M08), dimethylbenzazimide sulfide (M15) and anthranilic acid (M12) were found never or in a minor extent.
The proposed metabolic pathway (Figure B.7.4-1) of the degradation of azinphos-methyl in aquatic systems involves hydrolysis of the phosphorus ester, with or without a previous desmethylation, resulting in methylbenzazimide sulfonic acid and various benzazimides which undergo further reactions (e.g. oxidations) and ultimate cleavage of the heterocyclic and benzene ring structure and mineralisation.

B.7.4.7 Assessment

Azinphos-methyl is fairly degradable in aquatic systems. The degradation results mainly in carbon dioxide and in significant amounts of residues that are firmly fixed in the sediment matrix. The main degradation products are hydroxymethylbenzazimide and/or benzazimide, dimethylbenzazimide sulfide and anthranilic acid under conditions non-relevant for environment, and methylbenzazimide sulfonic acid under biologically active, aerobic conditions.
The hydrolysis has been sufficiently investigated.
The results on photolysis of as can be used only for orientation because of various deviations from the requirements (e.g. the range of 280 - 290 nm were not excluded, non-sterile conditions, natural sunlight or mercury lamp, higher temperature). The results cannot be accepted for Middle Europe. Furthermore, the results on photolysis of as are not sufficient for the final evaluation of the photolytic degradation of the relevant metabolites.

The results submitted on aerobic aquatic degradation are not sufficient for the decisive evaluation since the distribution of residues over the first days after application is not sufficiently described, the statistically significant analysis of degradation kinetics is not possible and one of the sediments could be possibly contaminated with pesticide residues.
Studies on the degradation in the saturated zone are not required because azinphos-methyl and its metabolites are not expected to leach into deeper soil layers.
Results on the ready biodegradability are not required for the classification of the active substance.

B.7.5 Impact on water treatment procedures (Annex IIIA 9.2.2)

Information on the impact on water treatment procedures is not given and is not necessary.
With regard to use pattern in Middle and South Europe, the existing results on mobility in soil are suited for conclusion that a risk for ground water contamination with residues of azinphos-methyl is not expected.

B.7.6 Predicted environmental concentrations in surface water and in ground water (PEC_{sw}, PEC_{gw}) (Annex IIIA 9.2.1, 9.2.3)

B.7.6.1 Predicted environmental concentrations in ground water (PEC_{gw}) (Annex IIIA 9.2.1)

Ref.: Schäfer and Borchers, 1995, BOD 95-50058
Method:
The translocation behaviour of azinphos-methyl in soil was estimated using the simulation model PELMO, version 1.0 (Klein, M.: PELMO, Version 1.0, Dez. 91 (modified PRZM), Fraunhofer-Institut für Umweltchemie und Ökotoxikologie Schmallenberg, 1992).
To ensure a conservative estimate, the following worst case conditions were used as input for the calculation:

	Northern Europe	Southern Europe
Maximum application rate	3 kg as/ha/a (4 x 0.75 kg as/ha) in apple orchards	7.5 kg as/ha/a (5 x 1.5 kg as/ha) in apple orchards
With 50 % ground cover:	4 x 0.375 kg as/ha	5 x 0.75 kg as/ha
Application dates:	1.5./15.5./1.6./15.6.	1.5./15.5./1.6./15.6./1.7.
Weather data from	Hamburg for the year 1961:	
- Accumulated annual rainfall	872 mm	
- Annual average temperature (on the basis of daily mean)	9 °C	
- Annual average relative humidity	62 % (at 14:00)	
Soil	scenario "Borstel":	

- Type	loamy sand				
- Horizon	1	2	3	4	5
- Thickness (cm)	30	30	15	15	20
- Bulk density (g/ml)	1.5	1.6	1.58	1.62	1.6
- Sand (%)	68.3	67.0	96.2	99.8	100
- Silt (%)	24.5	26.3	2.9	0.2	0
- Clay (%)	7.2	6.7	0.9	0	0
- Organic carbon (%)	1.5	1.0	0.2	0	0
- Hydrodynamic dispersion coefficient (cm^2/d)	0	0	0	0	0
Relative degradation rate of as (%):	100	16	9	13	0
Ground cover	grass, 50 %		grass, 50 %		
Plant uptake	0		0		
DT50 values	63 d (Gronberg et al., 1979) and 10.9 d (Grace and Cain, 1990)				
K_{OC} values	516 (Lenz, 1979) and 693 (Ziegler and Hallenbeck, 1987)				

Results:
- Annual amounts of leachate water: 434.5 mm
- In all cases under investigation (8 parameter combinations), the predicted concentrations of azinphos-methyl in ground water, which is assumed to be present already at a depth of 110 cm, were significantly below 0.1 µg/l over the whole simulation period of 10 years.

Comment: Accepted.

B.7.6.2 Predicted environmental concentrations in surface water (PEC_{sw}) (Annex IIIA 9.2.3)

- Direct application and spray drift

The initial maximum PEC_{sw} (PEC_i) were calculated by the applicant (Annex III, point 10, Bayer AG, April 1995) based on the spray drift rates established for different crops by the German BBA/UBA (Ganzelmeier et al.: Mitt. Biol. Bundesanst. Land-Forstwirtsch., Berlin-Dahlem, 1995) for water depths of 0.3 and 1.0 m (see Table B.7.6.2-1).

Table B.7.6.2-1: Exposure of aquatic organisms (ground application) (from Bayer AG)

Crop	Distance (m)	Drift (%)	Application rate (as) (kg/ha)	(mg/m²)	PEC_i (µg as/l) water depth (m) 0.3	1.0
Gusathion M EC 19.5						
arable crops	10	0.4	0.5	50	0.67	0.20
orchards	50	0.2	1.0	100	0.67	0.20

grapes	30	0.2	0.8	80	0.53	0.16
Gusathion M WP 25						
arable crops	10	0.4	0.7	70	0.93	0.28
orchards	50	0.2	1.5	150	1.00	0.30
grapes	30	0.2	1.0	100	0.67	0.20

Assuming first order kinetics for the decline of the concentration, longer term predicted environmental concentrations (PEC_t) were calculated as actual and as the time weighted average concentration (TWA) for the respective time interval according to the formula

$$PEC_{twa} = PEC_i \cdot DT50 \cdot (1 - e^{(-t \cdot \ln(2)/DT50)}) \cdot (t \cdot \ln(2))^{-1}$$

where PEC_{twa} = time weighted average concentration (TWA),
PEC_i = initial concentration,
DT50 = half-life of disappearance and
t = considered time period.

For these calculations (see Table B.7.6.2-2), a half-life of 1.2 d measured in a pond water with sediment (Flint et al., 1970, cf. Chapter B.7.4.1) was used by the applicant, assuming that this half-life is representative for natural conditions.

Table B.7.6.2-2: Time course of the PEC of azinphos-methyl in water after single application (half-life 1.2 d) (from Bayer AG)

(d)	actual concentration (% of initial)	time weighted average (% of initial) calculated by Bayer AG[1]
0	100.0	100.0
1	56.1	76.0
2	31.5	59.3
4	9.9	39.0
5	5.6	32.7
7	1.8	24.3
14	0.0	12.4
21	0.0	8.2
28	0.0	6.2
42	0.0	4.1
60	0.0	2.9
85	0.0	2.0
140	0.0	1.2

[1]: These values were calculated with the equation described above and with PEC_i = 100 and DT50 = 1.2 d.

Comment:
The calculation of PEC (Table B.7.6.2-2) on the basis of a half-life of 1.2 d is not accepted because this value resulted from a study which does not meet the requirements.
The PEC_{sw} (initial) in Table B.7.6.2-1 are not sufficient for the

assessment of exposure of aquatic organisms because of
- missing values on direct application
- missing PEC considering all fixed drift values for different buffer zone distances and growth stages, and
- missing PEC after multiple application.

Furthermore, some other recommended uses (worst case situations because of high application rates) should be also taken into consideration.

The results of these calculations are given in Tab. B.7.6.2-3. **According to the statements of the applicant, uses in berries and small fruit (except grapes), vegetable and sugar beet, and uses in Northern Europe (Denmark, Finland, Sweden) are not intended furthermore and therefore not considered.**

Tab. B.7.6.2-3: PEC_{sw} (initial) based on direct application and spray drift rates after single application of EC or WP formulation (model pond: 1 m² surface area, 30 cm deep)

Crop/Growth stage (Country)	Appl. rate kgas/ha	PEC_{sw}(initial) (µg as/l) Buffer zone distance (m) 0	5	10	15	20	30	40	50
cotton (Greece)	1.0	333	2.0	1.3	0.667	0.333	-	-	-
potatoe (Italy)	0.7	233	1.4	0.933	0.467	0.233	-	-	-
grapes/early (France)	1.0	333	5.3	1.3	0.667	0.333	-	-	-
grapes/late (France)	1.0	333	16.7	5.0	2.7	1.3	0.667	-	-
citrus/early (Spain)	3.0	1000	200	110	60	40	20	4.0	2.0
citrus/late (Spain)	3.0	1000	100	45	25	15	6.0	4.0	2.0
ornamentals/ (< 50 cm)	0.7	233	1.4	0.933	0.467	0.233	-	-	-
ornamentals/ (> 50 cm) (Italy)	0.7	233	11.7	3.5	1.9	0.933	0.467	-	-
carthamus/ (> 50 cm) (Portugal)	2.0	666	33.3	10	5.3	2.7	1.3	-	-

- : not defined or not relevant

A **preliminary** estimation of short- and long-term PEC_{sw} (actual concentration and time weighted averages) for selected uses of azinphos-methyl is summarized in Table B.7.6.2-4 for the water phase and the entire water/sediment system, based on the PEC_{sw} (initial) for direct application (0 m), assuming
1) a first order kinetics with a worst case DT50 value of 4 d for the water phase (water/sediment study, cf. Chapter B.7.4.3), and
2) a first order kinetics with a worst case DT50 value of 10 d for the entire water/sediment system (from water/sediment system, cf.

Chapter B.7.4.3).

Table B.7.6.2-4: Initial, short- and long-term PEC_{sw} after direct application of azinphos-methyl for selected uses (DT50 = 4 d in water; DT50 = 10 d in water/sediment system)
(actual concentration and time weighted averages(TWA))

Crop Country		Citrus Spain		Apple Italy	
Application rate (kg as/ha)		3.0		1.5	
Number of applications		1		5	
Intervall (d)		-		7	
Time (d)		PEC_{sw} (µg/l)			
		actual w/ws	TWA w/ws	actual w/ws	TWA w/ws[1]
Initial:					
0	1.appl.	1000/1000	1000/1000	500/ 500	500/500
	2.appl.	- -	- -	649/ 808	290/396
	3.appl.	- -	- -	693/ 997	333/518
	4.appl.	- -	- -	706/1114	356/609
	5.appl.	- -	- -	710/1186	369/677
after last application:					
Short-term:	1	841/933	918/966	597/1106	379/693
	2	707/871	845/934	502/1032	384/706
	4	500/758	721/873	355/ 899	387/722
long-term:	7	297/616	579/792	211/ 730	377/730
	14	88/379	376/640	63/ 449	335/704
	21	26/233	268/527	19/ 277	292/655
	28	8/144	204/441	6/ 170	257/600
	42	1/ 54	137/325	1/ 65	206/502
	365	0/ 0	16/ 40	0/ 0	37/ 92

[1]: w/ws = water/entire water/sediment system

Major degradation products which at any time during the investigations account for more than 10 % of the amount of as added, were:
- Benzazimide - during photolysis under natural sunlight and sterile conditions
- Methyl benzazimide sulfonic acid - in the water phase of one water/sediment system, in the dark.

Assuming the worst case condition of direct application on surface water with the highest single application rate of 3 kg as/ha (cf. Table B.7.6.2-4) and taking into account the mole mass of both metabolites, the following dimensions of PEC_{sw} (static water bodies) can be roughly and provisionally estimated:

Metabolite	Benzazimide	Methyl benzazimide sulfonic acid

Test conditions	sterile natural sunlight	dark water/sediment system
Time (d)	PEC_{sw} (µg/l, actual concentration)	
1.3	126	
2.3	135	
3		5
3.6	149	
34		37
62		65
91		87

PEC_{sw} for slow moving water bodies were not submitted.
The applicant argued that the worst case scenario is given with the PEC_{sw} for static water bodies because of the additional dissipation processes by transport and dilution in moving water bodies.

- Run-off

Ref.: Flint et al., 1970, BOD 95-50052
Material and Method:
A spray concentrate of azinphos-methyl was applied to the top 3 m of each lane (4.5, 6 and 9 m long, respectively, and sloped approximately 0.08) at a rate of approximately 22 kg as/ha. The runoff plots were constructed on 3 soils, a sandy loam (soil no. 1, Table B.7.2-1), a silt loam (soil no. 2), and a high organic silt loam (soil no. 3). Irrigation was applied to the entire plots. Aqueous samples and slurries of soil were extracted with chloroform; the extracts were purified, concentrated and analysed by GLC. Recoveries were 90 % or greater in most cases.

Results:

Soil	Days after appl.	Irrigation (mm)	1.5 m runoff (l)	1.5 m as (%)	3 m runoff (l)	3 m as (%)	6 m runoff (l)	6 m as (%)
sandy loam	2	33.0	18.9	1.41	18.9	0.58	18.9	0.63
	total[1]	93.5	109.9	1.51	154.6	0.64	142.1	0.64
silt loam	2	33.0	18.9	0.45	18.9	0.15	18.9	0.22
	total	55.6	223.6	0.76	212.2	0.38	168.7	0.30
high organic silt loam	2	33.0	18.9	0.21	37.9	0.16	18.9	0.16
	total	54.1	170.6	0.37	178.1	0.40	199.0	0.33

1: total after 37 d post application

Comment:
The results can be accepted as additional information.

Conclusion:
The highest residues in runoff water were found with sandy loam

soil, the lowest with high organic silt loam soil. The effect of distance between application and collection points is not great with azinphos-methyl beyond 3 m; that is, although a difference is seen between 1.5 m and 3 m with silt and sandy loam soils, there is generally little difference between the 3 m and 6 m points with any of the soils. At 6 m, azinphos-methyl was not detected after 9 d with one exception. No clear relationship is seen between residues and the amounts of irrigation applied. Overall, the amount of runoff recovered in these experiments is < 1 % AR in all cases except one.

Exposition via run-off cannot be excluded, but, based on use pattern, the low water solubility and the adsorption of as to organic matter, any significant run-off could be possible mostly by adsorbed azinphos-methyl and only during the first days after application because of the progressive degradation in soil.

- Other sources of entries

Not submitted.

Comment:
Not neccessary.
Entries of azinphos-methyl into surface water by leaching, interflow, discharge via drains and atmospheric deposition are not expected.

B.7.7 Fate and behaviour in air (Annex IIA 7.2.2; Annex IIIA 9.3)

B.7.7.1 Vapour pressure

Ref.: Seweko, 1974, LUF 95-50010
Method: OECD 104 Vapour pressure balance method (comparable with EC Test A4)
Material: OAL standard 1974

Results: values obtained from the vapour pressure curve:
70 °C $2.1 \cdot 10^{-4}$ hPa
100 °C $2.0 \cdot 10^{-3}$ hPa
values extrapolated from the vapour pressure curve:
20 °C $1.8 \cdot 10^{-6}$ hPa
25 °C $3.1 \cdot 10^{-6}$ hPa

Conclusion: Azinphos-methyl can be classified as semivolatile substance.

Comment:
The purity of test material is not specified.
However, the study fulfills the requirements, the results are accepted.

B.7.7.2 Volatility from water

Ref.: Krohn, 1994, WAS 95-50024
Method: Henry's law constant at 20 °C, calculated from vapour pressure and water solubility

Result: $2.0 \cdot 10^{-3}$ Pa · m³/mole

Conclusion: The potential of volatility from aqueous surfaces should be low.

B.7.7.3 Volatilization

Not submitted and not required according to German BBA Guideline, part IV, 6-1, since photolytic half-lives are expected to be < 4 d for the months of main use (cf. Table B.7.4-3).

B.7.7.4 Photolysis in air

Ref.: Hellpointner, 1994, LUF 95-50011
Method:
The estimation of photochemical oxidative degradation of azinphos-methyl in the troposphere was conducted by using the "Atmospheric Oxidative Program" (Meylan and Howard, 1993, LUF 96-50001) which is based on a calculation procedure by means of quantitative structure reactivity relations (QSAR) developed by Atkinson (1985, LUF 95-00019; 1988, LUF 95-00018). Based on the molecular structure of as and a comprehensive set of experimental data on the reaction of organic compounds with photochemically produced OH radicals and with ozone, a conservative estimation of rate constant, half-life and chemical lifetime in the troposphere was made.

Result:
On the basis of the molecular structure of azinphos-methyl it is apparent that reactions with photochemically produced OH-radicals and to a lesser extent ozone or direct photolysis contribute to degradation in air.
Main reactions are hydrogen abstraction at the methylen group and reaction at the P=S-group.
Based on a mean OH-radical concentration of $1.5 \cdot 10^6$ OH radicals/ cm³ (global 12 h day time concentration, excluding the night) and an calculated overall OH reaction rate of $145.22 \cdot 10^{-12}$ cm³/molecule/s, a half-life of 0.9 h corresponding to a chemical lifetime of 1.3 h was assessed, with respect to the OH radical reaction only.
A more conservative assessment using an accuracy factor of 2 resulted in a maximum half-life of < 2 h and a maximum chemical lifetime of < 3 h.

Conclusion:
On account of the short chemical lifetime of azinphos-methyl in the air, it is not to be expected that the as can be transported in gaseous phase over large distances or can accumulate in the air.

B.7.7.5 Summary

With a vapour pressure of $1.8 \cdot 10^{-4}$ Pa at 20 °C, azinphos-methyl can be classified as semivolatile substance. But, Henry's law constant reveals a low potential of volatility from aqueous

surfaces.
Based on the calculation according to Atkinson (1985, 1988) the chemical life-time of beta-cyfluthrin in the troposphere averages in the range of < 3 h, with respect to OH-radical reaction only, and lead to a half-life of < 2 h. A further reduction by ozone attack or direct photolysis is possible.
It is to be expected that these reactions result in the formation of various primary radicals and secondary oxidation products, which can be eliminated from the air by wet and/or dry deposition.

B.7.7.6 Assessment

It is assumed that the tendency of azinphos-methyl to volatilize should be low. Considering the short half-life in the air, it is not to be expected that azinphos-methyl can be transported in the gaseous phase over large distances or can accumulate in the air.
The results submitted are sufficient for evaluation and assessment of the fate in air.
Results on volatilization are not required.

B.7.8 Predicted environmental concentrations in air (PEC_a) (Annex IIIA 9.3)

There is no PEC_a for azinphos-methyl because of the short half-life in the troposphere.

B.7.9 Definition of the residue (Annex IIA 7.3)

From the studies on the metabolism of [^{14}C]azinphos-methyl in soil, it can be concluded that CO_2 will be the principal aerobic degradation product.
With exception of the degradation product methylsulfonylmethyl benzazimide with 10 - 11.5 % after 62 - 92 d of incubation in the course of only one (aged leaching study) of total 3 studies, no other degradate exceeded an amount of 7 % of applied under any of the incubation conditions tested (aerobic, anaerobic, sterile). Therefore, the parent compound only can be regarded as relevant residue in soil.

Based on results on the behaviour in water/sediment system (in the dark!) and photolysis under natural sunlight, the metabolites benzazimide and methyl benzazimide sulfonic acid should be provisionally regarded as relevant residue in surface water besides azinphos-methyl. Valid results on the fate and behaviour in water are required for the final decision on the definition of the residue.

The parent compound can be regarded as relevant residue in air.

B.7.10 References relied on

Annex point(s) (91/414/EEC):	year. author(s). title. source. report number registration number.	GLP GEP Y/N	publ. Y/N	owner	data prot
EG:AIIA-7.1.1	1976. Close, C.L. The stability of Guthion in silt loam soil under frozen storage. Generated by: Mobay Corporation, submitted by: Bayer AG. 7.1.1.2.4 /01 ! Bayer MR48473. BOD95-50051.	N	N	BAY	
EG:AIIA-7.1.1	1990. Grace, T.J. Freezer storage stability of azinphos-methyl and azinphos-methyl oxygen analogue in soil. Generated by: Mobay Corporation, submitted by: Bayer AG. 7.1.1.2.1 /02 ! Bayer MR100165. BOD95-50045.	Y	N	BAY	
EG:AIIA-7.1.1	1989. Gronberg, R.R. Photolysis of azinphos-methyl on soil - Abbreviated study. Generated by: Mobay Corporation, submitted by: Bayer AG. 7.1.1.1.2 /04 ! Bayer MR99777. BOD95-50043.	Y	N	BAY	
EG:AIIA-7.1.1	1976. Liang, T.T. and Lichtenstein, E.P. Effects of soils and leaf surfaces on the photo-decomposition of [14C]azinphos-methyl. Journal of Agricultural and Food Chemistry - Volume 24, Issue 6 (1976), p. 1205-1210, submitted by: Bayer AG, 1976. 7.1.1.1.2 /01 ! Bayer IM495. BOD95-50040.	N	Y		
EG:AIIA-7.1.1	1990. Morgan, J.G. The photodecomposition of Guthion-phenyl-UL-14C on soil (revision of 31.01.1990). Generated by: Mobay Corporation, submitted by: Bayer AG.	N	N	BAY	

Annex point(s) (91/414/EEC);	year. author(s). title. source. report number registration number.	GLP GEP Y/N	publ. Y/N	owner	data prot
	7.1.1.1.2 /03 ! Bayer MR94708. BOD95-50042.				
EG:AIIA-7.1.1	1986. Timme, G., Frehse, H. and Laska, V. Zur statistischen Interpretation und graphischen Darstellung des Abbauverhaltens von Pflanzenschutzmittel-Rückständen. II. Pflanzenschutz-Nachrichten Bayer, 39, 2, 1986, 188-204. BOD96-50001.	N	Y		
EG:AIIA-7.1.1	1988. Wagner, K. Behaviour of the pesticidal active ingredient in soil (laboratory degradation test); Active ingredient: Azinphos-methyl (revision of20.01.1988). Generated by: Bayer AG, submitted by: Bayer AG. 7.1.1.2.2 /01 ! Bayer RR4306/74. BOD95-50046.	N	N	BAY	
EG:AIIA-7.1.1	1979. Wargo, J.P. and Gronberg, R.R. An analytical residue method for the determination of Guthion, Guthion oxygen analog, and total Guthion and metabolite residues in soils (revision of 26.06.1979). Generated by: Mobay Corporation, submitted by: Bayer AG. 7.1.1.2.1 /01 ! Bayer MR67084. BOD95-50044.	N	N	BAY	
EG:AIIA-7.1.1	1979. Wilkes, L.C., Wargo, J.P. and Gronberg, R.R., Photodegradation of Guthion on a soil surface. Generated by: Analytical Development Corporation, Colorado, USA, submitted by: Bayer AG. 7.1.1.1.2 /02 ! Bayer MR67979. BOD95-50041.	N	N	BAY	
EG:AIIA-7.1.1;	1990.	Y	N	BAY	

Annex point(s) (91/414/EEC);	year. author(s). title. source. report number registration number.	GLP GEP Y/N	publ. Y/N	owner	data prot
EG:AIIIA-9.1	Grace, T.J. and Cain, K.S. Dissipation of azinphos-methyl in California soil. Generated by: Mobay Corporation, submitted by: Bayer AG. 7.1.1.2.3 /04 ! Bayer 100164. BOD95-50050.				
EG:AIIA-7.1.1; EG:AIIA-7.1.1.1	1995. Gronberg, R.R., Pollock, R.J., Wargo, J.P. The metabolism of Guthion in sandy loam soil (revision of 16.03.1995). Generated by: Mobay Corporation, submitted by: Bayer AG, 1979. 7.1.1.1.1 /01 ! Bayer MR68030. BOD95-50038.	N	N	BAY	
EG:AIIA-7.1.1; EG:AIIIA-9.1	1979. Morris, R.A. (residue form) Residue trials with Guthion 50 WP in soil in USA. Generated by: Mobay Corporation, submitted by: Bayer AG. 7.1.1.2.3 /02 ! Bayer 67807. BOD95-50048.	N	N	BAY	
EG:AIIA-7.1.1; EG:AIIIA-9.1	1979. Morris, R.A. (residue form) Residue trials with Guthion 2L in soil in USA. Generated by: Mobay Corporation, submitted by: Bayer AG. 7.1.1.2.3 /03 ! Bayer 67803. BOD95-50049.	N	N	BAY	
EG:AIIA-7.1.1; EG:AIIA-7.1.1.1	1995. Wagner, K., Jozefowski, P. and Oehlmann, L. Metabolism of azinphos-methyl (Guthion) in the soil (revision of 16.03.1995). Generated by: Bayer AG, submitted by: Bayer AG, 1982. 7.1.1.1.1 /02 ! Bayer RA221/82. BOD95-50039.	N	N	BAY	
EG:AIIA-7.1.1; EG:AIIIA-9.1	1974. Yaron, B., Bielorai, H. and Kliger, L.	N	Y		

Annex point(s) (91/414/EEC);	year. author(s). title. source. report number registration number.	GLP GEP Y/N	publ. Y/N	owner	data prot
	Fate of insecticides in an irrigated field: Azinphos-methyl and tetradifon cases. Journal of Environmental Quality - Volume 3, Issue 4 (1974), p. 413-417, submitted by: Bayer AG, 3, 4, 1974, 413-417. 7.1.1.2.3 /01 ! Bayer IM143. BOD95-50047.				
EG:AIIA-7.1.2	1979. Lenz, M.F. Soil adsorption and desorption of Guthion. Generated by: Mobay Corporation, submitted by: Bayer AG. 7.1.2 /02 ! Bayer MR66848. BOD95-50053.	N	N	BAY	
EG:AIIA-7.1.2	1987. Ziegler, D.A. and Hallenbeck, S.A. Adsorption of Guthion to silt loam, sandy loam, sand, and clay loam. Generated by: Analytical Development Corporation, submitted by: Bayer AG. 7.1.2 /03 ! Bayer MR95084. BOD95-50054.	N	N	BAY	
EG:AIIA-7.1.3	1976. Atwell, S. and Close, C. Leaching characteristics of Guthion on aged soil. Generated by: Mobay Corporation, submitted by: Bayer AG. 7.1.3.3 /01 ! Bayer MR48466. BOD95-50056.	N	N	BAY	
EG:AIIA-7.1.3	1988. Fritz, R. Leaching behaviour of azinphos-methyl aged in soil. Generated by: Bayer AG, submitted by: Bayer AG. 7.1.3.3 /02 ! Bayer PF3099. BOD95-50057.	N	N	BAY	
EG:AIIA-7.1.3	1976. Thornton, J.S., Hurley, J.B. and	N	N	BAY	

Annex point(s) (91/414/EEC);	year. author(s). title. source. report number registration number.	GLP GEP Y/N	publ. Y/N	owner	data prot
	Obrist, J.J. Soil thin-layer mobility of twenty four pesticides chemicals. Generated by: Mobay Corporation, submitted by: Bayer AG. 7.1.3.1 /01 ! Bayer MR51016. BOD95-50055.				
EG:AIIA-7.1; EG:AIIA-7.1.3; EG:AIIA-7.2.1; EG:AIIIA-9.1; EG:AIIIA-9.2	1970. Flint, D.R., Church, D.D., Shaw, H.R. and Armour II, J. Soil runoff, leaching and adsorption, and water stability studies with Guthion. Generated by: Chemagro Corporation, submitted by: Bayer AG. 7.1.2 /01 ! Bayer MR28936. BOD95-50052.	N	N	BAY	
EG:AIIA-7.2.1	1974. Heuer, B., Yaron, B. and Birk, Y. Guthion half-life in aqueous solutions and on glass surfaces. Bulletin of Environmental Contamination and Toxicology, 11, 6, 1974, 532-537. 7.2.1.1 /02 ! Bayer IM145. WAS95-50025.	N	Y		
EG:AIIA-7.2.1; EG:AIIIA-9.2	1994. Hellpointner, E. Determination of the quantum yield and assessment of the environmental half-life of the direct photodegradation of azinphos-methyl in water. Generated by: Bayer AG, submitted by: Bayer AG. 7.2.1.2 /03 ! Bayer PF3990. WAS95-50030.	Y	N	BAY	
EG:AIIA-7.2.1; EG:AIIIA-9.2	1987. Morgan, J.G. The aqueous photolysis of Guthion-phenyl-UL-14C. Generated by: Mobay Corporation, submitted by: Bayer AG. 7.2.1.2 /02 ! Bayer MR94709. WAS95-50029.	N	N	BAY	

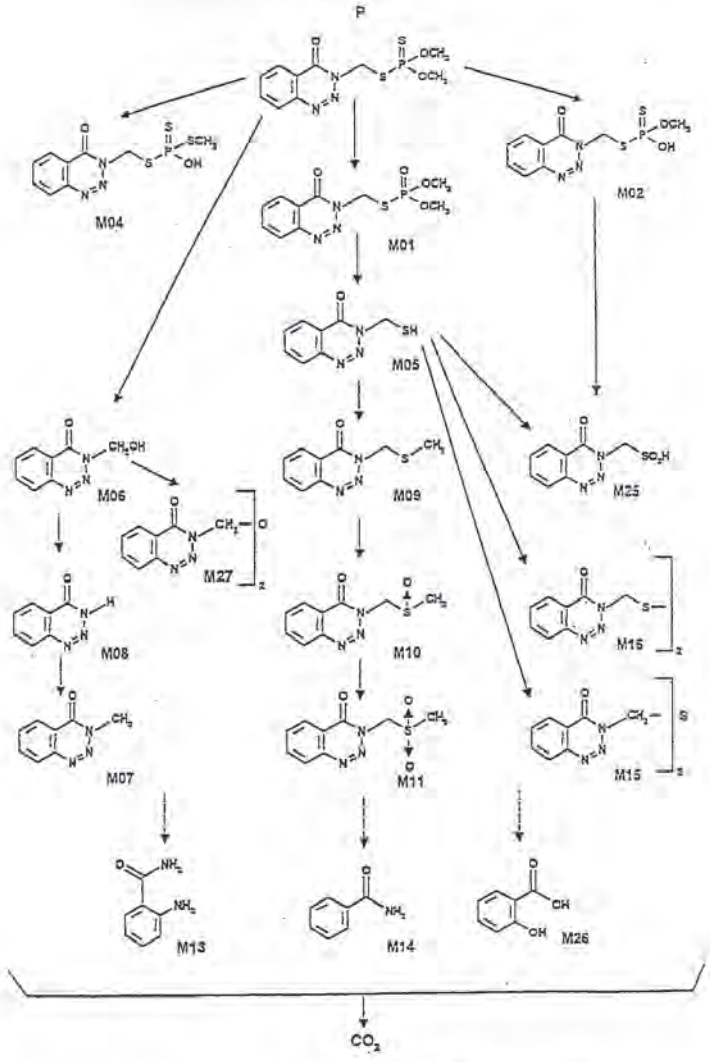
Annex point(s) (91/414/EEC);	year. author(s). title. source. report number registration number.	GLP GEP Y/N	publ. Y/N	owner	data prot
EG:AIIA-7.2.1; EG:AIIIA-9.2	1979. Wilkes, L.C., Wargo, J.P. and Gronberg, R.R. Dissipation of Guthion in buffered aqueous solution. Generated by: Mobay Corporation, submitted by: Bayer AG. 7.2.1.1 /03 ! Bayer MR67983. WAS95-50026.	N	N	BAY	
EG:AIIA-7.2.1; EG:AIIIA-9.2	1981. Wilkes, L.C., Wargo, J.P. and Gronberg, R.R. Photodegradation of Guthion in aqueous solution (revision of 13.08.1981). Generated by: Mobay Corporation, submitted by: Bayer AG. 7.2.1.2 /01 ! Bayer MR67980. WAS95-50028.	N	N	BAY	
EG:AIIA-7.2.1; EG:AIIA-7.2.2; EG:AIIIA-9.2; EG:AIIIA-9.3	1984. Wilmes, R. Properties of pesticides in water. Active ingredient: Azinphos- methyl (Guthion) (revision of 10.07.1984). Generated by: Bayer AG, submitted by: Bayer AG. 7.2.1.1 /04 ! Bayer M1039. WAS95-50027.	N	N	BAY	
EG:AIIA-7.2.2; EG:AIIIA-9.3	1985. Atkinson, R. Kinetics and mechanism of the gas phase: Reactions of the hydroxyl radical with organic compounds under atmospheric conditions. Chem. Rev., 85, 1985, 69-201. LUF95-00019.	N	Y		
EG:AIIA-7.2.2; EG:AIIIA-9.3	1988. Atkinson, R. Estimation of gas-phase hydroxyl radical rate constants for organic chemicals. Env. Tox. Chem., 7, 1988, 435-442. LUF95-00018.	N	Y		
EG:AIIA-7.2.2;	1994.	N	Y		

Annex point(s) (91/414/EEC);	year. author(s). title. source. report number registration number.	GLP GEP Y/N	publ. Y/N	owner	data prot
EG:AIIIA-9.3	Heilpointner, E. Calculation of the chemical lifetime of azinphos-methyl in the troposphere. Generated by: Bayer AG, submitted by: Bayer AG. 7.2.2.3 /01 ! Bayer PF3989. LUF95-50011.				
EG:AIIA-7.2.2; EG:AIIIA-9.3	1994. Krohn, J. Calculation of the Henry law constant of azinphosmethyl. BAYER FILE NO.: PC 157. WAS95-50024.	N	N	BAY	
EG:AIIA-7.2.2; EG:AIIIA-9.3	1993. Meylan, W. and Howard, P. Atmospheric Oxidation Program, Version 1.51 (March 13, 1993). Syracuse Research Corp., Syracuse, N.Y., USA, 1993. LUF96-50001.	N	Y		
EG:AIIA-7.2; EG:AIIIA-9.3	1974. Sewekow, B. Determination of vapour pressure of azinphos-methyl (Methylgusathion). Generated by: Bayer AG, submitted by: Bayer AG, 1974. 7.2.2.1 /01 ! Bayer PC143. LUF95-50010.	N	N	BAY	
EG:AIIA-7.2.1.3.2 ;	1988. Fritz, R. Aerobic metabolism of azinphos-methyl in the aquatic model ecosystem. Generated by: Bayer AG, submitted by: Bayer AG. 7.2.1.3 /02 ! Bayer PF3097. WAS95-50031.	N	N	BAY	
EG:AIIIA-9.2	1995. Schäfer, H. and Borchers, H. Predicted environmental concentration of azinphos-methyl in groundwater recharge based on the calculation of PELMO. Generated by: Bayer AG, submitted by:	N	N	BAY	

		GLP	publ.	owner data
Annex point(s) (91/414/EEC):	year.			
	author(s).	GEP		prot
	title.			
	source.			
	report number			
	registration number.	Y/N	Y/N	

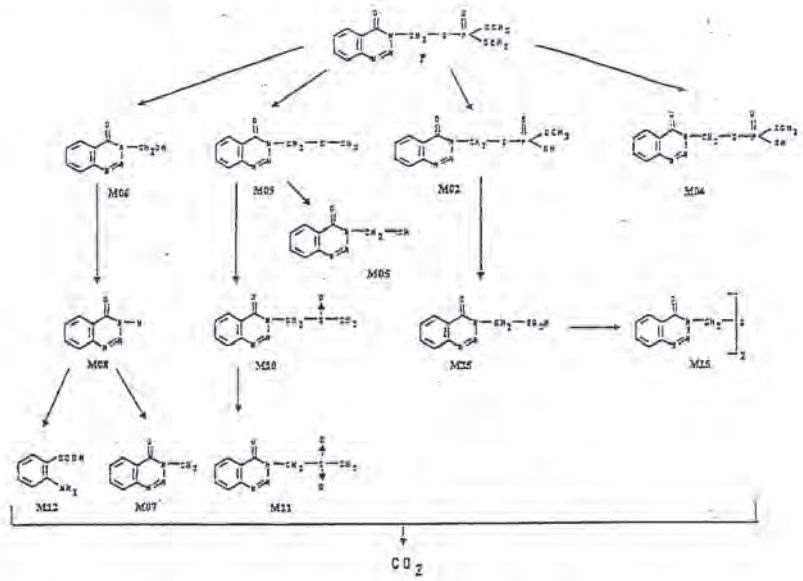
Bayer AG.
7.1.3.5 /01 : Bayer M9367.
BOD95-50058.

Figure B.7.1-1: Proposed degradation pathway of azinphos-methyl in soil



P	azinphos-methyl
M01	azinphos-methyl oxygen analogue
M02	desmethyl azinphos-methyl
M04	desmethyl azinphos-methyl isomer
M05	mercaptomethyl benzazimide
M06	hydroxymethylbenzazimide
M07	methyl benzazimide
M08	benzazimide
M09	methylthiomethylbenzazimide
M10	methylsulfinylmethyl benzazimide
M11	methylsulfonylmethyl benzazimide
M13	anthranilamine
M14	benzamide
M15	dimethylbenzazimid sulfide
M16	dimethylbenzazimide disulfide
M25	methyl benzazimide sulfonic acid
M26	salicylic acid
M27	dimethylbenzazimide ether

Figure B.7.4-1: Proposed metabolic pathway of the degradation of azinphos-methyl in aquatic systems



P	azinphos-methyl	M08	benzazimide
M02	desmethyl azinphos-methyl	M09	methylthiomethylbenzazimide
M04	desmethyl azinphos-methyl S-methyl isomer	M10	methylsulfinylmethylbenzazimide
M06	hydroxymethylbenzazimide	M11	methylsulfonylmethylbenzazimide
M07	methyl benzazimide	M25	methyl benzazimide sulfonic acid

Table B.7.1-3: Weather conditions/Soil dissipation studies of azinphos-methyl in USA

Location	SOIL NO.	DATE		AIR TEMP		SOIL TEMP 4-inch depth		PRECIPITATION/ IRRIGATION	SOLAR RAD
		YEAR	MONTH	[°F]	[°C]	[°F]	[°C]	[mm]	kW/sqm
Indiana	sandy loam/13	78	5			62	16,7		
		78	6			67	19,4	63,5	
		78	7			70	21,1	174,2	
Texas	sandy clay loam/14	77	11-12					73,7	
		77	11			72	22,2		
		77	12			67	19,4		
		78	1			58	14,4	79,5	
		78	2			59	15,0		
		78	3			71	21,7	188	
		78	4			79	26,1		
		78	5			87	30,6	220,5	
		78	6			91	32,8		
		78	7			93	33,9	277,9	
		78	8			90	32,2		
Florida	sand/12;silt loam/15	77	11-12					228,1	
		77	11			68	20,0		
		77	12			56	13,3		
		78	1			56	13,3	278,1	
		78	2			54	12,2		
		78	3			65	18,3	432,3	
		78	4			70	21,1		
		78	5			79	26,1	498,3	
		78	6			83	28,3		
		78	7			85	29,4		
		78	8			85	29,4	2042,2	

Continuation from Table B.7.1-3

Location	SOIL NO.	DATE		AIR TEMP		SOIL TEMP		PRECIPITATION/ IRRIGATION	SOLAR RAD [kW/sqm]
		YEAR	MONTH	[°F]	[°C]	[°F]	[°C]	[mm]	
Chualar	sand-sandy loam/17	89	6		17,6		17,9	94	
		89	7		16,3		18,7	96,5	
		89	8		(15,9)*		(19,4)*	160	(0,21)*
		89	9		(16,0)*		(19,0)*	109	(0,27)*
		89	10		14,5		16,4	134	

* Data on soil and air temperature were taken from USDA Station #37 (Salinas) in the period from 17.8. to 30.9.89

Location	SOIL NO.	DATE		AIR TEMP		SOIL TEMP*		PRECIPITATION/ IRRIGATION	SOLAR RAD [kW/sqm]
		YEAR	MONTH	[°F]	[°C]	[°F]	[°C]	[mm]	
Fresno	sand loam-loamy sand/16	89	5		19,6		22	184,6-204,6	0,30
		89	6		23,5		25,5	95	0,33
		89	7		25,5**		25,3**	108	0,35
		89	8		23,6		25,5	100,6	0,29
		89	9		20,6		22,4	36,1	0,24

* average soil temperatures were not reported in final report but are available in raw date
** air temperature data for 19.7 through 25.7.89 were lost during downloading

Annex B

Azinphos-methyl

B-8: Ecotoxicology

B.8 Ecotoxicology

B.8.1 Effects on birds (Annex IIA 8.1; Annex IIIA 10.1)

B.8.1.1 Acute oral toxicity (Annex IIA 8.1.1, Annex IIIA 10.1.1)

Stubblefield 1987 (AVS 95-142)
The acute oral toxicity of technical azinphos-methyl was tested in bobwhite quail according to EPA guideline no. 71-1. The test material was dissolved in corn oil and intubated at dose levels of 0/5.6/11.2/23/45/90 mg as/kg body weight. Five males and five females, about 17 weeks old, were treated per dose level. The LD50 is 32.2 (25-41) mg as/kg bw. The lowest dose causing mortality was 23 mg as/kg bw. The NOED is 5.6 mg as/kg based upon signs of intoxication at 11.2 mg as/kg bw.

Further LD50-values of birds are reported in the literature (table B.8.1.1-1). Some of these data are to be regarded as approximate LD50 as the number of individuals per test was often quite low.

Table B.8.1.1-1: Acute oral toxicity of technical azinphos-methyl to birds. Data are given as mg as/kg bw.

Species	Age	Sex	LD50	Lowest dose causing mortality	NOED	Ref.
Bobwhite quail	17 w	M+F	32	23	5.6	(1)
Bobwhite quail	24 m	M	60-120			(2)
Pheasant	3-5 m	M	75			(2)
Mallard duck	3-4 m	M	136			(2)
Chukar	3-4 m	M	84			(2)
Chicken	10-12 d	M	277			(3)
Redwing blackbird			8.5			(4)
Starling			27			(4)

References: (1) Stubblefield 1987; (2) Hudson et al. 1984; (3) Sherman et al. 1967; (4) Schafer 1972;

The signs of intoxication are typical for ChE inhibitors: ataxia, wing drop, lethargy, immobility, labored breathing, salivation, convulsions and anorexia. Signs appeared as soon as 15 min post-dosing, mortalities occurred mainly during the first two days (Stubblefield 1987, Hudson et al. 1984).

In a study by Lari et al. (1994) four groups of seven adult male Japanese quail orally received 0/0.5/5/50 mg/kg bw azinphos-methyl. A dose of 0.5 mg as/kg bw had a slight effect on plasma-BChE, but no effect on brain-AChE. 5 mg as/kg bw moderately inhibited brain-AChE; 50 mg as/kg bw caused a strong inhibition of brain-AChE and the death of one bird.

In conclusion the acute oral toxicity of azinphos-methyl to birds is high. The range of senitivities is rather large with the lowest LD50 at 8.5 mg as/kg bw (redwing blackbird).

B.8.1.2 Dietary toxicity (Annex IIA 8.1.2)

Data are only available from the literature (table B.8.1.2-1). The studies were not conducted in compliance with GLP, but nevertheless can be regarded as sound and valid.

Table B.8.1.2-1: Dietary toxicity of technical azinphos-methyl to birds. Data are given as ppm (mg as/kg feed).

Species	Age	LC50	Lowest conc. causing mortality	NOEC	Ref.
Japanese quail	14 d	935	671		(1)
Japanese quail	9 d	639			(2)
Bobwhite quail	14 d	488			(2)
Pheasant	22 d	1821			(2)
Mallard duck	10 d	1940			(2)

References: (1) Hill and Camardese 1986; (2) Hill et al. 1975;

In the study of Hill and Camardese (1986) Japanese quail are reported having had a food consumption of 10 g per bird per day at a concentration level of 448 ppm. Assuming a body weight of 30 g this corresponds to a daily dose of about 150 mg as/kg. As no bird died at that concentration level detoxification of azinphos-methyl must be rapid so that birds survive more than one LD50 per day when the substance is administered in the feed.

In conclusion azinphos-methyl is of moderate subacute dietary toxicity to birds.

B.8.1.3 Effects on reproduction (Annex IIA 8.1.3)

Beavers et al. 1989
A one-generation reproduction study with bobwhite quail was conducted according to EPA guideline no. 71-4. Technical azinphos-methyl was mixed into the feed for an exposure period of 20 weeks at nominal concentrations of 0/20/48/115 ppm. Effective concentrations were 0/15.6/36.5/87.4 ppm. At each concentration level 16 pairs were used, that were about 17 weeks old at onset of exposure. The NOEC is 36.5 ppm. At 87.4 ppm there was observed substance-related mortality in the parental generation (3 birds), signs of intoxication, reduced egg production and reduced survivability of offspring.

Toll 1988
A one-generation reproduction study with mallard duck was conducted according to EPA guideline no. 71-4. Technical azinphos-methyl was mixed into the feed for an exposure period of 20 weeks at nominal concentrations of 0/12/35/101 ppm. Effective concentrations were 0/10.5/32.5/96.3 ppm. At each concentration level 15 pairs were used, that were about 18 weeks old at onset of exposure. The NOEC is 10.5 ppm. At 32.5 ppm the body weight of the hens was affected. At 96.3 ppm also the body weight of the drakes was affected; furthermore the hatching

rate deviated from the control, however the difference was statistically not significant.

In conclusion the lowest NOEC for the subchronic parental toxicity is 10.5 ppm; the lowest NOEC for reproductive effects is 36.5 ppm.

B.8.1.4 Other studies (Annex IIIA 10.1.2, 10.1.3, 10.1.4)

Acceptance of bait, granules, or treated seeds by birds is not applicable, because azinphos-methyl formulations are to be applied exclusively as sprays.

Three field trials are reported in the literature.

(1) Lari et al. 1994
In Modena province (Northern Italy) a cherry grove was treated once with 3.75 kg/ha azinphos-methyl (Formulation "Lathion methyl"). Tree sparrows were captured 24 h before treatment and 24 h after treatment. Blood samples were taken for plasma butyrylcholinesterase (BChE) and carboxylesterase (CbE) assays. There was found no difference in the BChE activity; the CbE was significantly increased after treatment. The authors interpreted this response as an induction of the detoxifying process.

(2) Graham and DesGranges 1993
a) In Quebec province (Canada) potato fields received 1-4 azinphos-methyl treatments by tractor or by plane; the application rate was 0.25-0.5 kg as/ha per treatment. Treated fields were only occasionally visited by birds, so the degree of exposure probably was low. Plasma and brain ChE of captured song and vesper sparrows was not different from controls.
b) Also in Quebec province orchards were treated 2-3 times with 1.75 kg as/ha. Brain ChE activity was analyzed in chipping sparrows and song sparrows; in both species reduction compared to control birds was found (mean inhibition -38/-27 %). Plasma ChE activity was investigated in chipping sparrows, song sparrows and American robin; in two species inhibition was found (-33/-50 %). No dead or moribund birds were found at either site and no abnormal behaviour was observed. Monitoring of nests revealed no indication that the breeding success was affected by the treatment.

(3) Custer and Mitchell 1987
In Texas (USA) azinphos-methyl was aerially applied in sugarcane and cotton fields at rates of 0.28-1.21 kg as/ha. Great-tailed grackles and mourning doves were captured for brain AChE assay. In 4 out of 51 grackles AChE activity was found to be two standard deviations below control values (corresponding application rates were 0.56 and 1.21 kg as/ha). No birds were found dead.

B.8.1.5 Risk assessment for birds

Birds may be exposed to azinphos-methyl mainly by the consumption of contaminated feed. Depending on species this may be insects, fruits or herbs. Azinphos-methyl is not to be used in situations where

intensive grazing of birds is expected to occur. However, feeding on herbs in the understory of orchards or on field margins may occur.

For residues on fruits data are available. The initial residue on different kinds of fruits and on grapes is mainly in the range of 0.3-3.0 mg as/kg. The highest value found is 5.7 mg as/kg (see section B.6). The initial residues on herbs and insects are estimated according to Hoerger and Kenaga (1972). In orchards it is assumed that half of the applied rate reaches the vegetation on the ground; furthermore the Hoerger and Kenaga figures for residues in insects are halved because in orchard the surface of the target area is at least the double of the ground area. The use in grapes (max. 1 kg as/ha) is covered by the consideration of orchards. This leads to PEC-values between 20 and 23 mg as/kg fresh weight.

In order to consider the worst case condition it is assumed that birds feed exclusively on contaminated material and that herbivorous birds have a daily feed demand of 25 % of their body weight and small insectivorous birds of 35 % of their body weight. Then the maximum daily intake is between 5.4 mg as/kg bw and 7.6 mg as/kg bw.

Table B.8.1.5-1: Exposure assessment for birds

Use	Max. appl. rate (kg as/ha)	Feed	Typical maximum residue[1] (mg/kg)	Initial residue (mg/kg)	Rel. feed demand (%)	Maximum daily intake (mg/kg bw)
Arable crops	0.7	Herbs	31*R	22	25	5.4
		Insects	29*R	20	40	8.1
Orchards	1.5	Herbs	31*R/2	23	25	5.8
		Insects	29*R/2	22	40	8.7
		Fruits		6[2]	40	2.4

[1] according to Hoerger and Kenaga (1972); R = application rate in kg/ha
[2] measured value

Comparison of the acute toxicity (8.5 mg as/kg bw) with the maximum daily intake gives an acute toxicity/exposure ratio of 3.5 for frugivorous birds and around one (1.0-1.6) for herbivores and insectivores. Comparison of the LC50 from the 5-day-dietary feeding studies (488 ppm) with the estimated initial residues results in a short-term toxicity/exposure ratio of at least 21. The acute toxicity/exposure ratio indicates a high risk, however the kind of exposure under practical conditions is better simulated by feeding studies. Thus the short-term toxicity/exposure ratio is more relevant and this indicates a moderate risk. This view is supported by the results of field studies. These have demonstrated that following application of 0.7 kg as/ha in field cultures respectively 1.5 kg as/ha in orchards birds may be exposed to such a degree that brain-AChE will be moderately inhibited, but serious effects are not probable. No die-offs of birds are reported according to different sources from Germany, UK, Canada and USA.

Regarding the long-term risk the initial residues are above the NOEC

from the reproduction study. Therefore a more realistic exposure assessment is tried. It is assumed that the half-life for vegetation is about 3 days. With two applications 2 weeks apart the average residue over a period of 4 weeks is then 30 % of the initial residue, that is 23 · 0.30 = 7 mg/kg for herbs. (Considering 3 applications 2 weeks apart and a period of 6 weeks would not change this figure very much). If assuming that on the long-term a bird covers 25 % of its feed requirement from the treated area, the average contamination of the feed amounts to 7 · 0.25 = 1.75 mg/kg. The quotient NOEC/PEC then is 10.5/1.75 = 6 and is just above the Annex VI trigger of 5. It has to be stated that the empirical basis for a sound long-term exposure assessment of birds is poor (for insectivores it is practically impossible). However, it appears that in the case of azinphos-methyl the acute risk is of more concern than the long-term risk.

In conclusion, although the acute toxicity/exposure ratio does not meet the criteria provided in Annex VI of Directive 91/414/EEC the risk to birds under normal use conditions is considered as tolerable. However, the margin of safety is small and risk assessments should be carefully conducted when authorizing products containing azinphos-methyl.

B.8.2 Effects on aquatic organisms

B.8.2.1 Acute toxicity of azinphos-methyl and formulations (Annex IIA 8.2; Annex IIIA 10.2)

The data summarized in table B.8.2.1-1 indicate a high acute toxicity of azinphos-methyl to aquatic organisms.

The tests with the golden orfe and the more sensitive rainbow trout were conducted without analysis of the test compound in the test water. Therefore they do not fulfil the actual requirements of test guidelines. From this data, a high acute toxicity to fish can be concluded. For a final evaluation a valid acute toxicity test with rainbow trouts has to be required.

Aquatic crustaceans are the most sensitive organisms with an EC50 of 1.1 µg/l and a NOEC of 0.7 µg/l. As a precise analysis of the test substance was not done, the test results can only used for information. For a final evaluation a valid acute toxicity test with water fleas has to be required.

The results on Scenedesmus with a NOEC of 1.8 mg/l indicate a moderate toxicity to algae.

The acute toxicity data demonstrate a steep dose-response relationship.

The main degradation products under aqueous conditions were hydroxymethylbenzazimide and/or benzazimide and dimethylbenzazimide sulfide (2.8.2.2). These compounds exceeded 10 % of applied amount only under conditions not relevant for aquatic environment (high temperature and/or high or low pH). The major metabolite in

water/sediment system was methylbenzazimide sulfonic acid up to 11 % after 91 days in sediment. Further toxicity demands should be decided in relation to the test results with the active substance and sediment-dwelling organisms.

Table B.8.2.1-1: Acute toxicity of azinphos-methyl to aquatic organisms

Species	NOEC mg as/l	LOEC mg as/l	EC/LC50 mg as/l	Exposure durat./design		Remarks	Reference
Fish							
Leuciscus idus m.	<0.05	0.05	0.12	96 h	static	nominal	(1)°
Oncorhynchus mykiss	<0.01	0.01	0.02	96 h	static	nominal	(2)°
Oncorhynchus mykiss	0.001	0.004	0.003	96 h	static	nominal	(3)°
Marine Fish							
Cyprinodon variegatus	0.91	1.4	2.7	96 h	flow-thr.	measured	(4)
Invertebrates							
Daphnia magna	0.0007	0.001	0.0011	48 h	static	nominal	(5)°
Marine Invertebrates							
Mysidopsis bahia	<0.0001	0.0001	0.0002	96 h	flow-thr.	measured	(6)
Crassostrea virgin.	2.0	3.1	4.7	96 h	flow-thr.	measured	(7)
Algae							
Scenedesmus sub.	1.8	3.2	3.61	96 h	static	nominal	(8)°

Ref.: (1) Hermann, 1979, WAT95-50207; (2) Hermann, 1978, WAT95-50206; (3) Carlisle, 1984, WAT95-50208; (4) Surprenant, 1987, WAT95-50219; (5) Lamb, 1980, WAT95-50210; (6) Surprenant, 1988, WAT95-50221; (7) Surprenant, 1987, WAT95-50220; (8) Heimbach, 1985, WAT95-50212

°: no analytical determination of the test substance in test water

Acute toxicity test of different plant protection products containing the active substance azinphos-methyl show also a high toxicity to aquatic organisms (table B.8.2.1-2). In comparison to the data of the active substance products are less toxic three to four times. The test with rainbow trouts was conducted without an analysis of the test compound in the test water. Therefore the risk assessment is based on the data of the active substance.

The results of acute toxicity tests with water fleas under static and flow-through conditions were very similar to those of rainbow trouts. It results in the short-term effects and therefore it can be concluded that metabolites are not important for adverse effects.

Table B.8.2.1-2: Acute toxicity of plant protection products containing the active substance azinphos-methyl to water organisms (data are expressed as active substance)

Species	NOEC µg as/l	LOEC µg as/l	EC/LC50 µg as/l	Exposure durat./design		Remarks	Reference
Fish							
O. mykiss	1.5	-	5.3	96 h	static	measured	(1)[a]
O. mykiss	2.2	-	6,2	96 h	static	nominal°	(2)[b]
L. macrochirus	7.0	-	8.8	96 h	static	nominal°	(2)[b]
Invertebrates							

Daphnia magna	2.4	-	4.4	48 h	flow-thr.	measured	(3)[c]
Daphnia magna	<3.2	-	6.7	48 h	static	measured	(4)[d]

Ref.: (1) Dorgerloh, 1995, WAT95-50184; (2) Nelson, 1978, WAT95-50216; (3) Surprenant, 1987, WAT95-50217; (4) Surprenant, 1989, WAT95-50226
[c]: no analytical determination of the test substance in test water
[a]: Gusathion M WP 25 (25 % as)
[b]: Guthion 2S (22 % as)
[c]: Guthion 50 WP (50 % as)
[d]: Guthion 2E (23.8 % as)

B.8.2.2 Prolonged and chronic toxicity of azinphos-methyl to aquatic organisms (Annex IIA 8.2)

Prolonged and chronic toxicity data from fish and the water fleas with NOEC's of 0.23 µg as/l and 0.25 µg as/l are in the same order as acute toxicity values (table B.8.2.2-1), although they are a result of dynamic test conditions. These results also demonstrate the predominantly short-term effects of azinphos-methyl.

Table B.8.2.2-1: Chronic toxicity of azinphos-methyl to aquatic organisms

Species	NOEC µg as/l	LOEC µg as/l	Endpoint	Exposure durat./design		Remarks	Reference
Fish							
Oncorhynchus mykiss	0.39	0.85	symptoms	21 d	flow-thr.	measured	(1)
Oncorhynchus mykiss	0.44	0.98	survival	85 d	flow-thr.	measured	(2)
Oncorhynchus mykiss	0.23	0.44	behaviour	85 d	flow-thr.	measured	(2)
Invertebrates							
Daphnia magna	0.25	0.40	reproduct.	21 d	flow-thr.	measured	(3)
Algae							
Scenedesmus sub.	1,800	3,200	biomass	96 h	static	nominal	(4)[c]

Ref.: (1) Grau, 1989, WAT95-50223; (2) Surprenant, 1988, WAT95-50209; (3) Carlisle, 1984 WAT95-50211; (4) Heimbach, 1985, WAT95-50212
[c]: no analytical determination of the test substance in test water

B.8.2.3 Micro- or Mesocosm study

Field tests were conducted in accordance with the model ecosystem studies under natural conditions (Dortland, 1980, WAT95-50346). The volume of the four test containers varied between 2 to 3 m^3. The containers were provided with a bottom soil layer of 0.1 m. The pre-experimental phase was in 1976, about one vegetation period before starting the study. For the definitive study the containers were divided into two separate identical parts. Azinphos-methyl was applicated with a concentration of 1 µg as/l in one system in 1977 and in two systems in 1978. The concentrations were analysed two times a week. Depending on the results the concentrations were maintained by re-applications (similar to a semistatic test procedure) about the test duration of three months, from June to August. During the study the abundance of a wide range of different aquatic invertebrate taxa (16) and species (65) were observed.

As the test substance was kept on a constant level by re-dosing, this exposure model is especially suitable for the situation of multiple applications.

Simocephalus vetulus and Daphnia pulex were the most sensitive species in this experiment.

After application of azinphos-methyl (1 µg as/l) the caddy fly larvae decreased remarkably and an almost complete disappearance of the water flea took place. In the containers with the controls the population was constant during the total test duration. The population of Daphnia in the treated trial did not recover after the treatment was stopped whereas the Simocephalus larvae nearly completely recovered.

B.8.2.4 Bioaccumulation in fish (Annex IIA 8.2)

Because the log Po/w of azinphos-methyl was estimated to be 2.96 a biococentration study is not indicated. Because of the rapid metabolisation of azinphos-methyl in water, the quick uptake and the nearly complete elimination of the total group of organophosphorus compounds, a bioconcentration potential of azinphos-methyl cannot be expected. There are no concerns to adverse effects because no remarkable reproductive or teratogenic effects occurred in the early-life stage test.

B.8.2.5 Exposure and risk assessment for aquatic organisms

The acute NOEC of 0.7 µg as/l and EC50 of 1.1 µg as/l for Daphnia should be used for a preliminary short-term exposure and risk assessment. In cases of multiple application of azinphos-methyl, the chronic NOEC of 0.25 µg as/l for water fleas is representative for long-term exposure.

Depending on crops and cultures aquatic organisms in surface waters located near the treated target areas are highly endangered by poisoning resulting in mortalities, especially in crustaceans due to direct overspraying and drift exposure. The predicted initial environmental concentrations can reach effect concentrations from 65 to 1,000 µg as/l. The application rate varies from two to five times. A single application is indicated only in cotton.

Unacceptable adverse effects of plant protection products with the active substance azinphos-methyl have to be expected if the products are used near water bodies.
In all intended uses, especially in high-dose applications e. g. 15 kg as/ha in forest, 3 kg as/ha in citrus fruits, 1 kg as/ha in grapes and 0.7 kg as/ha in ornamentals as well as in low-dose applications of 0.196 kg as/ha in mustard unacceptable adverse effects can happen.

The risk to aquatic organisms cannot be reduced by keeping safety distances to surface water during application, e. g. 20 m in low-growing crops and 30 m or 50 m in tall-growing crops, (table B.8.2.5-1).

The calculated values for the relationship between acute toxicity and short-term exposure (TERst) of 0.1 - 20 fall considerable below the trigger value of 100 after annex VI of the guideline 91/414/EEC.

With respect to the range of DT50 from 1-10 days in surface water a TERsw calculation with a DT50 of 1.2 days is given for the lowest application rate of 0.196 kg as/ha for mustard in table B.8.2.5-1. The time-weighted TERst of 28.5 show that the influence of shorter half-live time is only marginal.

Results from field studies confirm the damage of aquatic invertebrates, especially of crustaceans and caddy-fly larvae.

Table B.8.2.5-1: Short-term exposure ratios (TERst) for aquatic organism (Daphnia magna: 48-h EC50 1.1 µg/l) for selected realistic worst case, single application rates in different crops and longest distances from surface water, calculated with a DT50 of 4 d according to the German drift model with an water depth of 30 cm.

Crop	Application g as/ha	PEC (µg/l) overspray	Buffer zone (m)	PEC (µg/l)	TERst	PEC-TWA° (µg/l)	TERst
forest	15,000	5,000	50	10.0	0.1	8.4	0.1
citrus	3,000	1,000	50	2.0	0.5	1.7	0.6
olive	1,800	600	50	1.2	0.9	1.0	1.0
orchards	1,500	500	50*	1.0	1.1	0.8	1.3
grapes	1,000	333	30	0.6	1.6	0.5	1.9
cotton	1,000	333	20	0.3	3.3	0.2	3.9
potatoe	700	233	20	0.2	4.7	0.2	5.6
tomatoe	500	166	20	0.1	6.6	0.1	7.8
soya	400	133	20	0.1	8.3	0.1	8.3
tobacco	380	126	20	0.1	8.7	0.1	10.3
rape	294	98	20	0.09	11.2	0.08	13.3
mustard	196	65	20	0.06	16.8	0.05	20.0
mustard	196	65	20	0.06	16.8	0.04	28.5*

°: time weighted average for the first two days
*: time weighted average for the first two days (DT50 1.2 d)

B.8.3 Effects on other terrestrial vertebrates (Annex IIIA 10.3)

B.8.3.1 Toxicity to mammals (Annex IIIA 10.3)

The acute oral toxicity of azinphos-methyl was determined for rats, mice, dogs and guinea pigs. The lowest LD50 value is 4.4 mg as/kg bw (see section B.5).

Besides data on laboratory species there are also some data on wild mammals available.

Mount et al. 1988

The 5-day-dietary toxicity of technical azinphos-methyl was tested in deer mice according to EPA guideline no. 71-3. The test material was mixed into the feed at concentrations of 0/312/625/1250/2500/5000 ppm. Effective concentrations ranged between 87 % and 96 % of nominal values. For each concentration level groups consisting of 5 males and 5 females were treated. The LC50 is about 5000 ppm; the lowest lethal concentration was 1250 ppm; a NOEC could not be determined as already in the 312 ppm group there was an effect on body weight and food consumption.

Meyers and Wolff 1994
In a comparative study the toxicity of technical azinphos-methyl was tested in adult laboratory mice, house mice, deer mice and gray-tailed voles. For each species the single dose oral LD50 was determined. With three species also a 10-day-dietary toxicity test was conducted. LC50's were calculated after 5 and 10 days of treatment (table B.8.3.1-1).

Table B.8.3.1-1: 10-day-dietary toxicity test with different mammalian species (LC50 calculated after 5 and 10 days)

	LD50 mg/kg bw	5-day-LC50 ppm	10-day-LC50 ppm
Laboratory mouse	11	543	277
Wild house mouse	10	-	-
Deer mouse	48	2425	1180
Gray-tailed vole	32	406	297

Activity of brain-AChE in dead animals was typically inhibited by about 50 %.

The LD50 for mule deer is reported to be 32-64 mg as/kg bw (Hudson et al. 1984).

In conclusion the acute oral toxicity of azinphos-methyl to mammals is high with the lowest LD50 being 4.4 mg as/kg bw. The subacute dietary toxicity is moderate (lowest 5-day-LC50 406 ppm). Regarding the long-term risk the assessment will be based on a NOEL of 5 ppm that was determined in a 4-week-subchronic study with rats (see section B.5).

B.8.3.2 Toxicity to other terrestrial vertebrates (Annex IIIA 10.3)

Hall and Clark 1982
The acute oral toxicity of technical azinphos-methyl was tested in green anole lizards (Anolis carolinensis). The test material was dissolved in corn oil and intubated at dose levels of 0/18/30/50/83/139 mg as/kg bw. Five field-caught animals were treated per dose level. The LD50 is 98 mg as/kg bw. The lowest dose causing mortality was 83 mg as/kg bw. The lowest dose causing significant brain AChE inhibition was 30 mg as/kg bw.

B.8.3.3 Other studies (Annex IIIA 10.3)

Custer and Mitchell 1987
In Texas (USA) azinphos-methyl was aerially applied in sugarcane and cotton fields at rates of 0.28-1.21 kg as/ha. White-footed mice and spiny pocket mice were captured for brain AChE assay. The enzyme activity was not different from control. No wildlife was found dead.

B.8.3.4 Risk assessment for mammals

Mammals may be exposed to azinphos-methyl mainly by the consumption of contaminated feed. The highest exposure will be for herbivorous species.

The initial residue on herbs is estimated according to Hoerger and Kenaga (1972). In orchards it is assumed that half of the applied rate reaches the vegetation on the ground. The use in grapes (max. 1 kg as/ha) is covered by the consideration of orchards. This leads to PEC-values between 22-23 mg as/kg fresh weight on herbs (table B.8.3.4-1).

In order to consider the worst case condition it is assumed that mammals feed exclusively on contaminated material and that a medium-sized grazing species (e.g. hare) has a daily feed demand of 25 % of its body weight.

Table B.8.3.4-1: Exposure assessment for mammals

Use	Max. appl. rate (kg as/ha)	Feed	Typical maximum residue[1] (mg/kg)	Estimated initial residue (mg/kg)	Rel. feed demand (%)	Maximum daily intake (mg/kg bw)
Arable crops	0.7	Herbs	31*R	22	25	5.4
Orchards	1.5	Herbs	31*R/2	23	25	5.8

[1]according to Hoerger and Kenaga (1972); R=application rate in kg/ha

Comparison of the acute toxicity (4.4 mg as/kg bw) with the maximum daily intake gives an acute toxicity/exposure ratio of about 0.8. Comparison of the LC50 from the 5-day-dietary feeding studies (406 ppm) with the estimated initial residues results in a short-term toxicity/exposure ratio of 17. The acute toxicity/exposure ratio indicates a high risk; however, the kind of exposure under practical conditions is better simulated by feeding studies. Thus the short-term toxicity/exposure ratio is more relevant and this indicates a moderate risk. There is one field study with mammals that indicates a low risk. However, as only two murid species were included in this study the result should not be overtaxed.

Regarding the long-term risk the initial residues are above the NOEC from the reproduction study. Therefore a more realistic exposure assessment is tried. It is assumed that the half-life for vegetation is about 3 days. With two applications 2 weeks apart the average residue over a period of 4 weeks is then 30 % of the initial

residue, that is 23 · 0.30 = 7 mg as/kg for herbs. (Considering 3 applications 2 weeks apart and a period of 6 weeks would not change this figure very much). If assuming that on the long-term a mammal covers 25 % of its feed requirement from the treated area, the average contamination of the feed amounts to 7 · 0.25 = 1.75 mg as/kg. The quotient NOEC/PEC then is 5/1.75 = 2.9. This figure formally does not reach the Annex VI trigger of 5. However, the NOEC in the 28-day study in rats is based on a very sensitive parameter (blood-ChE was inhibited at 20 ppm) and is distinctly below the concentration where the fitness is affected. So the ratio of 2.9 can be regarded as tolerable. It appears that in the case of azinphos-methyl the acute risk is of more concern than the long-term risk.

In conclusion, although the acute toxicity/exposure ratio does not meet the criteria provided in Annex VI of Directive 91/414/EEC the risk to mammals under normal use conditions is considered as tolerable. However, the margin of safety is small and risk assessments should be carefully conducted when authorizing products containing azinphos-methyl.

B.8.4 Effects on bees (Annex IIA 8.3.1, Annex IIIA 10.4)

To fulfil the requirements of Annexes II and III the results of one LD50-test (contact and oral) has been submitted using formulated azinphos-methyl (99 % purity). This study is considered appropriate because the formulated material is the most relevant in terms of bee exposure on field use. The study submitted is not in compliance with the principles of GLP.

The risk assessment is based on the uses and nominal field rates outlined in this monograph. Further studies that are considered as not valid or not appropriate or do not further contribute to the decision-making process are disregarded below.

B.8.4.1 Acute toxicity (Annex IIA 8.3.1, Annex IIIA 10.4.1)

The acute contact toxicity of formulated azinphos-methyl (Guthion) was reported by Davies (1987). The laboratory test was carried out in accordance with the procedures outlined in the Pesticide Safety Precaution Scheme Working Document D3. In this experiment at least five doses were given to four replicates of ten bees. The median lethal dose LD50 was calculated using a Probit Analysis (GLIM, 1977 Royal Statistical Society, London). In this test Guthion was used as a toxic standard, thus precise data on the dose response relationship are not available.

Table B.8.4.1-1: Acute toxicity of formulated azinphos-methyl to honeybees, laboratory test

Exposure	Effects azinphos-methyl LD50 [µg/bee]	Reference

con	0.1 24h	(1)
ora	0.1 24h	

Exposure: uptake via: con = 1-2 µl drop topically applied to the thorax (ventral, compound dissolved in acetone); ora = oral administration in aqueous 20 % sucrose solution (0.2 ml to groups of ten bees)
Effects: LD50 µg as per bee (evaluation according to ICPBR)
>100 = non-toxic; 10-100 = slightly toxic; <10 = toxic
Reference: (1) Davies (1987) BIE 96-00021

B.8.4.1.1 Bee brood feeding test (Annex IIA 8.3.1.2)

Not required.

B.8.4.2 Cage tests (Annex IIIA 10.4.3)

Not submitted.

B.8.4.3 Field tests (Annex IIIA 10.4.4)

Not submitted.

B.8.4.4 Residue test (Annex IIIA 10.4.2)

Not submitted.

B.8.4.5 Risk assessment for honeybees

Honeybees are likely to be exposed to formulated azinphos-methyl by direct spray, contact on fresh or dry residues or by oral uptake of contaminated pollen, nectar and honey dew.

Plant protection products containing azinphos-methyl are used in agriculture, horticulture (field and protected crops), citrus and viticulture. Horticulture comprises growing of fruit, ornamentals and vegetables.

Table B.8.4.5-1: Field rates for formulated azinphos-methyl (examples for approved uses)

Crop		Exposure			
		Field rate [g as/ha]	No.	PIEC Soil [g as/ha]	PIEC Plant [g as/ha]
Agriculture - field crops	WP 25	700	2	700	700
Horticulture - tree fruit	WP 25	1500	5	750	600
Horticulture - viticulture	WP 25	1000	3	500	400

Field rate: maximum nominal field rate [g as/ha] of formulation
No.: Number of applications per season
PIEC: predicted initial environmental concentration (see SETAC-

GUIDANCE DOCUMENT ON REGULATORY TESTING PROCEDURES FOR PESTICIDES WITH NON-TARGET ARTHROPODS)

Table B.8.4.5-2: Risk assessment according to the EPPO scheme for azinphos-methyl, eg tree fruit

Exposure Route	Effects LD50 [µg/bee]	Hazard quotient	Reference
con	0.1 24h	7500 24h	(1)
ora	0.1 24h	7500 24h	

Exposure: uptake via: con = 1-2 µl drop topically applied to the thorax (ventral, compound dissolved in acetone); ora = oral administration in aqueous 20 % sucrose solution (0.2 ml to groups of ten bees)
Effects: LD50 µg as per bee (evaluation according to ICPBR)
>100 = non-toxic; 10-100 = slightly toxic; <10 = toxic
Hazard quotient: HQ = field rate [g as per ha] / LD50 [µg as per bee] (according to the EPPO risk assessment scheme)
Reference: (1) Davies (1987) BIE 96-00021

Concerning azinphos-methyl the hazard quotient values are > 50 indicating a high toxicity for both, the oral and the contact route according to the EPPO/CoE risk assessment scheme.

B.8.5 Effects on other arthropod species (Annex IIA 8.3.2, Annex IIIA 10.5)

The results presented below are considered as valid. The risk assessment is based on the uses and nominal field rates outlined in this monograph. Further studies considered as not valid or not appropriate or do not further contribute to the decision-making process are disregarded below.

B.8.5.1 Acute toxicity (Annex IIA 8.3.2, Annex IIIA 10.5.1)

Tests on the side-effects of formulated azinphos-methyl to the parasitoid Trichogramma cacoeciae (Hymenoptera: Trichogrammatidae), the predatory mites Amblyseius potentillae, A. finlandicus and Typhlodromus pyri (Acari: Phytoseiidae), the plant dwelling predators Chrysopa carnea (Neuroptera: Chrysopidae) and Syrphus vitripennis (Diptera: Syrphidae) and the ground dwelling predator Bembidion lampros (Coleoptera: Carabidae) were conducted according to IOBC-Guidelines (EPPO Bulletin 15, 214-255, 1985). The choice of species is in agreement to the SETAC-GUIDANCE DOCUMENT ON REGULATORY TESTING PROCEDURES FOR PESTICIDES WITH NON-TARGET ARTHROPODS as laid down in Directive 91/414/EEC. These studies do not comply with GLP.

Table B.8.5.1-1: Acute toxicity of formulated azinphos-methyl (25 % as) to beneficial arthropods, basic

laboratory tests

Species	Exposure: Test substance	%	dose [g as/ha]		Effects: lethal(sublethal) [%]	Reference
T. cacoeciae (a)	25 WP	0.2	100	con	100	(1)
A. potentillae(c)	25 WP	0.2	100	con	100	(1)
T. pyri (c)	25 WP	0.2	100	con	100	(1)
C. carnea (l)	25 WP	0.2	100	con	100	(1)
S. vitripennis(l)	25 WP	0.2	75	con	100	(1)
B. lampros (a)	25 WP	0.2	300	con	100	(1)

Species: exposed developmental stage of the test species in the test (a = adult; c = lifecycle; e = egg; l = larvae; n = nymph; p = pupae)
Exposure: Exposure via different routes, con = contact; dip = dipping; ora = oral uptake; spr = direct spray; duration
Dose: calculated in g as/ha as specified in the relevant guidelines, concentration of the product tested [%]
Effects: Mortality and sublethal effects (in brackets) (classification of IOBC adapted to EPPO requirements):
<30 % = low risk; 30-80 % = medium risk; >80 % = high risk
Reference: (1) Hassan et al. (1987) ANA 96-00031

The acute toxicity of Gusathion (25 WP) was determined in laboratory tests for different ecological groups exposed. The lethal and sublethal effects found in the laboratory amount to 100 % thus indicating a high toxicity of the compound tested for species of these groups.

Table B.8.5.1-2: Acute toxicity of formulated azinphos-methyl to T. cacoeciae, semi-field test on persistence

Species	Exposure: Test substance	Dose	Crop		Effects: Duration [days]	Reference
T. cacoeciae (a)	25 WP	0.2	run-off vine	con	> 30	(1)

Species: exposed developmental stage of the test species in the test (a = adult; c = lifecycle; e = egg; l = larvae; n = nymph; p = pupae)
Exposure: Exposure via different routes, con = contact; dip = dipping; ora = oral uptake; spr = direct spray; duration
Dose: concentration tested [%], substrat used
Effects: Duration of harmful effects (classification according to IOBC): < 5 days = short lived; 5-15 days = slightly persistent; 16-30 days = moderately persistent; > 30 days = persistent
Reference: (1) Hassan et al. (1987) ANA 96-00031

The semi-field test does not represent a worst case situation (table B.8.5.1-2). However, this study indicates a high level of persistence with regards to the duration of harmful effects on the parasitoid wasp T. cacoeciae (Hymenoptera: Trichogrammatidae) in field situations. High risk for other non-target species of comparable sensitivity cannot be ruled out.

B.8.5.2 Field tests (Annex IIIA 10.5.2)

Further tests on the side-effects of formulated azinphos-methyl (Gusathion, 25 % as) to the predatory mites Amblyseius finlandicus and Typhlodromus pyri (Acari: Phytoseiidae) were conducted on field conditions. The studies do not comply with GLP.

Table B.8.5.2-1: Toxicity of formulated azinphos-methyl (25 % WP) to predatory mites, field-test

Species	Exposure: Test substance	%	Dose	Crop	Effects: lethal(sublethal) [%]	Reference
A. finlandicus (c)	25 WP	0.2	run-off	cherry	>75	(1)
T. pyri (c)	25 WP	0.2	run-off	vine	>75	(1)

Species: exposed developmental stage of the test species in the test (a = adult; c = lifecycle; e = egg; l = larvae; n = nymph; p = pupae)
Dose: concentration tested [%], crop
Effects: Abundance (calculation according to Abbott; adapting IOBC classification to EPPO):
<25 % = low risk; 25-50 % = medium risk; >50 % = high risk
Reference: (1) Hassan et al. (1987) ANA 96-00031

B.8.5.3 Risk assessment for beneficial arthropods

Beneficial arthropods may be exposed to formulated azinphos-methyl by direct spray, contact on fresh or dry residues or by oral uptake of contaminated prey, nectar and honey dew or via host organisms.

Plant protection products containing azinphos-methyl are used in agriculture, horticulture (field and protected crops), citrus and viticulture. Horticulture comprises growing of fruit, ornamentals and vegetables.

Table B.8.5.3-1: Field rates for formulated azinphos-methyl (examples for approved uses)

Crop	Field rate [g as/ha]		No.	Exposure: PIEC Soil [g as/ha]	PIEC Plant [g as/ha]
Agriculture - field crops	WP 25	700	2	700	700

Horticulture - tree fruit	WP 25	1500	5	750	600
Horticulture - viticulture	WP 25	1000	3	500	400

Field rate: maximum nominal field rate [g as/ha] of formulation
No.: Number of applications per season
PIEC: predicted initial environmental concentration (see SETAC-GUIDANCE DOCUMENT ON REGULATORY TESTING PROCEDURES FOR PESTICIDES WITH NON-TARGET ARTHROPODS)

Parasitoids
Parasitization capacity of the parasitoid T. cacoeciae (Hymenoptera: Trichogrammatidae) exposed to dried deposits of Gusathion (100 g as/ha) was reduced by 100 %. The semi-field test indicates a high level of persistence with regards to the duration of harmful effects on T. cacoeciae (Hymenoptera: Trichogrammatidae) in field situation. According to the EPPO/CoE risk assessment scheme risk is classified 'high risk'.

Predatory mites
Tests on the side-effects of formulated azinphos-methyl to the predatory mites Amblyseius potentillae and Typhlodromus pyri (Acari: Phytoseiidae) indicated a high sensitivity of these species in basic laboratory tests. These findings are approved in field tests, where abundance of A. finlandicus and T. pyri was reduced by > 75 %. According to the EPPO/CoE risk assessment scheme risk is classified 'high risk'.

Ground dwelling predators
In laboratory tests on the side-effects of formulated azinphos-methyl to the ground dwelling predator Bembidion lampros (Coleoptera: Carabidae) dried deposits of the product proved to be harmful increasing mortality of the beetle to 100 %. According to the EPPO/CoE risk assessment scheme risk is classified 'high risk'.

Plant dwelling predators
Basic laboratory tests on the side-effects of formulated azinphos-methyl to larvae of the plant dwelling predators Chrysopa carnea (Neuroptera: Chrysopidae) and Syrphus vitripennis (Diptera: Syrphidae) indicate a high toxicity of the product. Larval mortality of these species increased to 100 % when exposed on dried deposits. According to the EPPO/CoE risk assessment scheme risk is classified 'high risk'.

Summary
With regards to the effects found for the species tested all of the intended uses of products containing azinphos-methyl outlined in this monograph pose a 'high risk' to 'parasitoids', 'predatory mites', 'plant dwelling predators' and 'ground dwelling predators' according to the EPPO/CoE risk assessment scheme.

B.8.6 Effects on earthworms (Annex IIA 8.4; Annex IIIA 10.6.1)

The results presented below are considered valid. Further studies that are not considered valid, not appropriate or do not further contribute to the decision making process are disregarded below. The risk assessment is based on the use pattern and dosage outlined in this monograph. For a better comparison of the data all results are expressed in mg as/kg dry weight substrate.

B.8.6.1 Acute toxicity (Annex II 8.4.1; Annex III 10.6.1.1)

The acute toxicity of the active substance was tested according to OECD-guideline no. 207 (table B.8.6.1-1). The LC50 of the active substance was 59 mg as/kg. There were effects on body weight at 10 mg as/kg, the lowest concentration tested. A decrease in body weight of about 30 % compared to control was observed at this concentration.

Table B.8.6.1-1 Acute toxicity to earthworms

Test substance	Species	LC50 [mg as/kg dry wt substrate]	NOEC	LOEC	Ref.
as	Eisenia fetida	59	< 10	10	(1)

Ref.: (1) Heimbach, 1986, ARW 95-00089

B.8.6.2 Other studies

The effects on reproduction were tested in the laboratory using a 200 EC formulation (table B.8.6.2-1).

Table B.8.6.2-1 Effect of Gusathion M EC 200 on reproduction

Application rate [kg as/ha]	Mortality [%]	Number of Juveniles/ test box	Weight [% of initial weight]
Control	0	203.5 ± 42.8	158.9 ± 17.9
1.0 kg as/ha	0	55.8 ± 22.4 *	106.6 ± 12.5 *
1.5 kg as/ha	0	35.0 ± 4.8 *	93.2 ± 9.8 *
6.0 kg as/ha	3 ± 5	7.3 ± 1.7 *	58.6 ± 2.9 *

* sign. $p < 0.05$; Williams-test

Ref.: Heimbach, 1995, ARW 95-00090

In this test the lowest concentration resulted in a decrease of juveniles to 27 % of the control values. The number of juveniles

found was highly reduced in all concentrations tested. Additionally there was an effect on body weight of the adults. Body weight decreased in all application rates tested. The reduction amounted to 67 % of the control values in 1.0 kg as/ha, the lowest concentration tested.

A field study was done on a 200 EC formulation with a 1-fold rate of 2 x 1.5 kg as/ha and a 4-fold rate of 2 x 6.0 kg as/ha applied on grassland (Heimbach, 1988, ARW 95-00091). The effects on earthworm populations were evaluated 6 weeks after application, half a year later in autumn and one year later in spring of the following year.

After six weeks there was an overall reduction in abundance to 68 % of the control values in the 1-fold rate and to 30 % of the control values in the 4-fold rate. On the species level some species were even more affected than the overall reduction showed. Half a year later the reduction was lower than after 6 weeks in the 1-fold rate, but still amounted to 44 % of the control value in the 4-fold rate. In the 1-fold rate there was no overall reduction and an effect of about 80 % of the control values on two species was not significant. Some species showed an increase in abundance. One year after the application there was no overall reduction in the 1-fold rate, but an increase of the tanylobous species and a reduction of the epilobous species of 20 to 35 % of the control which was not significant. In the 4-fold rate a reduction to 76 % of the control values was found. This reduction was not significant. The reduction on the species level amounted up to 54 % of the control values, which was also not significant.

These data demonstrate that effects on populations of earthworms are present, but due to the general problems of field studies, e.g. low numbers of some species and high patchiness the effects may be high but not statistically significant and it is not clear whether the effects are related to the chemical tested.

B.8.6.3 Risk assessment for earthworms

For risk assessment the lowest LC50 and NOEC from both studies is used (table B.8.6.1-1). According to the EPPO risk assessment scheme the toxicity data from laboratory tests in artificial soil are divided by the factor of two when logPow > 2, to take into account the organic matter content of the artificial soil substrate. For chemicals with log Pow > 2 sorption is expected to be linearly related to soil organic matter content.

Based on an application of 1.5 kg as/ha (apple) according to table 1.5.3-2 the short-term PEC amounts to 1.1 mg as/kg substrate for one application (assumptions: 5 cm soil depth, 50 % of applied amount reaches the soil surface). For 3.0 kg as/ha (citrus) the PEC amounts to 2.1 mg as/kg.

For one application the calculation of the short-term toxicity/exposure ratio (LC50/PEC) leads to a factor of 27 (29.5/1.1) for apples. The factor for short-term NOEC/PEC is < 4.5 (< 5/1.1). For citrus the ratio LC50/PEC leads to a factor of 14 (29.5/2.1). The factor for short-term

NOEC/PEC amounts to < 2.4 (< 5/2.1).

Up to five applications in one season can be done in apple. For these applications with 5 x 1.5 kg as/ha exposure estimation according to the EPPO risk assessment scheme leads to a PEC of 3.2 mg as/kg. The toxicity/exposure ratio LC50/PEC amounts to 9.2 (29.5/3.2).

A reproduction test was done with 1.0, 1.5 and 6.0 kg as/ha. This amount was fully applied to the soil surface. There was an effect on juvenile numbers and on body weight development of adults at the lowest concentration tested. Therefore the NOEC was lower than 1.0 kg as/ha, the lowest concentration tested. According to the EPPO risk assessment scheme the toxicity data should be divided by a factor of two when using artificial soil as test substrate. NOEC then is lower than 0.5 kg as/ha. To compare the NOEC given in kg as/ha with PEC values given in mg as/kg substrate, the NOEC is recalculated to a soil concentration for 5 cm soil depth according to EPPO to < 0.72 mg as/kg dry wt substrate. The long-term NOEC/PEC ratio using a long-term PEC (100 days) of 1.2 mg as/kg (see chapter B.7.3, table B.7.3-4) for 5 applications in apple with 1.5 kg as/ha per application amounts to < 0.6 (< 0.72/1.2). Using a long-term PEC of 0.57 mg as/kg for 365 days (see table B. 7.3-4) the factor amounts to < 1.75. As in the reproduction test the product is applied on the soil surface without any plant cover, the exposure and TER estimation is a worst case assumption. Assuming a plant cover of 50 % and therefore reducing the PEC values by 50 % does not change the order of magnitude in the risk assessment.

From these comparisons of toxicity data and exposure a high risk concerning effects on earthworms is given. The acute toxicity/exposure ratio and the ratio NOEC reproduction/long-term exposure for several applications are in the order of magnitude or below the acceptable trigger for the toxicity/exposure ratios provided in Annex VI of Directive 91/414/EEC.

A field test underlines that on the given field conditions a risk for earthworm species is present. This effect was biggest at the sampling date 6 weeks after application and included several species. It was probably still present one year after application in some species. This field test shows that the effects in the field might not be as high as predicted from the laboratory data, but it also shows that under realistic exposure situations an effect on earthworm populations is probable. The highest rate tested in the field was 2 x 6.0 kg as/ha whereas the highest intended use amounts to 5 x 1.5 kg as/ha. The lower application rate tested in the field was 2 x 1.5 kg as/ha. This is in a range of several intended uses. As an effect in this range of application is probable and no more data on this application rate in the field exist, more than 1.0 kg as/ha has to be judged critical. Comparing this value with the NOEC from the laboratory reproduction test, the laboratory NOEC was lower than 1.0 kg as/ha. Taking into account that the laboratory data are a worst case assumption, a critical application rate of 1.0 kg as/ha is assumed to be justified.

The conclusion from the data available is that depending on the application rate there is a high risk for earthworms.

B.8.7 Effects on other soil non-target macro-organisms (Annex IIIA 10.6.2)

Concerning effects on other soil non-target macro-organisms no data are available.

B.8.8 Effects on soil non-target micro-organisms (Annex IIA 8.5; Annex IIIA 10.7)

Laboratory tests were performed to examine the effect of azinphos-methyl on microbial activities in soil. The tests were carried out with the active substance in 1986.

B.8.8.1 Carbon conversion (Annex IIA 8.5; Annex IIIA 10.7)

The effects on long-term respiration were tested in two different soils (loamy sand and sandy silt) with 0.8 kg as/ha and 8.0 kg as/ha.

Table B.8.8.1-1: Results of long-term respiration

type of soil	loamy sand		sandy silt	
application rate in kg as/ha	single 0.8	ten-fold 8.0	single 0.8	ten-fold 8.0
with lucerne meal				
in % to untreated:	104	103	99	109
influence tolerable:	yes	yes	yes	yes
after days:	28	28	28	28
without lucerne meal				
in % to untreated:	96	91	103	103
influence tolerable:	yes	yes	yes	yes
after days:	28	28	28	28

Ref.: (1) Anderson, J.P.E., 1986, BMF 95-00055

B.8.8.2 Nitrogen conversion (Annex IIA 8.5; Annex IIIA 10.7)

Nitrification was tested in two different soils (loamy sand and sandy silt) with the single rate of application (0.8 kg as/ha) and the ten-fold rate (8.0 kg as/ha). Compared with the untreated component the results showed no negative effects after a test period of 28 days and also of 56 days.

Table B.8.8.2-1: Results of nitrification

type of soil	loamy sand		sandy silt	
application rate in kg as/ha	single 0.8	ten-fold 8.0	single 0.8	ten-fold 8.0
nitrification				
in % to untreated:	100	101	98	99
after days:	28	28	28	28
in % to untreated:	95	96	98	90
after days:	56	56	56	56
influence tolerable:	yes	yes	yes	yes

Ref.: (2) Anderson, J.P.E., 1986, BMF 95-00056

B.8.8.3 Risk assessment for soil micro-organisms

The results presented show that on microbial activities no negative effects are to be expected when applying azinphos-methyl according to the amounts of intended uses.

B.8.9. Effects on other non-target organisms (flora and fauna) believed to be at risk (Annex IIA 8.6)

No additional data are submitted. It must be assumed that other species are affected than those which have been tested, because the active substance azinphos-methyl inhibits the acetylcholinesterase.

B.8.10 Effects on biological methods of sewage treatment (Annex IIA 8.7)

Due to the low bacteriostatic potential it can be assumed from studies concerning soil micro-organisms that the expected effect is minimal.
A study concerning possible effects on biological sewage treatment processes is available according to ISO/ETAD/OECD-guideline no. 209 with an EC50 of >10 g as/l (Kanne, 1988, WAT96-50149).
The water/sediment study showed that azinphos-methyl is rapidly degradable, because the active substance was degraded with more than 70 % in 28 days from the system (according to Directive 91/325/EEC).

B.8.11 References relied on

Annex point(s) (91/414/EEC);	year. author(s). title. source. report number registration number.	GLP GEP Y/N	publ. Y/N	owner	data prot
EG:AIIA-8.1.1	1984. Hudson, R.H., Tucker, R.K. and Haegele, M.A. Handbook of toxicity of pesticides to wildlife. USDI Fish Wildl Service, Resource Publ, 153, 1984. AVS96-00071.	N	Y		
EG:AIIA-8.1.1	1972. Schafer, E.W. The acute oral toxicity of 369 pesticidal, pharmaceutical and other chemicals to wild birds. Toxicol Appl Pharmacol, 21, 1972, 315-330. AVS96-00069.	N	Y		
EG:AIIA-8.1.1	1987. Stubblefield, W.A. Guthion (technical grade) - Acute LD50 to bobwhite quail. 87-015-01. AVS95-00142.	Y	N	BAY	
EG:AIIA-8.1.1; EG:AIIA-8.1.2	1967. Sherman, M., Herrick, R.B., Ross, E. and Chang, M.T.Y. Further studies on the acute and subacute toxicity of insecticides to chicks. Toxicol Appl Pharmacol, 11, 1967, 49-67. AVS96-00072.	N	Y		
EG:AIIA-8.1.2	1986. Hill, E.F. and Camardese, M.B. Lethal dietary toxicities of environmental contaminants and pesticides to Coturnix. USDI Fish Wildl Service, Fish Wildlife Technical Report, 2, 1986. AVS96-00078.	N	Y		
EG:AIIA-8.1.2	1975. Hill, E.F., Heath, R.G., Spann, J.W. and Williams, J.D. Lethal dietary toxicities of	N	Y		

Annex point(s) (91/414/EEC);	year. author(s). title. source. report number registration number.	GLP GEP Y/N	publ. Y/N	owner	data prot
	environmental pollutants to birds. USDI Fish Wildl Service, Special Scientific report, 191, 1975. AVS96-00079.				
EG:AIIA-8.1.3	1988. Beavers, J.B., Marselas, G. and Jaber, M.J. Guthion technical insecticide: A one-generation reproduction study with the bobwhite (Colinus virginianus). PROJ.NO.149-140A. AVS95-00144.	Y	N	BAY	
EG:AIIA-8.1.3	1989. Grace, T.J. and Toll, P.A. Effects of technical guthion on mallard duck reproduction. 98328-1. AVS95-00145.	Y	N	BAY	
EG:AIIA-8.1.3	1988. Toll, P.A. Effects of technical Guthion on mallard duck reproduction. 87-675-03. AVS95-00143.	Y	N	BAY	
EG:AIIA-8.2	1988. Kanne Gusathion M (Azinphos-methyl), 92,4 % PT. KEINE ANGABE. WAT96-50162.	N	N	BAY	
EG:AIIA-8.2.1	1984. Carlisle, J.C. Acute toxicity of Azinphos-Methyl (Guthion) technical to Rainbow trout. BAYER FILE NO: 491. WAT95-50208.	N	N	BAY	
EG:AIIA-8.2.1	1978. Hermann, G. Fish Toxicity, R 1582 (Gusathion active ingredient), Rainbow trout. FF-52. WAT95-50206.	N	N	BAY	

Annex point(s) (91/414/EEC);	year. author(s). title. source. report number registration number.	GLP GEP Y/N	publ. Y/N	owner	data prot
EG:AIIA-8.2.1	1979. Hermann, G. Fish Toxicity, Azinphos-methyl, Golden Orfe. FO-236. WAT95-50207.	N	N	BAY	
EG:AIIA-8.2.2	1988. Surprenant, D.C. The toxicity of technical grade Azinphos-methyl (trade name Guthion) to Rainbow trout (Salmo gairdneri) Embryos and Larvae. BAYER FILE NO: 95662. WAT95-50209.	Y	N	BAY	
EG:AIIA-8.2.5	1980. Lamb, D.W. Azinphos-methyl (Guthion) technical - acute toxicity to Daphnia magna. BAYER FILE NO: 66085. WAT95-50210.	N	N	BAY	
EG:AIIA-8.2.6	1984. Carlisle, J.C. Chronic toxicity of 14C-Guthion to Daphnia magna under flow- through test conditions. BAYER FILE NO: 558. WAT95-50211.	Y	N	BAY	
EG:AIIA-8.2.7	1985. Heimbach, F. Growth inhibition of green algae (Scenedesmus subspicatus) by Azinphos-methyl (Technical). BAYER FILE NO: HBF/AL 10. WAT95-50212.	N	N	BAY	
EG:AIIA-8.3.1	1987. Davies, L.G. Report on a laboratory investigation into the toxicity of HWG to honey bees (Apis mellifera). BAYER 8701. BIE96-00021.	N	N	BAY	

Annex point(s) (91/414/EEC);	year. author(s). title. source. report number registration number.	GLP GEP Y/N	publ. Y/N	owner	data prot
EG:AIIA-8.3.2	1989. Eichhorn, K.W. Ergebnisse der Prüfung von Präparaten gegen Raubmilben 1989. BAYER 890777. ANA96-00037.	N	N	BAY	
EG:AIIA-8.3.2	1983. Englert, W.D. Untersuchungen zur Auswirkung von Pflanzenschutzmitteln auf Raubmilben im Weinbau. 12/81. ANA96-00033.	N	N	BAY	
EG:AIIA-8.3.2	1987. Hassan, S.A. et al. Results of the third joint pesticide testing programme by the IOBC/WPRS-Working Group "Pesticides and Beneficial Organisms". j. Appl. Ent., 103, 1987, 92-107. 03. ANA96-00031.	N	Y		
EG:AIIA-8.3.2	1986. Heimbach, F. Acute toxicity of azinphos-methyl (technical) to earthworms. HBF/RG 58. ARW95-00089.	N	N	BAY	
EG:AIIA-8.3.2	1990. Holst, H. Versuchsbericht für die Prüfung im Zulassungsverfahren. Auswirkung von Pflanzenschutzmitteln auf Raubmilben im Weinbau. BAYER 900574. ANA96-00039.	N	N	BAY	
EG:AIIA-8.3.2	1990. Lipps, H.P. Versuchsbericht der Prüfstelle KH für die Prüfung im Zulassungsverfahren. Auswirkung auf Raubmilben. BAYER 900649. ANA96-00041.	N	N	BAY	

Annex point(s) (91/414/EEC);	year. author(s). title. source. report number registration number.	GLP GEP Y/N	publ. Y/N	owner	data prot
EG:AIIA-8.3.2	1990. Louis, F. Ergebnisse der Prüfung von Präparaten gegen Raubmilben 1990. BAYER 900676. ANA96-00043.	N	N	BAY	
EG:AIIA-8.3.2	1989. Mader, H. Prüfbericht im Zulassungsverfahren der amtlichen Mittelprüfung 1989 auf Raubmilbenbeeinflussung (Typhlodromus pyri). BAYER 890734. ANA96-00035.	N	N	BAY	
EG:AIIA-8.3.2	1983. Schropp, A. Results from laboratory and field testing on the influence of different fungicides and insecticides on predatory mites (E 605 500 EC / E 120 40 WP / & E 1582 25 WP & E 158 7,5). N176. ANA96-00027.	N	N	BAY	
EG:AIIA-8.3.2	1984. Zoebelein, G. Effects of Gusathion M 25 WP on the predatory mite Phytoseiulus persimilis. ZOE/PP 6/84. ANA96-00029.	N	N	BAY	
EG:AIIA-8.3.4	1986. Anderson, J.P.E. Influence of Gusathion M (azinphos-methyl) on the Microbial Mineralization of Carbon in Soils. AJO/21286. BMF95-00055.	N	N	BAY	
EG:AIIA-8.3.4	1986. Anderson, J.P.E. Influence of Gusathion M (azinphos-methyl) on the microbial mineralization of nitrogen in soils. AJO/21586.	N	N	BAY	

Annex point(s) (91/414/EEC);	year. author(s). title. source. report number registration number.	GLP GEP Y/N	publ. Y/N	owner	data prot
	BMF95-00056.				
EG:AIIIA-10.1.3	1987. Custer, T.W. and Mitchell, C.A. Exposure to insecticides of brushland wildlife within the lower Rio Grande Valley, Texas, USA. Environmental Pollution, 45, 1987, 207-220. AVS96-00075.	N	Y		
EG:AIIIA-10.1.2	1959. Dahm, P.A., Gurland, J., Hibbs, E.T., Orgell, W.H., Pfaeffle, W.O.and Lee, I. Field sampling of alfalfa for the estimation of guthion residues. J Econ Entomol, 52, 1959, 791-798. AVS96-00080.	N	Y		
EG:AIIIA-10.1.2	1993. Graham, D.J. and DesGranges, J.-L. Effects of the organophosphate azinphos-methyl on birds of potato fields and apple orchards in Quebec, Canada. Agric Ecosystem Environ, 43, 1993, 183-199. AVS96-00074.	N	Y		
EG:AIIIA-10.1.2	1994. Lari, L., Massi, A., Fossi, M.C., Casini, S., Leonzio, C. and Focardi, S. Evaluation of toxic effects of the organophosphorus insecticide azinphos-methyl in experimentally and naturally exposed birds. Arch Environ Contam Toxicol, 26, 1994, 234-239. AVS96-00073.	N	Y		
EG:AIIIA-10.3	1989. Grace, T.J., Toll, P.A. Guthion technical: Subacute dietary LC50 to Deer mice. ID 98333-1. AVS95-00147.	N	N	BAY	
EG:AIIIA-10.3	1982.	N	Y		

Annex point(s) (91/414/EEC);	year. author(s). title. source. report number registration number.	GLP GEP Y/N	publ. Y/N	owner	data prot
	Hall, R.J. and Clark, D.R. Responses of the iguanid lizard Anolis carolinensis to four organophosphorus pesticides. Environ Pollut Ser A, 28, 1982, 45-52. AVS96-00076.				
EG:AIIIA-10.3	1994. Meyers, S.M. and Wolff, J.O. Comparative toxicity of azinphos-methyl to house mice, laboratory mice, deer mice and gray-tailed voles. Arch Environ Contam Toxicol, 26, 1994, 478-482. AVS96-00077.	N	Y		
EG:AIIIA-10.3	1988. Mount, D.R., Bergman, H. and Bobbitt, Guthion technical: Subacute dietary LC50 to deer mice. 98333. AVS95-00146.	Y	N	BAY	
EG:AIIIA-10.3.4	1989. Eichhorn, K.W. Ergebnisse der Prüfung von Präparaten gegen Raubmilben 1989. BAYER 890777. ANA96-00038.	N	N	BAY	
EG:AIIIA-10.3.4	1983. Englert, W.D. Untersuchungen zur Auswirkung von Pflanzenschutzmitteln auf Raubmilben im Weinbau. 12/81. ANA96-00034.	N	N	BAY	
EG:AIIIA-10.3.4	1987. Hassan, S.A. et al. Results of the third joint pesticide testing programme by the IOBC/WPRS-Working Group "Pesticides and Beneficial Organisms". j. Appl. Ent., 103, 1987, 92-107. 03. ANA96-00032.	N	Y		

Annex point(s) (91/414/EEC);	year. author(s). title. source. report number registration number.	GLP GEP Y/N	publ. Y/N	owner	data prot
EG:AIIIA-10.3.4	1990. Holst, H. Versuchsbericht für die Prüfung im Zulassungsverfahren. Auswirkung von Pflanzenschutzmitteln auf Raubmilben im Weinbau. BAYER 900574. ANA96-00040.	N	N	BAY	
EG:AIIIA-10.3.4	1990. Lipps, H.P. Versuchsbericht der Prüfstelle KH für die Prüfung im Zulassungsverfahren. Auswirkung auf Raubmilben. BAYER 900649. ANA96-00042.	N	N	BAY	
EG:AIIIA-10.3.4	1990. Louis, F. Ergebnisse der Prüfung von Präparaten gegen Raubmilben 1990. BAYER 900676. ANA96-00044.	N	N	BAY	
EG:AIIIA-10.3.4	1989. Mader, H. Prüfbericht im Zulassungsverfahren der amtlichen Mittelprüfung 1989 auf Raubmilbenbeeinflussung (Typhlodromus pyri). BAYER 890734. ANA96-00036.	N	N	BAY	
EG:AIIIA-10.3.4	1983. Schropp, A. Results from laboratory and field testing on the influence of different fungicides and insecticides on predatory mites (E 605 500 EC / E 120 40 WP / & E 1582 25 WP & E 158 7,5). N176. ANA96-00028.	N	N	BAY	
EG:AIIIA-10.3.4	1984. Zoebelein, G. Effects of Gusathion M 25 WP on the predatory mite Phytoseiulus persimilis. ZOE/PP 6/84.	N	N	BAY	

Annex point(s) (91/414/EEC);	year. author(s). title. source. report number registration number.	GLP GEP Y/N	publ. Y/N	owner	data prot
	ANA96-00030.				
EG:AIIIA-10.3.5	1988. Heimbach, F. Influence of Gusathion M 200 EC on the earthworm fauna of a grassland area. HBF/RGF 12. ARW95-00091.	N	N	BAY	
EG:AIIIA-10.3.5	1990. Heimbach, F. Toxicity of Gusathion MS to Earthworms. HBF/RG 121. ARW95-00088.	N	N	BAY	
EG:AIIIA-10.3.5	1995. Heimbach, F. Influence of Gusathion M EC 200 on the Reproduction of Earthworms (Eisenia fetida). HBR/RG 206. ARW95-00090.	Y	N	BAY	

02

MONOGRAPH – ADDENDUM 3

11 May 1999

Azinphos-methyl

Volume 3

Annex B-8

Ecotoxicology

Rapporteur Member State: Germany

B.8.2 Effects on aquatic organisms

B.8.2.2 Prolonged and chronic toxicity of azinphos-methyl on aquatic organisms (Annex IIA 8.2.5)

extended laboratory study on *Daphnia magna:*

An additional laboratory study concerning the effects of azinphos-methyl on *Daphnia magna* in a static system was submitted. One week before the beginning of exposure sediment was added to the systems and three days before exposure a model population of 20 daphniae was added. The nominal concentrations tested reached from 0.2 to 12.8 µg a.i./l. The measured concentrations decreased from 30 % of the nominal concentration after 7 days to 7 % after 14 days. The abundances of the model populations in the lower concentrations (0.2 and 0.4 µg/l) did not differ significantly from the controls. Concentration of 0.8 µg/l and 1.6 µg/l lead to significant effects on the populations within 4 and 2 days, respectively. In 3.2 µg/l and higher all the daphniae died within 48 hours from exposure start. Within 14 days after the single application of azinphos-methyl no survival of added juveniles was possible.

The resulting NOEC of the study is 0.4 µg/l and the calculated EC_{50} 1.0 µg/l. These data were useful to confirm the results from the standard acute test (EC_{50}: 1.1 µg/l) and the standard reproduction test (NOEC of 0.25 µg/l). However, they did not demonstrate the possibility of recovery. Hence, the risk assessment using the results of testing acute toxicity with Daphnia magna proposed by the RMS within the monograph and by ECCO 47 is confirmed by the study.

Risk assessment

For a single application of azinphos-methyl (intended are 3 applications in total) in orchards and field and application rates of 1200 and 600 g a.i./ha, respectively, and using the EC_{50} resulting from the acute test evaluated with *Daphnia magna* (1.1 µg/l) the calculated TER-values were as follows:

distance	orchards 1.2 kg a.i./ha			field crops 0.6 kg a.i./ha		
(m)	Drift (%)	PEC_{ini} (µg/l)	TER	Drift (%)	PEC_{ini} (µg/l)	TER
5	10	40	0.03	0.6	1.2	0.9
10	4.5	18	0.06	0.4	0.8	1.4
15	2.5	10	0.1	0.2	0.4	2.8
20	1.5	6.0	0.2	0.1	0.2	5.5
30	0.6	2.4	0.5	0.1	0.2	5.5
40	0.4	1.6	0.7	0.1	0.2	5.5
50	0.2	0.8	1.4	0.1	0.2	5.5

Assuming the lowest application rate of 0.5 kg a.i./ha as intended for the use in sugar beet the TER-value for daphniae even in a distance of 20 m is 6.7. As it is indicated by the calculated TER-values the risk for aquatic invertebrates is unacceptable high, even after a single application and when considering risk management measures to protect aquatic ecosystems.

Azinphos-methyl – Addendum to the Monograph, Volume 3, Annex B.8: Ecotoxicology

B.8.2.2 Effects on sediment-dwelling organisms (Annex IIA 8.2.7)

To assess the risk for sediment-dwelling organisms a static test using *Chironomus riparius* was submitted to the RMS (Rep. No. HBF/Ch 29). During the 28 days of exposure the initial measured concentration decreases from 79 -106 % to 0 - 41 % after day 7. After 28 days no azinphos was analysed in the system. However, the nominal EC_{50} resulting from the test was 0.00055 mg/l and the EC_{15} 0.0003 mg/l. No NOEC was evaluated. Therefore the EC_{15} is used to assess the risk for sediment-organisms instead.

Risk assessment for sediment-dwelling organisms

The risk for sediment-dwelling organisms is assessed using the initial concentration in surface water (PEC_{ini}). Following a single application of azinphos-methyl with the intended rates of 1.2 kg a.i./ha in orchards, 0.6 kg a.i./ha in field uses, the initial concentrations in surface water and the resulting TER-values were as follows.

distance	orchards 1.2 kg a.i./ha			field crops 0.6 kg a.i./ha		
(m)	Drift (%)	PEC_{ini} (µg/l)	TER	Drift (%)	PEC_{ini} (µg/l)	TER
5	10	40	0.01	0.6	1.2	0.3
10	4.5	18	0.03	0.4	0.8	0.4
15	2.5	10	0.03	0.2	0.4	0.8
20	1.5	6.0	0.05	0.1	0.2	1.5
30	0.6	2.4	0.1	0.1	0.2	1.5
40	0.4	1.6	0.2	0.1	0.2	1.5
50	0.2	0.8	0.4	0.1	0.2	1.5

Assuming the lowest application rate of 0.5 kg a.i./ha as intended for the use in sugar beet the TER-value for sediment-dwelling organisms in a distance of 20 m is 1.3. As it is indicated by the calculated TER-values the risk for sediment-dwelling organisms is unacceptable high, even after a single application and when considering risk management measures to protect aquatic ecosystems.

B.8.2.3 Mesocosm studies (Annex IIA 8.2, IIIA, 10.2.2)

The following studies were taken into account for a higher tier risk assessment after the ECCO-Meetings:

TANNER, D. K. AND M. L. KNUTH (1995). Effects of Azinphos-methyl on the Reproductive Success of the Bluegill Sunfish, Lepomis macrochirus, in Littoral Enclosures. *Ecotoxicology and Environmental Safety* **32**, 184-193.

SIERSZEN, M. E. AND S. J. LOZANO, S. J. (1998). Zooplankton Population and Community Responses to the Pesticide Azinphos-methyl in Freshwater Littoral Enclosures. *Environmental Toxicology and Chemistry* **17(5)**, 907-914.

HEIMBACH, F. (1999). Extended Laboratory Study on Effects and Recovery of a Daphnia magna Population in a Water-Sediment System after Applikation of Azinphos-methyl (tech.). BAYER Rep. No. HBF/eDM02.

The results of the studies and the assessment of the RMS were as follows:

Enclosure studies

The enclosure study „Effects of Azinphos-methyl on the Reproductive Success of the Bluegill Sunfish, *Lepomis macrochirus*" is not useful to support the position of the notifier. The responses measured were adult behaviour and spawning, embryo hatchability, larval survival, young-of-the-year growth, and total biomass. The given information is insufficient to validate the study, because the test-parameters relevant to assess the risk were not published. Considering that the main focus of the study was the reproduction success of fish, the information especially concerning the aquatic invertebrate species is inclomplete. A further assessment of the study might be possible after validating and evaluating the original raw data.

Although the identified deficiencies of the study concerning the risk assessment, some findings should be noted:

Within a preliminary test using tanks of stainless steel all the adult fishes exposed to 6 and 8 µg/l azinphos-methyl died within 8 days. In the main study using the two concentrations 4.0 and 1.0 µg/l with 4 ponds for each concentration and 4 control ponds adult mortality and larval deformities were the most sensitive endpoints. At 4 µg azinphos-methyl/l the spawning of the adult fish was reduced for 90 %. However spawning and viability of the embryos in the ponds varied extremely. In addition to that in one pond with 4.0 µg/l scoliosis-like deformities of 30 % of the *Lepomis macrochirus* larvae were observed on day 12. At the end of the study these deformities were not longer detectable.

In addition to that, the abundance of the aquatic invertebrate species was reduced during exposure. However, this effect is not significant, because the abundances of the observed invertebrate populations showed a very high degree of variation. Hence, the determination of a reliable NOEC is not possible.

A further study published but not submitted concerning the effects of Azinphos-methyl zooplankton population and community responses was taken into account by the RMS for a higher tier risk assessment. The effects were studied after single applications in enclosures of 5 x 10 m using 4 concentrations of the active substance (0.2, 1.0, 4.0 and 20.0 µg/l) and different zooplankton taxa. The invertebrates populations in the different enclosures showed a very high degree of variation. The results were analysed by principal component analysis (PCA) showing no differences between the enclosures of 0.2 µg/l and the control. Within the discussion of the studies, the authors proposed short-term effects at the nominal concentration of 1 µg/l and long-term effects at 4.0 µg/l. However, the validation of the study without further information concerning the surroundings and the raw data is not possible yet. Therefore, the determination of a reliable NOEC is not possible.

It needs to be mentioned that the effects of azinphos-methyl in the enclosures were studied only after a single application, whereas up to three applications are intended.

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B.8.5 Effects on other arthropod species

B.8.5.2 Extended laboratory tests (Annex IIA 8.3.2, IIIA, 10.5.1)

The following additional studies and statements of the notifier were taken into account for a higher tier risk assessment after the ECCO-Meetings:

MOLL, M. (1998). Effects of Gusathion M 25 WP on the Ladybird Beetle *Coccinella septempunctata* L. - Extended Laboratory Study. BAYER Rep. No. 3992013.

MOLL, M. (1998). Effects of Gusathion M 25 WP on the Parasitoid Aphidius rhopalosiphi - Extended Laboratory Study. BAYER Rep. No. 3991002.

SCHMUCK, R. (1999). Risk Assessment and Proposal for risk Mitigation on Gusathion M 25 WP for Terrestrial Non Target Arthropods.

Two studies concerning the effects of the 25 % WP preparation („Gusathion M" on the arthropod species *Aphidius rhopalosiphi* and *Coccinella septempunctata* were submitted in april 1999. Within a first study the populations were exposed to apple leaves sprayed 1 -3 hours before. The concentrations tested on *A. rhopalosiphi* were 0.002 to 0.048 kg preparation/ha (0.5 to 12 g a.i./ha), corresponding to amounts of 0.05 to 1 % of the use rates intended for orchards. At 6 g a.i./ha the mortality of the aphids was 85 % and at 2.5 g a.i./ha 30 %. No significant lethal effects were observed at a concentration of 1 g a.i./ha, corresponding to an amount of 0.1 % of the application rate intended for a single use in orchards. The LC_{50} and LC_{10} calculated for *A. rhopalosiphi* were 3.4 g a.i./ha and 1.8 g a.i./ha respectively.

The effects of azinphos-methyl on *C. septempunctata* were tested using concentrations reaching from 0.048 to 4.8 kg preparation (0.012 to 1.2 kg a.i./ha) corresponding to 1 - 100 % of the amount intended for a single use in orchards. The beetles were exposed to fresh residues (1-3 hours). Within 96 days following a single application of the lowest tested concentration (12 g a.i./ha), the mortality was 60 %. The mortality increases concentration-dependend up to 90 % using the concentration of 1.2 kg a.i./ha. No NOEC was determined. The LC_{50} and LC_{10} calculated from this test were 4.9 g a.i./ha and 0,01 g a.i./ha, respectively. Within an additional trial the mortality following three applications with 12 g a.i/ha each was 50 % (corrected).

Within two further tests the possibility of recolonisation should be evaluated. For that three times 1.2 kg a.i./ha were applicated in appletrees within an interval of 7 days. The leaves were picked several weeks following these applications and used as test substrate. The mortality of *A. rhopalosiphi* decreased from 100 % on 6-days old residues to 70 % after 10 weeks. On residues aged for 13 weeks the mortality was 3 % and hence recolonization might be possible.

In the test using *C. septempunctata* no survival on the treated apple leaves was possible for the first 6 weeks. After 10 weeks no significant effects on the survival of the beetles were observed and hence recolonization might be possible after this period. However, the reproduction and hatching were reduced.

Risk assessment

With regard to the german drift model, currently used for the aquatic risk assesment, the TER-values using the LC_{50} evaluated with *Aphidius rhopalosiphi* (3.4 g a.i./ha) were as follows:

distance	orchards 1.2 kg a.i./ha			field crops 0.6 kg a.i./ha		
(m)	Drift (%)	PEC_{ini} (g/ha)	TER	Drift (%)	PEC_{ini} (g/ha)	TER
1 and 3	16	192	0.02	5	30	0.1
5	10	120	0.03	0.6	3.6	0.9
10	4.5	54	0.06	0.4	2.4	1.4
15	2.5	30	0.1	0.2	1.2	2.8
20	1.5	18	0.2	0.1	0.6	5.6
30	0.6	7.2	0.5			
40	0.4	4.8	0.7			
50	0.2	2.4	1.4			

With regard to the german drift model, the TER-values using the LC_{50} evaluated with *Coccinella septempunctata* (4.9 g a.i./ha) were as follows:

distance	orchards 1.2 kg a.i./ha			field crops 0.6 kg a.i./ha		
(m)	Drift (%)	PEC_{ini} (g/ha)	TER	Drift (%)	PEC_{ini} (g/ha)	TER
1 and 3	16	192	0.03	5	30	0.2
5	10	120	0.04	0.6	3.6	1.4
10	4.5	54	0.09	0.4	2.4	2.0
15	2.5	30	0.2	0.2	1.2	4.1
20	1.5	18	0.3	0.1	0.6	8.2
30	0.6	7.2	0.7			
40	0.4	4.8	1.0			
50	0.2	2.4	2.0			

As it is indicated by these TER-values the risk for non-target arthropods is classified unacceptable.According to the SETAC/ESCORT Guidance Document (Barrett et al., 1994) the acceptability of the effects for in-crop non-target arthropods is indicated by these results. Taking into account the duration of the vegetation period and the strong effects on arthropods in the off-crop area, the possibility of recolonization and recovery of arthropod populations following the uses as intended for this active ingredient needs to be addressed by an appropriate field study. Studies referred to (e.g. Sechser) should be made available to the RMS.

In addition to that, the usefulness of risk management measures, i.e. the use of appropriate wind breaks as described by Van Vliet and Tas (1996) for orchard applications and proposed by the notifier, and the use of a fixed no spray zone as described by Forster and Rothert (1998)

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for uses in arable crops, to protect arthropods in the off-crop area needs be demonstrated by appropriate field tests. At the moment the risk for non-target arthropods is classified to be unacceptable.

It should be noted that the drift model was developed to estimate the exposure of surface waters. However, estimates of the exposure of populations in a three dimensional terrestrial habitat might be less realistic, because, the data were measured using two dimensional collectors on bare ground. In addition to that, no account could be made for the filtering capacity of the crop nor the distribution in a natural occurring heterogenous environment. This aspect needs to be addressed by further discussions, e.g by the focus group.

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8 June 2001

Azinphos-methyl

Volume 3

Annex B-9

Ecotoxicology

Rapporteur Member State: Germany

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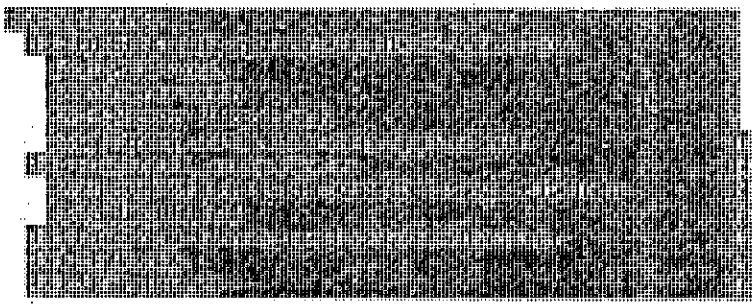

B.9 Ecotoxicology

B.9.2 Effects on aquatic organisms (Annex IIA 8.2; Annex III 10.2)

B.9.2.1 Acute toxicity

Study title:
Hommen, U.(2000): Calculation of a HC_5 for the acute toxicity of azinphos-methyl to freshwater fish. Bayer AG, Final Report from 2000-09-01 (WAT 2001-225).

Objective:
For a refined risk assessment of possible effects of azinphos-methyl on freshwater fish species a sensitive distribution can be calculated to allow the estimation of a concentration protecting 95 % of the species (HC_5 = Hazardous Concentration for 5 % of species). Because only the acute toxicity of azinphos-methyl is under consideration here, this evaluation restricts to the acute toxicity on freshwater fish only.

Data used for calculation:
The US-EPA data base of aquatic toxicity data, Aquire (http://www.epa.gov/ecotox/), was searched for records concerning effects of azinphos-methyl (trade name in the US: Guthion, CAS No. 86500) on freshwater animals. Only records with complete documentation were considered. 94 records were found for fish. Possible duplications per species were identified if mean concentrations and test duration were equal. Four possible duplicates were excluded from the analysis. In addition, data from the open literature not inclnded in the Aquire database were used, too. Seven additional 96 h LC_{50} values were found including two additional fish species.

Method for HC_5-calculation:
The HC_5 was calculated according the following formula:

$$\log(HC_5) = \bar{x} - k \cdot s$$

where $\bar{x}$ and s are mean and standard deviation of the log transformed toxicity data and k is a factor depending on the number of data, the protection level (e.g. 95 % for the HC_5) and the confidence level (e.g. 50 % for the median estimation of the HC_5). HC_5 values were calculated for acute toxicity, in particular for test duration of 24, 48 and 96 hours. If more than one record was available for a species, the geometric mean of the reported concentrations was used for further analysis.

Result:
The HC_5 according Aldenberg & Jaworska (2000) declined with test duration from 2 µg as/L after 24 hours down to 0.535 µg as/L after 96 hours. Exposure time and sample size (based on 21 species represent both the most reliable and the safest HC_5 estimation for the acute toxicity of azinphos-methyl to freshwater fish with a 96 h-HC_5 of 0.535 µg as/L.

Comment of the RMS:
Because the original data used for the probabilistic approach of HC_5 calculation were not submitted an evaluation of validity and plausibility of the values is not possible. In addition to that other fish toxicity data (available in the UBA data base) are relevant for risk assessment, too, but not considered in the HC_5 calculation. For this reason the result of the HC_5 calculation is not accepted.

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B.9.2.2 Chronic toxicity

Study title:
Carlisle, J. C. (1985): Toxicity of Azinphos-methyl (®Guthion Technical) to Rainbow Trout Early Life Stages. Mobay Chemical Corporation, Study No. 84-666-02)

Guideline:
Not reported

GLP compliance:
A statement of compliance that the study was conducted in accordance with Good Laboratory Standards and an quality assurance statement for periodically inspections by the Quality Assurance Unit are available.

Material and methods:
The study reported is the second of two studies. The first study was not used completely because of technical problems call its validity into question. In that study on test day 18 and 19 a diluter malfunction caused delivery of pure stock solution to the test vessels for up to 16 hours. This event was followed by much higher mortality in the replicate "A" of the second and third lowest concentrations (0.31 and 0.58 µg as/L) during the following three days. Consequently the study was repeated.

The 47 d ELS-test was conducted between September 1984 and October 1985. The test materials was azinphos-methyl (Guthion technical) with a content of active substance of 87.3%. The test vessels consisted of 20-liter stainless steel tanks Fifty fertilised eggs from *Oncorhynchus mykiss* were used for each of two replicates per test concentration. The nominal concentrations were 0.25, 0.5, 1, 2 and 4 µg as/L. The water temperature was maintained between 9.4 to 14.2 °C. Temperature and dissolved oxygen content of the test solutions were measured daily. Dead fish were removed and counted as well as batched fry and swimups daily. On test termination all survivors in each chamber were sacrificed and weighted to the nearest milligram. The concentrations of active substance in the test solutions was determined by liquid chromatographic analysis. The percent hatch, survival to the swim up stage, survival to term, final mean weight and bio mass in each concentration were compared with that in the control group using analysis of variance.

Results:
The mean measured concentrations ranged from 94 to 154% of nominal concentrations. The results are given on basis of mean measured concentrations. There was no concentration-related effect on hatching or survival to swim up stage. Final survival was significantly less than control survival in the 1.14 to 4.75 µg as/L exposure groups. Therefore the highest no-effect concentration (NOEL) for survival was 0.47 µg as/l. Final mean fish weight and bio mass (as product of survival and mean weight) were reduced in the 0.29 to 4.75 µg as/L exposure groups. Only one replicate of the 0.29 µg as/L group was statistically separable from the controls, and the 13 % difference in that group is considered a marginal or threshold effect. In summary the predicted EC10 for bio mass was 0.29 µg as/L.

Comment of the RMS:
Because of technical problems in the first test and appearance of effects in the lowest concentration group resulting in no definitive NOEC in the second study, both studies are assessed to be invalid. Summarising the results of both studies the 47 d - NOEC of 0.18 µg as/L from the first study seems to be plausible and the value is in the same order as valid ELS-Test with a 85 d - NOEC of 0.23 µg as/L (see Volume 3, Annex B.8.2.2-1 of the monograph).

B.9.2.3 Micro- or Mesocosm study

Study title:
Dorgerloh, M. and Sommer, H. (2001): Gusathion M WP 25 – Indoor Microcosm (Water/Sediment) with Rainbow Trout (*Oncorhynchus mykiss*) Simulating Multiple Applications. Bayer AG, Report No.: DOM 20081 (WAT 2001-226).

Guidelines:
This indoor microcosm study was specially designed and the basic technical procedures followed as much as possible the internationally accepted guidelines Directive 92/69/EEC, C1 (1992) and OECD No. 203 (rev. 1992).

GLP compliance:
Yes (certified laboratory)

Material and methods:
The test was conducted between January 10 and March 02 in 2001. The test material was "Gusathion M WP 25" with a content of active substance of 24.2 % azinphos-methyl. Young rainbow (*Oncorhynchus mykiss*) with a mean body wet weight of 1.57 g (range 0.6 to 2.6 g) were exposed under static conditions at water temperature of 13.2 to 14.6 °C to simulate repeated applications via spray drift. Aqueous solutions of "Gusathion M WP 25" were sprayed three times in weekly intervals directly on the surface of the indoor microcosm aquaria (total volume: 100 L, water column about 34 cm, sediment layer about 2 cm) in weekly intervals. Four concentrations levels with the following nominal concentrations (µg as/L) after each application were tested (A: 0.44, 0.44, 0.5; B: 0.88, 0.88, 1.0; C: 1.76, 1.76, 2.0 and D: 3.52, 3.52, 4.0). The 21 d test was implemented with 20 fish per test level and one replicate. The test included a water/sediment control and a water only control. Fish were fed daily with 24 h old larvae of *Artemia salina*. The test aquaria were aerated to avoid oxygen depletion below 60 % of the saturation value. Water quality parameters (pH, dissolved oxygen concentration and temperature) were measured daily, concentrations of nitrate and nitrite were determined weekly throughout the test. Observations regarding mortality and any adverse sublethal effects were made 4 – 6 hours after each pray application and then daily. Analytical determination of the active substance concentration were made in each newly prepared stock solution. Water samples were taken from the aquaria 6 hours and 7 days after each of the three applications.

Results:
Water quality parameters as criteria for the validity of the test were within the appropriate range according recommendations for fish testing (O_2-saturation: 72 to 90 %; pH: 6.7 to 7.4; temperature: 13.2 to 14.6 °C; nitrate: <1 mg/L; nitrite: <0.05 to 0.17 mg/L). Analytical determinations of the active substance in all freshly prepared stock solutions resulted in

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recovery rates of 78.5 to 111 % of nominal. The measured active substance concentrations in the test water taken from the aquaria before and after each application were in accordance with the predicted concentrations in relation to the fate of the substance in water. In both of the control groups neither adverse effects nor mortality occurred. No sublethal and lethal effects were observed during the exposure time of 21 d in the lowest test level A. In test level B no lethal effects were observed during the test, but sublethal effects were observed starting on day 13, six days after the second application. Based on this observations and taken into account the residue before last treatment (0.144 µg as/L) and the higher application rate at the last treatment (0.5 µg as/L) a 21d NOEC of 0.64 µg as/L for three applications and a LOEC of 1.32 µg as/L were derived on basis of maximum initial concentration immediately after the last application.

Comment of the RMS:
With respect to the short half-life of azinphos-methyl in water/sediment system RMS do not follow notifiers original interpretation in the report of a NOEC of 1. 38 µg as/L as a cumulative nominal concentration of three applications.

Study title:
Heimbach, F.; Hendel, B. & Sommer, H. (2001): Biological effects and fate of Azinphos-methyl WP 25 in outdoor microcosm ponds. Bayer AG, Report No.: HBF/Bt 03 (WAT 2001-227).

GLP compliance:
This study was conducted in compliance with the Principles of Good Laboratory Practice (Chemikaliengesetz, dated July 25, 1994, current version of Anhang 1 and the current OECD Principles of Good Laboratory Practice (GLP). The test facilities have been inspected and certified as working in compliance with the Principles of Good Laboratory Practice by the competent authorities (Institute for Environmental Biology: File IV C 4 – 31.11.60.03, 4th March 1999, and Institute for Metabolism and Residue Analysis: File IV C 4 – 31.11.62.03, 4th March 1999).

Material and methods:
The objective of this study was to investigate the ecological effects of Azinphos-methyl WP 25 (Fl.-No. F995120410, TOX-No. 05301-00) in outdoor microcosms serving as an aquatic model ecosystem for lentic freshwater ecosystem with different trophic levels, and the fate of the test substance in the test system. The twelve test tanks (6 m^3 water, 1m water depth) used in this study are especially designed systems which allow the establishment of almost identical conditions at the start of a study. The bottoms of the artificial tanks were covered with natural sediment (approximately 10 cm in height) seven months prior to study start. The water was composed of local ground water. Natural communities developed spontaneously from seeds and roots of aquatic plants as well as from air borne and naturally transferred stages of planktonic, benthic and filamentous algae organisms during the months before study start. In general, the artificial ponds are representative of a small standing water. The test substance was applied during the early growing season in April, May and June 2000 three times at an interval of 20 days onto the water surface of nine test ponds. The treatment levels were 0.10, 0.32, 1.0, 3.2 and 10 µg as/L per application (2 replicates 0.10 to 3.2 µg as/L, 1 replicate for 10 µg as./L). Three further tanks were used as untreated controls. The microcosms were investigated for a period of 14 days before and 103 days after first treatment. Several times during the study period water and sediment samples were taken and analysed to investigate the

concentration of the test substance in water and sediment. Further parameters studied were the taxonomic composition of zooplankton, macroinvertebrates and emergence of insects. The physico-chemical water parameters and the content of chlorophyll-a and phaeophytin of phytoplankton were also evaluated. The coverage of the sediment with macrophytes and filamentous algae were estimated. Two diurnal cycles of oxygen concentration, water temperature and pH were recorded during the study.

Results:
An average of 86.5 % of the total applied amount of azinphos-methyl was analysed in the water four hours after the first application, confirming nominal concentrations. 4 hours after the second and the third application 94.9 % and 109.2 % were analysed, respectively. The active substance disappeared after all applications constantly. Even at the highest treatment of 10 µg as/L less than 1 % of the substance was detected 21 days after the third application.

The average half-life of azinphos-methyl in water for the test concentrations of 0.32 to 10 µg as/L was 4.9 days (minimum: 1.8 days, maximum: 7.8 days). During the whole time of the study the amount of azinphos-methyl in the sediment was below the limit of quantification or even below the limit of detection, respectively.

In general, direct and indirect effects of the application of Azinphos-methyl WP 25 to the chemical and physical parameters of the pond water as well as phytoplankton have not been observed at any test concentration. The values determined for all physico-chemical parameters in this study are located in the range of natural ponds.

During the last weeks of the study some differences were observed between the individual basins. These fluctuations are caused by an increasing difference in the composition of the biocoenosis of the test ponds over time. It has been shown in previous experiments that the biocoenosis of individual ponds can be considered as quite similar for about 10 weeks after separation of the ponds; thereafter, the ponds diverge more and more from one another. In conclusion, the minor differences in the final part of the study reported here cannot be considered to be an effect caused by the test substance.

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The biological data showed some effects on a small group of organisms only. No effects on the coverage of the ponds and the biomass of macrophytes and filamentous algae were observed at any treatment level.

	Test concentration [µg as/L]				
ASS SAMPLES	0.1	0.32	1.0	3.2	10.0
Total numbers					
Oligochaeta					
Mollusca					
Hirudinea					
Nematocera (Larvae of Chironomidae)					
Ephemeroptera (Baetidae)					
Shannon-Weaver Index (Diversity)					
Evenness (Diversity)					
Stander's Index (Similarity) *)					
Principal Response Curves *)					
BENTHIC MACROINVERTEBRATES					
Benthos: total numbers					
Oligochaeta (Tubificidae)					
Nematocera (Chironomidae)					
Mollusca					
Hirudinea					
Shannon-Weaver Index (Diversity)					
Evenness (Diversity)					
Stander's Index (Similarity)					
Principal Response Curves					
Emerged Insects					
Emergence: total numbers					
Nematocera					
Chironomidae					
Cricotopus spec.					
Micropsectra spec.					
Chaoboridae					
Ephemeroptera (*Cloeon* spec.)					
Trichoptera					
Shannon-Weaver Index (Diversity)					
Evenness Index (Diversity)					
Stander's Index (Similarity)					
Principal Response Curves					
Zooplankton					
Rotatoria					
Keratella cochlearis		+		+	
Keratella quadrata				+	
Polyarthra spec.					
Synchaeta spec.					
Phyllopoda					
Daphnia longispina					
Acroperus harpae					
Chydorus sphaericus					
Simocephalus vetulus					
Copepoda					
Nauplius		+			
Calanoida					
Cyclopoda					
Ostracoda					
Shannon-Weaver Index (Diversity)					
Evenness (Diversity)					
Stander's Index (Similarity)					
Principal Response Curves					
Community-NOEC		X			
lowest species-NOEC		X			

\+ Increase in numbers

	No effect
	Weak and/or short effects with recovery
	Medium effects with recovery
	Distinct effects with recovery
	Distinct effects without recovery until end of study

Data of the artifical substrate samplers (ASS) evaluated showed no effects, neither at species nor at community level, up to the highest test concentration of 10 µg as/L. Also no reaction to the test substance applied could be found for the benthic macro-invertebrates.

Data on emerged organisms displayed weak to medium effects only at the highest test concentration of 10 µg as/L for several species: Ephemeroptera and Trichoptera have been proofed to be less sensitive than Chironomidae and Chaoboridae. In any case there was a full recovery till study termination even at the highest test concentration.
At the community level (Diversity and Similarity Indices, Principal Response Curves) weak to medium effects only have been evaluated for the highest test concentration with a full recovery before the end of the study. The Principal Response Curves indicated an effect also for the test concentration of 3.2 µg as/L, also with full recovery.

For zooplankton Phyllopoda have been shown to be the most sensitive group of organisms in this study. There was no effect up to a test concentration of 0.32 µg as/L, but at 1.0 µg as/L *Daphnia longispina* was affected by the test substance. A full recovery could be demonstrated at the end of the study at this test concentration. At the test level of 3.2 µg as/L Phyllopoda were affected more with a distinct effect on *Daphnia longispina* showing no recovery. All other Phyllopoda recovered. At the highest test concentration effects expanded to other taxonomic groups: also some rotatoria and copepoda were influenced, definitely showing a full recovery till study termination.

Obviously, there was a strong response of the zooplankton community level-indices, reflecting weak to distinct effects for the highest test concentration. Also at the test concentration of 3.2 µg as/L the Stander's Index and the Principal Response Curve showed distinct effects without recovery. The effects at 1.0 µg as/L have been proofed to be medium and with a full recovery of the Principal Response Curve. No effects have been observed at the lower test concentrations.

Conclusion:
An overall DT_{50} of 4.9 days was observed for azinphos-methyl in water of the microcosms. No effect (NOECcommunity), neither on biocoenosis nor single species, have been identified up to a concentration of 0.32 µg as/L. At 1.0 µg as/L slight effects on Phyllopda, i.e. *Daphnia longispina*, have been observed. Apparently these effected populations recovered completely during the study. At higher test concentrations of 3.2 µg as/L the effects on *Daphnia longispina* were distinct without a full recovery until study termination. In the highest test concentration also other organism groups, i.e. Rotatoria and Copepoda have been affected by the test substance. Thus, taking into account all data on single species as well the community parameters, the ecological acceptable concentration (EAC) is stated to be 1.0 µg as/L due to a full recovery of all organism groups at this test concentration.

Comment of the RMS:
The study is accepted and evaluated to be valid. The RMS do not agree with the conclusion of the notifier that a full recovery of *Daphnia longispina* occurred at 1 µg as/L. Because the

control values were not reached only a tendency of recovery can be stated. For this reason the EAC is not used for risk assessment.

B.9.2.4 Exposure and risk assessment for aquatic organisms

Study title:
Heimbach, F. (2001): Studies for a Refined Risk Assessment of azinphos-methyl to Earthworms, Aquatic Invertebrates and Fish. Unpublished report (WAT 2001–239).

Risk assessment:
The base set data show a high acute toxicity of azinphos-methyl to fish with a steep dose-response curve, and chronic endpoints are in the same range as acute ones.

Assessment factors, which are used on standard acute and chronic studies, consider the ACR (acute chronic relationship) for acute studies, differences in species sensitivity, multiple applications, and natural exposure conditions. Since the degradation of aziuphos-methyl is not pH-dependent under natural conditions, laboratory studies already reflect the worst case exposure scenario.

The assessment factor for azinphos-methyl based on the two higher tier microcosm studies (Fish: NOEC 0.64 µg as/L; Invertebrates: NOEC 0.32 µg as/L, EAC 1.0 µg as/L) should be adjusted accordingly. Since fish might be considered with more care than other organisms as invertebrates and algae, an assessment factor of two based on the fish microcosm NOEC seems advisable for a final conclusion following the HARAP recommendations. This means, an environmental concentration of 0.3 ppb can be considered as a maximum initial PEC to avoid unacceptable effects on fish populations under natural conditions.

Comment of the RMS:
Higher tier mono-species indoor microcosm study demonstrate that fish under more realistic exposure conditions are very sensitive, too with a nominal 21 d - NOEC of 0.64 µg as/L for repeated applications. This value is supported by a provisionally but not validated calculation of a 96 h - HC_5 = 0.535 µg as/L on basis of toxicity data for 21 species. With respect to the extensively database including higher tier studies and the short term impact only, the uncertainty for the risk assessment is lowered considerably. Thus, the RMS agree with the conclusion of the notifier that the nominal $NOEC_{community}$ of 0.32 µg as/L from multi-species outdoor microcosm can considered as a safe concentration for protection of aquatic invertebrate and fish.

Therefore, the definitive and refined risk assessment is based on the nominal $NOEC_{community}$ 0.32 µg as/L for invertebrates and the $NOEC_{water/sediment}$ 0.64 µg as/L for fish. An accumulation of the active substance in water phase is not expected because dissipation time in outdoor microcosm study was short with a DT_{50} of 4.9 d and multiple applications in indoor water/sediment toxicity study with fish did not result in concentrations higher than initial.

The first tier TER calculation according to the still actual Guidance Paper for Aquatic Ecotoxicology (8075/VI/97 rev. 7, 2000-07-08) on basis of 95th drift percentile indicate a high unacceptable risk for aquatic organisms by the maximum intended application rates of 750 g

as/ha with a TER below 1 even at the highest distance of 50 m to surface water (tables 1 - 3). Only at the lowest application rate of 440 g as/ha the initially predicted environmentally concentrations do not reach the relevant NOEC from invertebrates and fish with TER > 1 to 2.

A second tier TER calculation according to the actual German model (Bundesanzeiger Nr. 100 vom 26.05.2000. S. 9878 – 9881; basis for the new Draft Guidance Paper for Aquatic Ecotoxicology) using 90th drift percentile results in TERs of up to 21 at the highest application rate of 750 g as/ha (tables 3-4).

Tables 1 - 2: TER calculation according to the still actual Guidance Paper for Aquatic Ecotoxicology (8075/VI/97 rev 7, 2000-07-08)

Treatment and Application rate: 1 x 750 g as/ha
Scenario: Orchards (worst case, 95 Percentile for single application)

Distance (m)	Conventional technique		$TER_{chronic}$-values	
	Drift rate (%)	$PEC_{init.}$ (µg as/L)	Invertebrates: $NOEC_{community}$ = 0.32 µg as/L	Fish: 21 d $NOEC_{water/sediment}$ = 0.64 µg as/L
0	100.000	250.000	0.0	0.0
3	29.600	74.000	0.0	0.0
5	20.000	50.000	0.0	0.0
10	11.000	27.500	0.0	0.0
15	6.000	15.000	0.0	0.0
20	4.000	10.000	0.0	0.1
30	2.000	5.000	0.1	0.1
40	0.400	1.000	0.3	0.6
50	0.200	0.500	0.6	1.3

Treatment and Application rate: 1 x 440 g as/ha
Scenario: Orchards (worst case, 95 Percentile for single application)

Distance (m)	Conventional technique		$TER_{chronic}$-values	
	Drift rate (%)	$PEC_{init.}$ (µg as/L)	Invertebrates: $NOEC_{community}$ = 0.32 µg as/L	Fish: 21 d $NOEC_{water/sediment}$ = 0.64 µg as/L
0	100.000	146.667	0.0	0.0
3	29.600	43.413	0.0	0.0
5	20.000	29.333	0.0	0.0
10	11.000	16.133	0.0	0.0
15	6.000	8.800	0.0	0.1
20	4.000	5.867	0.1	0.1
30	2.000	2.933	0.1	0.2
40	0.400	0.587	0.5	1.1
50	0.200	0.293	1.1	2.2

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Tables 3 and 4: TER calculation according to the actual German risk assessment model (Bundesanzeiger Nr. 100 vom 26.05.2000. S. 9878 – 9881; basis for the revised new Draft Guidance Paper for Aquatic Ecotoxicology)

Treatment and Application rate: 1 x 750 g as/ha
Scenario: Orchards (worst case, 90 Percentile for single application)

Distance (m)	Conventional technique		$TER_{chronic}$-values	
	Drift rate (%)	$PEC_{init.}$ (µg as/L)	Invertebrates: $NOEC_{community}$ = 0.32 µg as/L	Fish: 21 d $NOEC_{water/sediment}$ = 0.64 µg as/L
0	100.000	250.000	0.0	0.0
3	29.200	73.000	0.0	0.0
5	19.890	49.725	0.0	0.0
10	11.810	29.525	0.0	0.0
15	5.550	13.875	0.0	0.0
20	2.770	6.925	0.0	0.1
30	1.040	2.600	0.1	0.2
40	0.520	1.300	0.2	0.5
50	0.300	0.750	0.4	0.9
75	0.110	0.275	1.2	2.3
100	0.060	0.150	2.1	4.3
125	0.030	0.075	4.3	8.5
150	0.021	0.053	6.1	12.2
175	0.015	0.038	8.5	17.1
200	0.011	0.028	11.6	23.3
225	0.008	0.020	16.0	32.0
250	0.006	0.015	21.3	42.7

Treatment and Application rate: 1 x 440 g as/ha
Scenario: Orchards (worst case, 90 Percentile for single application)

Distance (m)	Conventional technique		$TER_{chronic}$-values	
	Drift rate (%)	$PEC_{init.}$ (µg as/L)	Invertebrates: $NOEC_{community}$ = 0.32 µg as/L	Fish: 21 d $NOEC_{water/sediment}$ = 0.64 µg as/L
0	100.000	146.667	0.0	0.0
3	29.200	42.827	0.0	0.0
5	19.890	29.172	0.0	0.0
10	11.810	17.321	0.0	0.0
15	5.550	8.140	0.0	0.1
20	2.770	4.063	0.1	0.2
30	1.040	1.525	0.2	0.4
40	0.520	0.763	0.4	0.8
50	0.300	0.440	0.7	1.5
75	0.110	0.161	2.0	4.0
100	0.060	0.088	3.6	7.3
125	0.030	0.044	7.3	14.5

150	0.021	0.031	10.4	20.8
175	0.015	0.022	14.5	29.1
200	0.011	0.016	19.8	39.7
225	0.008	0.012	27.3	54.5
250	0.006	0.009	36.4	72.7

References relied on :

Annex Point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner
AIIA-8.2.1	Hommen,U.	2000	Final Report Calculation of a HC_5 for the acute toxicity of azinphos-methyl to freshwater fish unpublished WAT2001-225	Y	BAY
AIIIA-10.2	Carlisle, J. C.	1985	Toxicity of Aziophos-methyl (®Guthion Technical) to Rainbow Trout Early Life Stages. Mobay Chemical Corporation, Study No. 84-666-02) unpublished WAT2001-.....	N	BAY
AIIIA-10.2.2	Dorgerloh,M. and Sommer,H.	2001	Gusathion M WP 25 - Indoor microcosm (water/sediment) with rainbow trout (Oncorhynchus mykiss) sumulating multiple applications GLP, unpublished WAT2001-226	Y	BAY
AIIIA-10.2.2	Heimbach,F.; Hendel,B. and Sommer, H.	2001	Biological effects and fate of Azinphos-methyl WP 25 in outdoor microcosm ponds GLP, unpublished WAT2001-227	Y	BAY
AIIA-8.2, AIIIA-10.2	Heimbach, F.	2001	Studies for a Refined Risk Assessment of azinphos-methyl to Earthworms, Aquatic Invertebrates and Fish unpublished WAT2001-239	Y	BAY
	Anonym	2000	Bekanntmachung des Verzeichnisses risikomindernder Anwendungsbedingungen für Nichtzielorganismen. Bundesanzeiger Nr. 100 vom 27.04.2000, S. 9878		
	Anonym	2000	Bekanntmachung über die Abtrifteckwerte, die bei der Prüfung und Zulassung von Pflanzenschutzmitteln herangezogen werden. Bundesanzeiger Nr. 100 vom 08.05.2000, S. 9879 – 9881		

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B.9.5 Effects on other arthropod species

B.9.5.1 Extended laboratory tests (Annex IIA 8.3.2, Annex IIIA 10.5.1)

The following additional studies and statements currently made available by the notifier were taken into account for a refined higher tier risk assessment:

BAXTER, I. (2000).Toxicity of Azinphos-methyl WP 25 to the parasitic wasp *Aphidius rhopalosiphi* (Hymenoptera, Braconidae) iin a semi-field test. Rep. No. BAY-00-8.

MOLL, M. (2001). Effects of Azinphos-methyl WP 25 on Adults of the Parasitoid *Trichogramma cacoeciae* Marchal (Hymenoptera, Trichogrammatidae) - Extended Laboratory Study. Project No. 8731026.

SCHMUCK, R. (2001). Refined Risk Assessment for Azinphos-methyl and Non-target Arthropod Species, 7 pages.

BAXTER, I. (2000). Three application rates of Azinphos-methyl WP 25 (24.2 % azinphos-methyl; 5, 10, 50 g as/ha) were tested under semi-field conditions on treated barley plants (one- to two-leaf growth stage, 10 to 15 cm tall, sowing density based on rate of 175 kg seed/ha) using max. 48 h old adult *Aphidius rhopalosiphi* (10 females into each of 4 replicates per treatment for fecundity assessments; 40 wasps of mixed sex ratio into each of 4 replicates per treatment for activity assessments). Plant were maintained outdoors under UV-permeable rain protection. Bioassays commenced within 1 h of application. Tap water was used as control treatment and dimethoate 40 (0.85 L/ha) as a toxic reference treatment. Endpoints were parasitation efficiency (numbers of aphid mummies after 24 and 48 hrs) and activity (numbers of wasps trapped on yellow sticky traps after 24 and 48 hrs). The study was conducted under GLP and is considered valid.
Findings: No statistically significant effects ($p > 0.05$) were observed on either wasp activity (98 %, 108 %, 93 % compared to the control) or on the fecundity (134 %, 110 %, 90 % compared to the control) for all rates tested with Azinphos-methyl WP 25 (5, 10, 50 g as/ha).

MOLL, M. (2001). Azinphos-methyl WP 25 (24.2 % azinphos-methyl) was tested under extended laboratory conditions using app. 24 h old adult *Trichogramma cacoeciae* (38 to 162 wasps per cage; 4 replicates per treatment). Wasps were exposed to dried spray deposits of 0.4 to 31.25 g as/ha (diluted in 200 L water/ha) on treated apple leaves (singularly treated using a laboratory spraying equipment). Deionized water was used as control treatment and Perfekthion EC (6 mL/ha) as a toxic reference treatment. The duration of the test was 11 d (7 d exposure + 4 d incubation of host eggs). Endpoint was parasitation efficiency. The toxic standard caused 76.2 % reduction compared to the control. The study was conducted under GLP and is considered valid.
Findings: Effects on parasitation efficiency was not statistically significant for all rates tested with Azinphos-methyl WP 25. However, data showed a consistent dose-response-relationship and ER_{50} was calculated 11.0 g as/ha (CL 95 %: 5.2 to 48.5 g as/ha).

SCHMUCK, R. (2001). The refined risk assessment for Azinphos-methyl proposed by the notifier is mainly supported by a number of publications which indicate that under field conditions the effects caused by Azinphos-methyl on populations of hymenopteran species are considerably lower than observed under laboratory conditions and indicated by deterministic risk assessment models currently used for *Aphidius rhopalosiphi*. In order to verify these

assumptions the notifier conducted two studies which are reported above in order to address i) differences in sensitivity between species and ii) differences in sensitivity of *Aphidius rhopalosiphi* tested under more natural exposure conditions compared to former laboratory tests.

Conclusions:

i) The ER_{50} for *Trichogramma cacoeciae* was considerably higher than the LR_{50} for *Aphidius rhopalosiphi* (11 g as/ha compared to 1.2 g as/ha).

ii) ii) Under more realistic exposure conditions the ER_{50} for *Aphidius rhopalosiphi* must be considerably higher than 50 g as/ha. According to the notifier both findings support the view that under field conditions there is no unacceptable risk on the population level for sensitive species such as hymenopteran parasitoids. In order to protect non-target arthropods the notifier proposed buffer zones and spray drift reducing application technique.

Comment of the RMS:
Findings reported by the notifier are conclusive. However, RMS does not follow notifiers original conclusion in all details. Based on the data submitted the recolonisation of treated crops by sensitive species is still questionable.

B.9.5.2 Risk assessment

A In accordance with SETAC, Annexes IIA and IIIA of 91/414/EEC

According to the latest list of uses currently supported by the notifier (2001-04-10) maximum rates for orchards are reduced to 0.750 kg as/ha for a single application with a maximum number of 3 applications per year. Because specification of the pest species was not given by the notifier drift values for early applications are used in the calculations:

Max. Number of Treatments and Application rate: 3 x 750 g as/ha
Scenario: Orchards (worst case, 95th Percentile, early)

	PEC-calculation		TER- calculation related to NOEC[2] for *Aphidius rhopalosiphi* 50 g as/ha
Distance (m)	Drift rate (%)	PEC[1] (g as/ha)	Conventional technique
0	100	750.0	0.07
3	30	225	0.22
5	20	150	0.33
10	11	82.5	0.61
15	6	45	1.11
20	4	30	1.67

1) PEC for 1 application, drift values according to Ganzelmeier et al. 1995
2) According to BAXTER, I. (2000) no statistically significant effects ($p > 0.05$) were observed on either wasp activity (98 %, 108 %, 93 % compared to the control) or on the fecundity (134 %, 110 %, 90 % compared to the control) for all rates tested with Azinphos-methyl WP 25 (5, 10, 50 g as/ha).

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According to the TER-calculations a very high risk for non-target arthropods within the treated fields must be anticipated (TER = 0.07). The potential for recolonisation of treated fields within one season – as demonstrated in former aged-residue studies - is low.

Off-field TER-values indicate also a very high, unacceptable risk for populations of non-target arthropods due to spray drift into habitats next to treated crops. No agreements have been met on EU-level whether buffers of more than 5 m are practicable. No further information are available which are necessary to estimate the potential of recovery of the off-field- populations.

B In accordance with current scientific knowledge

Max. Number of Treatments and Application rate: 3 x 750 g as/ha
Scenario: Orchards (worst case, 90th Percentile for MAF of three applications, early)

	PEC-calculation		TER- calculation related to NOEC[2] for *Aphidius rhopalosiphi* 50 g as/ha
Distance (m)	Drift rate (%)	PEC_{MAF}[1] (g as/ha)	Conventional technique
0	100.000	1035.0	0.05
3	29	300.15	0.17
5	20	207	0.24

1) MAF = Multiple Application Factor (GONZALES-VALERO et al. 2001), MAF = 1.38 (DT_{50} leaves 11.3 d, spraying interval 20 d), drift values according to BBA (2000) for MAF = 1
2) According to BAXTER, I. (2000) no statistically significant effects ($p > 0.05$) were observed on either wasp activity (98 %, 108 %, 93 % compared to the control) or on the fecundity (134 %, 110 %, 90 % compared to the control) for all rates tested with Azinphos-methyl WP 25 (5, 10, 50 g as/ha).

According to the TER-calculations on the basis of the actual German drift values (Bundesanzeiger Nr. 100 vom 26.05.2000. S. 9878 – 9881) the results of the risk assessment are similar to that on the basis of the older drift values. Based on German agricultural conditions buffers of more than 5 m are not considered practicable.

References relied on :

Annex Point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner
AIIA-8.3.2, AIIIA-10.5	Baxter, I.	2000	Toxicity of Azinphos-methyl WP 25 to the parasitic wasp, Aphidius rhopalosiphi (Hymenoptera: Braconidae), in a semi-field test GLP, unpublished ANA2001-195	Y	BAY

AIIA-8.3.2, AIIIA-10.5	Moll, M.	2001	Effects of Azinphos-methyl WP 25 on adults of the parasitoid Trichogramma cacoeciae Marchal (Hymenoptera, Trichogrammatidae) - extended laboratory study GLP, unpublished ANA2001-196	Y	BAY
AIIA-8.3.2, AIIIA-10.5	Schmuck, R.	2001	Refined Risk assessment for Azinphos-methyl and Non-target Arthropod Species unpublished ANA2001-197	Y	BAY

B.9.6 Effects on earthworms (Annex IIA 8.4, Annex IIIA 10.6.1)

B.9.6.1 Field study (Annex IIIA 10.6.1.3)

Title:
Effects of Azinphos-methyl WP 25 on the earthworm biocoenosis of a grassland area in northern Italy one month and six month after application (Vighi and Heimbach, ARW2001-36)

Report-No.: HBF/RgF55

Guideline: ISO 11268-3, 1999 and BBA VI, 2-3, 1994

GLP: yes

Year of study: 2000

Study site:
Grassland site at Brugherio, near Milano (Italy). The site was not treated with chemical fertilisers or pesticides for about 10 years. The area had a size of 11 x 140 m. Irrigation was performed from the end of May to mid-September 2000 in weekly intervals by overflowing an irrigation channel adjacent to the test field. The first irrigation took place in the night following to the second application of azinphos-methyl. After the third application the field was irrigated one week after the application. No information about the amount of water used for irrigation is given. The site was mown 6 times in 2000. The cuttings were partly removed to avoid a thick layer of grass on the soil surface.

The study was designed in a randomised block design. Each plot (= replicate) was 100 m^2 in size and was replicated four times. The pre-treatment earthworm density was checked on 14 April 2000. The mean density was about 200 individuals/m^2.

Soil:
sandy loam, pH 5.65, organic carbon content 2.4 %

Vegetation:

Poa pratensis (approximate coverage 50 %), *Lolium perenne* (30 %), *Arrenatherum elatior* (10 %), *Bromus mollis* (10 %), *Anthoxanthum odoratum* (5 %), *Trifolium pratense* (20 %), *Plantago lanceolata* (5 %), *Rumex* sp.

Application:
Azinphos-methyl WP 25 was applied 3 times at 1 kg as/ha with 20 days intervals. The first application was done on may 5, the second on may 25 (with an irrigation in the following night) and the third application on June 14 (the next irrigation was done on June 21). Benomyl 50 WP was applied as reference substance at 4 kg as/ha once on may 5.

Sampling:
Four samples per plot and sampling date were taken using the formaldehyde-extraction method (10 L of 0.2 % formaldehyde solution). A 30 minutes extraction time was chosen. The sampling dates were one month after the last application on 14 July and about 4.5 month after the last application on 3 November. Directly after treatment the soil surface was checked for dead and alive earthworms.

Earthworm species:
Lumbricus terrestris, Lumbricus castaneus, Lumbricus rubellus, Aporrectodea caliginosa, Aporrectodea rosea, Allolobophora chlorotica, Allolobophora georgii, Octolasion tyrtaeum

Results:

Soil surface search:
4 and 7 days after the first application some dead or alive earthworms were observed on the azinphos-methyl and the benomyl-treated plots. Whereas in the control plots in total 2 dead and 3 alive earthworms were found, in the benomyl plots 12 dead and 12 alive earthworms, and in the azinphos-methyl plot 8 dead and 5 alive earthworms were observed. After the second and the third application no surface activity in the control and the azinphos-methyl was observed. In the benomyl plots this was not checked, because the treatment was only done once on May 5.

Formaldehyde extraction:

One month after treatment the overall earthworm density and extraction efficiency were low (mean earthworm density 37 /m^2). The extraction efficiency was between 0 and 33 %. In general the earthworm densities on the site were uneven distributed depending on the plot. Because of the low densities and the timing of the sampling, the results of this sampling date are not evaluated.

In autumn the mean density was 138 earthworm/m^2. The extraction efficiency was between 64 and 84 %.

Table 1: Effects of azinphos-methyl and benomyl on earthworms numbers and biomass six month after treatment in % compared to control (control = 100 %) (Vighi and Heimbach 2001, ARW2001-36)

	Azinphos-methyl (6 x 1 kg as) Abundance (% of control)	Azinphos-methyl (6 x 1 kg as) Fresh weight (% of control)	Benomyl (1 x 4 kg as) Abundance (% of control)	Benomyl (1 x 4 kg as) Fresh weight (% of control)
Overall abundance	89	101	73 *	92
Overall juveniles	79	91	64 *	77
Overall adults	95	102	81	95
Juvenile epilobous	76	84	68	83
Juvenile *Lumbricus terrestris*	/	/	/	/
Juvenile *Lumbricus castaneus*	71	76	29	27
Other juvenile tanylobous	100	97	41 *	54 *
Adult *Lumbricus terrestris*	83	106	17 *	16
Adult *Lumbricus castaneus*	107	124	53 *	31 *
Adult *Lumbricus rubellus*	100	231	200	697
Adult *Aporrectodea caliginosa*	95	101	86	104
Adult *Aporrectodea rosea*	83	100	100	104
Adulte *Allolobophora chlorotica*	200	768	233	753
Adult *Allolobophora georgii*	0	0	20	14
Adult *Octolasion tyrtaeum*	100	115	13 *	15 *

* sign. $p < 0.05$; t-test/Mann-Whitney U-test

B.9.6.2 Risk Assessment

The effects of benomyl on earthworms after the first application demonstrate that the reference substance has worked. The conditions of the first application indicate that the test substance reached the soil and has been done in a phase of earthworm activity (effects of benomyl, surface activity after the application). For the other two applications (May 25 and June 14) this may not have been the case due to the summer season conditions. As benomyl has not been applied in these dates again, and as there was an irrigation in the night after the

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second application on May 25 with an unknown amount of water, there is uncertainty about the conditions of these applications.

The guidelines require a sampling date one year after application. The results of this date are missing yet (study is ongoing). The sampling in June is not taken into account because of a low extraction efficiency. This means that only one evaluation date is available in the moment.
The results from this evaluation (about six month after application) show that there are slight, not significant reductions in some species, especially in the juveniles. There were some species with a low density (e.g. *Allolobophora chlorotica, A. georgii* and *A. rosea* with in total 3 to 6 individuals in 16 samples in the control). For these species a risk assessment cannot be done.

Comment of the rapporteur member state:
The previous field test according to Heimbach, 1988; ARW 95-00091 was done with application rates of 2 x 1.5 kg as/ha and 2 x 6 kg as/ha. The present field test is done with 3 x 1 kg as/ha, so the overall amount for the lower rate is comparable. A final risk assessment is not possible because the test has not been finished yet. The completion of the test should be awaited.

References relied on :

Annex Point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner
AIIIA-10.6.1.3	Heimbach, F.	1988	Influence of Gusathion M 200 EC on the earthworm fauna of a grassland area, GLP, published ARW9500091	Y	BAY
AIIIA-10.6.2.3	Vighi, M., Heimbach, F.	2001	Effects of azinphos-methyl WP 25 on the earthworm biocoenosis of a grassland area in northern Italy one month and six month after application, GLP, published ARW2001-36	Y	BAY

04

Addendum 8

to the Monograph

of 18 September 1996

Azinphos-methyl

30 April 2002

Rapporteur Member State: Germany

Contents

To Volume 1:

Level 1 Statement of subject matter and purpose for which the monograph was prepared

1.5 Use of the plant protection product

The final list of intended uses supported by available data was submitted by the notifier in February 2002. Risk assessment is based on these uses.

List of intended uses supported by available data

	Crop	Country	Number of Treatments	Application Interval (d)	Rate per Treatment (kg as / ha)	Currant PHI	Intended PHI	Currant Water rate	Intended Water rate
Orchards	Pears	Italy	3	20	0.60 - 0.75	20	21	1200 L/ha	500 L/m plant height up to a maximum of 1500 L/ha
		Portugal	1 - 3	20	0.50 - 0.60	28	21	1000 L/ha	
		Spain	1 - 3	20	0.50 - 0.75	28	21	1500 L/ha	
	Apples	Italy	3	20	0.60 - 0.75	20	21	1200 L/ha	
		France	1 - 3	20	0.44 - 0.50	21	21	1000 L/ha	
		Portugal	1 - 3	20	0.50 - 0.60	28	21	1000 L/ha	
		Spain	1 - 3	20	0.50 - 0.75	28	21	1500 L/ha	
		Greece	1 - 3	14 - 21	0.60 - 0.75	14	21	1000 L/ha	
Field crops	Potato	Spain	1 - 2	8	0.12 - 0.40	28	21	1000 L/ha	800 L/ha
		Italy	2	8	0.40 - 0.50	20	21	1000 L/ha	
		Portugal	1 - 2	21	0.24	21	21	1000 L/ha	

Comment of the RMS: The minimum application rate intended for the use in potatoes in Spain is only 0.12 kg a.i/ha. Considering that in the Dossier originally an amount of 0.6 kg a.s./ha was intended for this use and is still intended as use-rate for potatoes in Italy, it seems to be questionable that using only 0.12 kg a.i./ha, the full effectivity of the a.s. is reached.

2.2 Physical and chemical properties

(Corrections are marked in bold type.)

Azinphos-methyl has a melting point of 73 °C. Its water solubility is 28 mg/l. The log of the partition coefficient n-octanol/water lies within the range of 2.87 to 3.02 with a mean of 2.96. The vapour pressure of azinphos-methyl was determined from $1.8 \cdot 10^{-4}$ ~~$1.8 \cdot 10^{-4}$~~ to $5 \cdot 10^{-7}$ Pa at 20 °C and the Henry's law constant from $2.0 \cdot 10^{-3}$ to $1.1 \cdot 10^{-4}$ Pa · m^3/mole.

The substance does not have acid or alkaline properties.

The hydrolytic half-lives of azinphos-methyl at 22 °C amount to 87, 50, and 4.1 d at pH 4, 7, and 9, respectively. The major hydrolysis products are bis-(benzazimide-N-methyl)sulfide (3,3'-[thiobis(methylene)]bis-[1,2,3-benzotriazin-4(3H)-one]), benzazimide and/or hydroxymethylbenzazimide (1,2,3-benzotriazin-4(3H)-one and/or 3-(hydroxymethyl)-1,2,3-benzotriazin-4(3H)-one), and anthranilic acid (2-aminobenzoic acid).

Photolytic half-lives were determined to be in the range of 9.4 **21** – 76.7 h **at 25 °C after artificial irradiation representative for solar conditions of Phoenix (Arizona) in June and natural sunlight of Kansas City, Missouri, during January through March, respectively.** ~~However, the results on photolysis can be used only for orientation because of various deviations from the requirements (e.g. the range of 280 – 290 nm were not excluded, non-sterile conditions, natural sunlight, higher temperature).~~

The **only** major photolysis products **are is** benzazimide ~~and/or hydroxymethylbenzazimide~~.

The environmental half-lives concerning direct photolysis are expected in the range between 1 and 4 d, for the months of main use.

Concerning indirect photolysis and based on the reactivity against OH radicals in the troposphere, a short half-life of < 2 h corresponding to a chemical lifetime of < 3 h was assessed conservatively.

Its flammability, explosive or oxidizing properties are not critical.

Gusathion M EC 19.5 is not oxidising, its pH is within the range that naturally occurs e.g. in soil. Its stability allows storage under practical and commercial conditions. At low temperatures the risk of crystallization exists. Its technical properties indicate that no particular problems are to be expected when it is used as recommended. The formulation is classified as flammable.

Gusathion M WP 25 is not explosive, not oxidising, its pH is within the range that naturally occurs e.g. in soil. Because of its limited stability at elevated and ambient temperatures an expiring date on the label is necessary for storage under practical and commercial conditions. Its technical properties indicate that no particular problems are to be expected when it is used as recommended.

Several studies on residues, toxicity etc. were performed with the Gusathion M WP 35 formulation instead of the WP 25 formulation. All the physical and chemical properties are comparable.

For Cotnion Methyl 20 SC no data are available.

2.5 Methods of analysis

2.5.3 Methods for residue analysis

Analytical methodology for azinphos-methyl is available for plants, soil, water, air, milk and blood.
No new validation data were submitted for eggs and meat. The requirement for a further validation data for eggs and meat (muscle) remains to be not fulfilled.

2.7 Residues

2.7.1 Residues relevant to consumer safety

All residue supervised trials data submitted (state February 2002) were re-evaluated with regard to the intended uses (state February 2002).

The re-evaluation of all residue trials received for the current intended uses of azinphos-methyl shows that MRLs could be recommended for pome fruit (0.5 mg/kg), potatoes (0.05* mg/kg), milk (0.02* mg/kg) and other animal products (0.05* mg/kg).

The dietary risk assessment on a national basis (NTMDI) and international level (TMDI, WHO, European diet) is based on an ADI value of 0.005 mg/kg bw for azinphos-methyl. Where an MRL could not be recommended, the LOQ of 0.05 mg/kg (or 0.1 mg/kg for tea and hops) is used in the dietary risk calculation. Based on the recommended MRLs and the LOQs, the NTMDI and the TMDI were calculated as 70% and 35% of the ADI.
The NEDI (German model) and IEDI (WHO, European diet) were calculated based on the STMR values for the intended uses. The IEDI/NEDI was estimated at about 2-10% of the ADI. It can be concluded that the long-term intake of residues of azinphos-methyl resulting from uses that have been considered is unlikely to present a public health concern.

The short-term dietary intake was calculated according to the deterministic model and is based on the acute reference dose (ARfD) of 0.075 mg/kg bw proposed by the RMS and UK consumption data. The NESTI represented 0 – 10 % of the ARfD for adults and 0 – 40 % of the ARfD for toddlers. It can be concluded that the short-term intake of residues of azinphos-methyl, resulting from uses that have been considered, is unlikely to present a public health concern.

2.9 Effects on non-target species

2.9.1 Effects on aquatic organisms

The refined risk assessment is based on the nominal $NOEC_{community}$ of 0.32 µg as/L derived from a mesocosm study with invertebrates and the $NOEC_{water/sediment}$ of 0.64 µg as/L from a prolonged fish test with sediment. In addition to that the risk for early life stages is assessed using the NOEC from an ELS, because some sensitive endpoints were not integrated in the prolonged fish test. An accumulation of the active substance in water phase is not expected because dissipation time in outdoor microcosm study was short with a DT_{50} of 4.9 d and multiple applications in indoor water/sediment toxicity study with fish did not result in concentrations higher than initial. However, accumulation in sediment cannot be excluded.

In orchards TER calculations indicate an unacceptable high risk for aquatic organism by the intended application rates of 500, 600 and 750 g as/ha with TER-values below 1 even at the highest distance of 50 m to surface water.

In potatoes TER values of > 1 to 2 for invertebrates and adult fish are reached for application rates of 500 and 400 g as/ha at 15 m, for 240 g as/ha at 10 m and for 120 g as/ha at 5 m distance to surface water.

2.9.2 Effects on non-target arthropods

According to the submitted studies and the calculated TER-values, for the uses intended by the notifier at the latest in February 2002 the risk for non-target arthropods living within and outside the treated fields is unacceptable high. The potential for recolonisation of treated fields is questionable.

2.9.3 Effects on earthworms

A new field study was submitted using 3 x 1 kg as/ha to test long-term effects on earthworms. However, there is an uncertainty about the exposure conditions for at least one application (irrigation in the night after application on May 25 with an unknown amount of water) in the test. Therefore, it is not possible to do a risk assessment for earthworms for 3 kg as/ha from the results available. It is concluded that on the data base available 2 kg as/ha reaching the soil surface might lead to a risk for earthworm populations in the field. As this amount is not part of the intended uses, no additional risk management is considered necessary. For every rate above 2 kg as/ha a proper risk assessment is needed. Thus, no long-term risk for earthworms are to be expected.

Level 3 Proposed decision with respect to the application for inclusion of the active substance in Annex I

3.1 Background to the proposed decision

Azinphos-methyl is an insecticide with a broad spectrum of activity used mainly in agriculture and horticulture.

According to the submitted studies and the calculated TER-values, the risk for non-target arthropods is unacceptable high. Risk assessement for aquatic organisms show also an unacceptablehigh risk in the case of the high field rates. Risk for earthworms is acceptable up to a field rate of 2 kg as/ha.

3.2 Proposed decision concerning inclusion in Annex I

On the basis of the submitted data it is proposed not to include the active substance azinphos-methyl in Annex I of Directive 91/414/EEC.

To Volume 3:

B.2 Physical and chemical properties

B.2.1 Physical and chemical properties of the active substance (Annex IIA 2)

B.2.1.3 Vapour pressure; volatility (Annex IIA 2.3)

B.2.1.3.1 Vapour pressure

Krohn, J.: Vapour pressure curve of azinphos-methyl. Bayer AG, Report Nos.: PC895, 14 215 0869, 27 June 1995, LUF98-50019

Guideline: OECD Guideline No. 104, EEC Guideline A4, EPA §63-9

GLP: yes

Test substance:	non-labelled azinphos-methyl, purity: 99.5 %
Test system:	gas saturation method
Findings:	The vapour pressure curve was recorded in the 30 – 70 °c range. Using the Claudius Clapeyron equation, vapour pressures of $5 \cdot 10^{-7}$ Pa for 20 °C and $1 \cdot 10^{-6}$ Pa for 25 °C were calculated by linear regression analysis and extrapolation.
Comment:	The study is acceptable. The Henry's law constant amounts to $1.1 \cdot 10^{-4}$ Pa · m^3 /mole at 20 °C based on this vapour pressure.

B.2.1.9.2 Photochemical degradation

(c) Sneikus, J.: Photolysis of [phenyl-UL-^{14}C]Azinphos-methyl in sterile water at pH 7 under controlled light conditions. Bayer AG, Report Nos.: MR-133/00, MO-01-001879, 1 February 2001, WAS2002-75

Guideline: SETAC (1995)

GLP: yes

Test substance:	[phenyl-UL-^{14}C]azinphos-methyl, radiochemical purity: >99 %, 3.24 MBq/mg, non-labelled azinphos-methyl, purity: 99.5 %
Test system:	
Irradiation:	®Suntest unit (Heraeus Original Hanau) fitted with a Xenon lamp (Nxe 1500B) and a special UV glass filter (lower limit of wavelength: 290 nm), continuous irradiation up to 30 h

	The spectral light energy distribution in the wavelength range of 295 - 400 nm) and the light intensity (mean 596 W/m^2) of the Xenon lamp is similar to midday sunlight at 40 ° latitude
Temperature:	25 ± 1 °C
Buffer:	Sterile 0.01 M phosphate buffer, pH 7
Concentration:	1 mg/l
Sampling:	Duplicate samples were taken 2, 5, 8, 24 and 30 hours post-treatment (dark controls after 24 and 48 h).
Analysis:	TLC, LSC, LC-MS/MS for identification

Findings:
The mass balances were in the range of 94.2 % and 100.4 % (mean 97.7 %). During the total irradiation period an amount equivalent to 1 % AR was photomineralized to carbon dioxide. Volatile organic compounds were not detected (< 0.01 % AR).
Photolysis resulted in rapid separation of the phosphorodithioic moiety and formation of the only major metabolite benzazimide (max. 50.8 % after 24 h; 47.4 % at termination) and some minor metabolites like methylbenzazimide (max. 1.9 % after 24 h) and anthranilic acid (max. 0.6 % after 24 h).
The experimental half-life of the direct photolysis of azinphos-methyl was calculated with 7.2 hours (rate constant = 0.0968/h; r^2 = 0.999) of continuous irradiation corresponding to an environmental half-life of e.g. 21 hours under solar conditions of Phoenix (Arizona) in June. Benzazimide degraded with an experimental half-life of 39 hours (rate constant = 0.0178/h; r^2 = 0.999, ®ModelManager) corresponding to an environmental half-life of 112 hours (4.7 days) at Phoenix.
The degradation of azinphos-methyl in the dark controls was much slower than in the irradiated samples. A portion of 94.1 % AR was recovered as parent compound at termination (48 hours).

Comment: The study is acceptable.
Direct photolysis of azinphos-methyl is a major route of degradation in aquatic systems, especially under Southern European conditions, as already predicted in the draft monograph, Volume 3, points B.2.1 and B.7.4.

B.2.3 References relied on

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner
AIIA-2.3	Krohn, J.	1995	Vapour pressure curve of azinphos-methyl. Bayer AG, Report Nos.: PC895, 14 215 0869, 27 June 1995, LUF98-50019	Y	BAY
AIIA-2.9.2	Sneikus, J.	2001	Photolysis of [phenyl-UL-^{14}C]Azinphos-methyl in sterile water at pH 7 under controlled light conditions. Bayer AG, Report Nos.: MR-133/00, MO-01-001879, 1 February 2001, WAS2002-75	Y	BAY

B.4 Methods of analysis

B.4.2 Analytical methods (residue) for food and feed (II 4.2.1; III 5.2.1)

B.4.2.1 Analytical methods (residue) for plants and plant products

The applicability of the DFG Method S 19 for the determination of Azinphos-methyl residues in plant material (apple, potato, olive) was examined (Report 69093/98, Weeren, R.D.; Pelz, S.; 1998). The residues in apple and potato were analysed using modified extraction (Fres. J. Anal. Chem. 1995; 353, 183 – 190) and the extraction from olive was performed according to Ernst (Fres. J. Anal. Chem. 1974; 372, 358 – 363). Azinphos-methyl was determined by gas chromatography using flame photometric detection and conforming mass selective detection. Control samples were analysed in duplicate and fortified samples were analysed in quintuplet for each fortification level of 0.05 mg/kg and 0.5 mg/kg.
Mean recoveries per fortification and sample material were between 86 % and 107 % with single values between 76 % and 109 %. The relative standard deviations ranged from 1.9 % to 8.7 %. The limit of quantification was 0.05 mg/kg for apple, potato and olive.

B.4.2.2 Analytical methods (residue) for food of animal origin

No new methods were submitted.

B.4.3 Analytical methods (residue) for soil, water and air (II 4.2.2-II4.2.4; III 5.2.2-III 5.2.4)

Water
The method 00581 (Sommer, 1999) for determination of azinphoz-methyl in drinking water and surface water was developed in accordance to the multi residue methods of the Deutsche Institut für Normung (DIN; Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlamm-Untersuchung, Physikalische, chemische, biologische und bakteriologische Verfahren, Band V, VCH Weinheim, Beuth Verlag, Berlin 1996, Gruppe F: Gemeinsam erfassbare Stoffgruppen) and of the European Committee for Standardization (CEN) under special consideration of the gas chromatographic method F6 and F14.
Azinphos-methyl is concentrated from water samples by solid phase extraction. After drying of the cartridges azinphos-methyl is eluted, concentrated to dryness and reconstituted. Identification and quantitative determination is done by gas chromatography using PND detection.
Control samples were analysed in duplicate and fortified samples were analysed in quintuplet for each fortification level of 0.03 µg/L and 0.3 µg/L. For confirmation of positive results of azinphos-methyl a second GC column with different polarity was used.
The mean recoveries for the different columns used, the different matrices and fortification levels ranged between 90 % and 110 % with relative standard deviations between 1.6 % and 7.3 %.The limit of quantitation was 0.03 µg/L for drinking water and surface water.

Air
A method 00555 (Riegner, 1999) was developed on basis of method 00391 (MR-328/95) in order to support a LOQ on basis of toxicological parameters. Azinphos-methyl was adsorbed on Tenax tubes, extracted by acetonitrile and determined after liquid chromatographic

separation by means of a UV-detector. In addition, the analytical method was confirmed by using GC-MS as a second method (m/z = 77, 132, 160).
For method validation 2 control samples, 5 samples fortified at 0.5 µg/m³ and 2 samples fortified at 0.3 mg/m³ were analysed. The mean recovery was 92 % for the LOQ of 0.5 µg/m³ with a relative standard deviation of 4 %. The mean recovery for samples fortified at 0.3 mg/m³ was 96 %.

B.4.4 Analytical methods (residue) for human and animal body fluids and tissues (II 4.2.5; III 5.2.5

Method EM F-05/98 (Report No. A 67646, Frenzel, T. et al., 1998) was developed to determine azinphos-methyl in blood. The method was validated by an independent laboratory (MR-918/98, Brennecke, R., 1998).Whole blood was hemolysed and then deproteinised. After extraction of the supernatant, blood levels were determined by GC-MS in the SIM-mode. Quantification was performed by the ratio of the peak height of azinphos-methyl to that of the internal standard (bromophos-methyl). For validation, 5 samples at each fortification level were analysed. Recoveries for the different fortification levels ranged from 82 % to 99 % with standard deviations in the range of 3.7 % to 13 %. The limit of quantification was 0.05 mg/L for blood.

B.4.6 References relied on

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner
AIIA-4.2.1	Weeren, R.D.; Pelz, S.	1998	Validation of DFG Method S 19 with modified extraction (with extraction according to Ernst for Olive) for the determination of Azinphos-methyl in plant material (apple, potato, olive) MET2000-2		BAY
AIIA-4.2.3	Sommer, H.	1999	Enforcement and confirmatory method for determination of Azinphos-methyl in drinking water and surface water by GC/PND; Method 00581 / MR-220/90 MET2000-4		BAY
AIIA-4.2.4	Riegner, K.	1999	Method for the determination of Azinphos-methyl in air by HPLC-UV and confirmation of the method by GC-MS Method 00555 / MR-880/98 MET2000-5		BAY
AIIA-4.2.5	Frenzel, T; Sochor, H.; Speer, K.; Uihlein, M.	1998	Rapid multimethod for determination fo toxic pesticides in whole blood by means of capillary GC-MS Method EM F-05/98, Report No. A67646 MET2000-6		BAY

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner
AIIA-4.2.5	Brennecke, R	1998	Independent laboratory validation of method EM F-05/98-0 " Rapid multimethod for determination of toxic pesticides in whole blood by means of capillary GC-MS" according to European guidelines Report No. MR918-98 MET2000-3		BAY

B.6 Residue data

B.6.4 Use pattern

The evaluation in the monograph (18 September 1996) was based on registered uses, status June 1994. The evaluation in the addendum is based on intended uses of azinphos-methyl reported in Table B.6.4-1.

Table B.6.4-1 Uses intended in the European Community (state February 2002)

Crop	Area	Country	Application rate [kg as/ha]	No. of applications per season	Water rate [l/ha]	Pre-harvest interval [days]	Spray interval [days]
Apples	N/S	France	0.44 – 0.50	1 – 3	500 l /m plant	21	20
	S	Greece	0.60 – 0.75	1 – 3	height up to a	21	14 - 21
	S	Italy	0.60 – 0.75	3	maximum of	21	20
	S	Portugal	0.50 – 0.60	1 – 3	1500 l/ha	21	20
	S	Spain	0.50 – 0.75	1 – 3		21	20
Pears	S	Italy	0.60 – 0.75	3	500 l /m plant	21	20
	S	Portugal	0.50 – 0.60	1 – 3	height up to a	21	20
	S	Spain	0.50 – 0.75	1 – 3	maximum of 1500 l/ha	21	20
Potatoes	S	Italy	0.40 – 0.50	2	800	21	8
	S	Portugal	0.24	1 – 2	800	21	21
	S	Spain	0.12 – 0.40	1 – 2	800	21	8

B.6.5 Identification of critical GAPs

From the intended uses the critical ones are selected in Table B.6.5-1.

Table B.6.5-1 List of critical intended uses (state Februar 2002)

Crop	Area	Application rate [kg as/ha]	No. of applications per season	Water rate [l/ha]	Pre-harvest interval [days]	Spray interval [days]
Apples	N	0.50	3	500 l /m plant height	21	20
	S	0.75	3	up to a maximum of	21	14 - 21
Pears	S	0.75	3	1500 l/ha	21	20
Potatoes	S	0.50	2	800	21	8

B.6.6 Residues resulting from supervised trials (Annex IIA 6.3; Annex IIA 8.2)

B.6.6.1 Residue analytical methods – analytical storage stability

Study title:
Lenz, C. A. (1995): **Azinphos-methyl – Freezer Storage Stability Study in Crops.** Bayer Study No. GU131601, Bayer Report No. 106796.
BBA reference no. RIP 2002-225.

Guideline:
No information received.

GLP compliance:
A statement of compliance that the study was conducted in accordance with Good Lahoratory Standards and quality assurance statement for periodical inspections by the Quality Assurance Unit are available.

Material and methods:
A freezer storage stability study was conducted using 29 matrices: green alfalfa, alfalfa hay, almond nutmeat, almond hull, apple, blackeyed pea, cherry, cucumber, cotton seed, cotton seed hull, cotton seed meal, cotton seed crude oil, cotton seed refined oil, cotton seed soapstock, cantaloupe, pepper, orange, orange dried pulp, orange juice, orange molasses, orange oil, potato, potato chip, potato dry peel, potato granule, potato wet peel, strawberry, sugarcane and tomato. Forty samples (10 g) of each matrix were weighed, 18 from them were fortified with azinphos-methyl at 1 mg/kg and were held in frozen storage (-23 to –27°C). Three samples of each matrix were fortified and analyzed immediately as the 0-time samples. The remaining 19 samples of each matrix were not fortified and served as concurrent recoveries for the 0-time and each subsequent sampling interval.
At 3, 6, 12, 18 and 24 months, two unfortified samples and two fortified samples were removed from frozen storage and analyzed. Prior to analysis, one of the unfortified samples was fortified with azinphos-methyl and used as concurrent recovery. The other unfortified sample was analyzed as a control.
Samples were extracted by blending with acetone and water and then partitioned with chloroform. Additional sample clean-up was conducted using silica gel chromatographic column for almond nutmeat, cotton seed (soapstock, crude oil, refined oil) and orange oil. The residues were quantitated using GLC/FPD in the phosphorus mode. The lowest fortification level for recoveries was 0.3 mg/kg. Control interferences observed in all of the 29 crop matrizes were <0.3 mg/kg. Therefore, the LOQ of all the crop matrices analysed was 0.3 mg/kg.

Results:
With 24 months of frozen storage, the average percent decomposition for each matrix was as follows: green alfalfa (0%), alfalfa hay (11%), almond nutmeat (9%), almond hull (1%), apple (2%), blackeyed pea (19%), cherry (27%), cucumber (2%), cotton seed (7%), cotton seed hull (0%), cotton seed meal (0%), cotton seed crude oil (0%), cotton seed refined oil (0%), cotton seed soapstock (9%), cantaloupe (6%), pepper (4%), orange whole fruit (7%), orange dried pulp (9%), orange juice (0%), orange molasses (0%), orange oil (4%), potato tuber (12%), potato chip (0%), potato dried peel (16%), potato granule (6%), potato wet peel (0%), strawberry (5%), sugarcane (0%) and tomato whole fruit (3%). The individual results for the intended uses pome fruit (apple) and potatoes are reported in Tables B.6.6-1 and B.6.6-2.

Table B.6.6-1 Summary of freezer stability data for azinphos-methyl in apple

Storage period [days]	Azinphos-methyl residues found in fortified sample (mg/kg)	Corresponding control (mg/kg)	% Remaining after storage[1]	% Concurrent analytical recovery[1]	% Remaining after storage corrected by recovery[2]	% Average decomposition
0	1.03	0.07	96	96	100	
0	1.09	0.07	102	102	100	
0	1.08	0.07	101	101	100	0
91	1.0	0.08	92	104	88	
91	1.0	0.08	93	104	89	11
183	0.91	0.06	85	86	99	
183	0.97	0.06	91	86	106	0
414	1.08	0.06	102	102	100	
414	1.08	0.06	103	102	101	0
546	1.49	0.09	139	115	121	
546	1.28	0.09	118	115	103	0
730	1.06	0.05	101	103	98	
730	1.05	0.05	100	103	97	2

1) All recovery values corrected for corresponding control values
2) Corrected for concurrent recovery

Table B.6.6-2 Summary of freezer stability data for azinphos-methyl in potato

Storage period [days]	Azinphos-methyl residues found in fortified sample (mg/kg)	% Remaining after storage[1]	% Concurrent analytical recovery[1]	% Remaining after storage corrected by recovery[2]	% Average decomposition
0	1.06	106	106	100	
0	1.07	107	107	100	
0	1.02	102	102	100	0
92	0.97	97	84	115	
92	1.03	103	84	123	0
186	0.87	87	92	95	
186	0.88	88	92	96	4
414	0.88	88	89	99	
414	0.88	88	89	99	1
553	0.92	92	92	100	
553	0.94	94	92	102	0
739	0.82	82	94	87	
739	0.84	84	94	89	12

1) Control values were not detected
2) Corrected for concurrent recovery

Comment of the RMS:
The study is accepted.

Study title:
Lemke, V. J. (1996): **Addendum 1. Azinphos-methyl – Freezer Storage Stability Study in Crops. Data for 36 months.** Bayer Study No. GU131601, Bayer Report No. 106796-1.
BBA reference no. RIP 2002-311.

Guideline:
No information received.

GLP compliance:
A statement of compliance that the study was conducted in accordance with Good Laboratory Standards and quality assurance statement for periodical inspections by the Quality Assurance Unit are available.

Material and methods:
For study design and analytical methods see study above by Lenz (1995, BBA reference no. RIP 2002-225).

In the study by Lenz (1995) the storage stability data of 29 matrices for 24 months are described. In the addendum by Lemke (1996) the same 29 matrices fortified with 1 mg/kg azinphos-methyl were analyzed at a nominal 36 months of frozen storage (-12 to -27°C).

Results:
With 36 months of freezer storage, the average percent decomposition for each matrix was as follows: green alfalfa (1%), alfalfa hay (0%), almond nutmeat (0%), almond hull (0%), apple (0%), blackeyed pea (0%), cherry (4%), cucumber (0%), cotton seed (0%), cotton seed hull (7%), cotton seed meal (0%), cotton seed crude oil (6%), cotton seed refined oil (3%), cotton seed soapstock (0%), cantaloupe (0%), pepper (0%), orange whole fruit (0%), orange dried pulp (0%), orange juice (0%), orange molasses (0%), orange oil (1%), potato tuber (0%), potato chip (2%), potato dried peel (14%), potato granule (2%), potato wet peel (0%), strawberry (0%), sugarcane (0%) and tomato whole fruit (0%).

Comment of the RMS:
The addendum shows that the % remaining is in some matrices higher after 36 than after 24 months of freezer storage (e. g. decomposition cherry 4% *vs* 27%, potato tuber 0% *vs* 12%). This could be an artefact based on the loss of water by freeze-drying. Nevertheless, all in all the results of the 24-months-study were confirmed by the results of the 36-months-study.

B.6.6.4 Residues in pome fruit

Six (South) and eight (North) new residue trials on apples as well as two on pears (South) conducted in 1999/2000 in France, Greece, Italy, Belgium and Germany were received (see Tables B.6.6-3 and B.6.6-4). The apple and pear samples were held in deep-frozen storage until analysis for a minimum of 4 and a maximum of 17 months. Nevertheless, the freezer storage stability shows that azinphos-methyl residues in apples are stable for at least two years (see B.6.6.1). Underlined residues are from treatments according to GAP (± 25%) and are valid for estimating maximum residue levels. The further older trials on apples and pears reported in Tables B.6.6.4-1 and B.6.6.4-2 of the 1996 monograph did not match GAP.

Table B.6.6-3 Residues of azinphos-methyl in apples

APPLES country, year (variety)	Application Form	kg ai/ha	kg ai/hl	water, l/ha	no.	interval, days	PHI days	Residues, mg/kg	Storage until analysis	Reference BBA reg. no.
South Greece, 2000 (Starkrimson)	WP	0.74	0.068	1100	3	21 21	0 21	0.40 0.09	7 months	Sur, R. RA-2135/00, R 2000 0072/3 RIP 2002-145
South Spain, 2000 (Golden)	WP	1.01	0.068	1500	3	21 20	control control 0 21	0.19 <0.04 0.78 0.38	7 months	Sur, R. RA-2135/00, R 2000 0391/9 RIP 2002-145
South Spain, 1999 (Suprema)	WP	1.01	0.068	1500	3	21 22	0 8 14 21 28 35	0.69 0.30 0.25 0.15 0.10 0.07	17 months	Spiegel, K., Hoffmann, M. RA-2135/99, R 1999 0559/9 RIP 2002-143
South France, 1999 (Braeburn)	WP	0.84 0.84 0.79	0.068	1250 1250 1175	3	21 21	0 7 14 21 28 35	1.0 0.92 0.54 0.19 0.13 0.15	17 months	Spiegel, K., Hoffmann, M. RA-2135/99, R 1999 0560/2 RIP 2002-143
South Italy, 1999 (Florina)	WP	0.84 0.84 1.01	0.068	1250 1250 1500	3	21 22	0 7 14 21 28 35	0.35 0.06 <0.04 <0.04 <0.04 <0.04	17 months	Spiegel, K., Hoffmann, M. RA-2135/99, R 1999 0561/0 RIP 2002-143
South Greece, 1999 (Granny Smith)	WP	1.01	0.068	1494 1500 1500	3	22 20	0 7 14 21 28 35	0.34 0.15 0.19 0.11 0.06 <0.04	17 months	Spiegel, K., Hoffmann, M. RA-2135/99, R 1999 0562/9 RIP 2002-143
North Germany, 2000 (Jonagold)	WP	0.675	0.068	1000	3	20 21	0 21	0.94 0.21	4 months	Sur, R., Hoffmann, M. RA-2136/00, R 2000 0085/5 RIP 2002-144
North Germany, 2000 (Kent)	WP	0.675	0.068	1000	3	20 21	0 21	0.64 0.11	4 months	Sur, R., Hoffmann, M. RA-2136/00, R 2000 0388/9 RIP 2002-144
North Belgium, 2000 (Jonagold)	WP	1.01	0.068	1500	3	22 20	0 21	0.49 0.15	4 months	Sur, R., Hoffmann, M. RA-2136/00, R 2000 0389/7 RIP 2002-144
North France, 2000 (Reine des Reinettes)	WP	1.01	0.068	1500	3	21 21	0 21	0.91 0.24	4 months	Sur, R., Hoffmann, M. RA-2136/00, R 2000 0390/0 RIP2002-144

APPLES country, year (variety)	Application Form	kg ai/ha	kg ai/hl	water, l/ha	no.	interval, days	PHI days	Residues, mg/kg	Storage until analysis	Reference BBA reg. no.
North Germany, 1999 (Jonagold)	WP	0.675	0.068	1000	3	21 21	0 7 14 21 28 35	0.49 0.23 0.20 0.10 0.09 0.06	15 months	Spiegel, K., Hoffmann, M. RA-2136/99, R 1999 0563/7 RIP 2002-142
North Germany, 1999 (Kent)	WP	0.675	0.068	1000	3	21 21	0 7 14 21 28 35	0.47 0.12 0.09 0.05 <0.04 <0.04	15 months	Spiegel, K., Hoffmann, M. RA-2136/99, R 1999 0564/5 RIP 2002-142
North UK, 1999 (Golden Delicious)	WP	0.675	0.068	1000	3	21 21	0 7 14 21 28 35	0.37 0.35 0.18 0.20 0.13 0.14	15 months	Spiegel, K., Hoffmann, M. RA-2136/99, R 1999 0565/3 RIP 2002-142
North France, 1999 (Idared)	WP	0.675	0.068	1000	3	21 21	0 6 14 21 28 35	0.70 0.45 0.24 0.15 0.16 0.12	15 months	Spiegel, K., Hoffmann, M. RA-2136/99, R 1999 0566/1 RIP 2002-142

Table B.6.6-4 Residues of azinphos-methyl in pears

PEARS country, year (variety)	Application Form	kg ai/ha	kg ai/hl	water, l/ha	no.	interval, days	PHI days	Residues, mg/kg	Storage until analysis	Reference BBA reg. no.
South Italy, 2000 (William)	WP	1.01	0.068	1500	3	23 21	0 21	0.52 <0.04	7 months	Sur, R. RA-2135/00, R 2000 0392/7 RIP2002-145
South France, 2000 (Guyot)	WP	0.81	0.068	1200	3	21 21	control control 0 21	0.05 <0.04 0.59 0.19	7 months	Sur, R. RA-2135/00, R 2000 0393/5 RIP2002-145

Findings

North EC (France, apples only)

GAP:

0.5 kg as/ha, 1500 l water/ha, 3 applications, interval 20 days, PHI 21days

Supporting residue data: 0.05, 0.1, 0.11, 0.15, 0.2, 0.21 mg/kg

South EC

GAP:

0.75 kg as/ha, 1500 l water/ha, 3 applications, interval 20 days, PHI 21 days

Supporting residue data (apples): <0.04, 0.09, 0.11, 0.15, 0.19, 0.38 mg/kg

Supporting residue data (pears): <0.04, 0.19 mg/kg

All residue data in rank order: <0.04, <0.04, 0.09, 0.11, 0.15, 0.19, 0.19, 0.38 mg/kg

MRL calculation (North, apples only)
R_{max}: 0.366
R_{cal}: 0.405

STMR: 0.13 mg/kg
HR: 0.21 mg/kg
$MRL_{proposal}$: 0.5 mg/kg

MRL calculation (South, apples and pears)
R_{max}: 0.501
R_{cal}: 0.380

STMR: 0.13 mg/kg
HR: 0.38 mg/kg
$MRL_{proposal}$: 0.5 mg/kg

Assessment
The critical intended use is supported by residue supervised trials data. Based on these data, an MRL of 0.5 mg/kg is recommended for pome fruit.

B.6.6.9 Residues in potatoes

No new residue data were received. Four Italian trials (1992) reported in Table B.6.6.9-1 of the 1996 monograph matched GAP. No further trials were required for evaluation because it is the overground green plant which is treated, and there is no translocation of azinphos-methyl into the tubers.

Findings
South EC
GAP:
0.5 kg as/ha, 800 l water/ha, 2 applications, interval 8 days, PHI 21 days
Supporting residue data: <0.04 (4) mg/kg

STMR: 0 mg/kg
HR: 0 mg/kg
$MRL_{proposal}$: 0.05* mg/kg

Assessment
The results of all trials show that the residues in potatoes are lower than the LOQs (<0.04 (4) mg/kg). Based on the 'nil-residue situation' an STMR of 0 and an MRL of 0.05* mg/kg is recommended for potatoes.

B.6.7 Effects of industrial processing and/or honsehold preparation (Annex IIA 6.5; Annex IIIA 8.4)

Apples. The following processing factors were estimated in the 1996 monograph (Table B.6.7-1): apple juice 0.35, wet pomace 2.1, dry pomace 5.9. For dietary intake calculation, the STMR-P of apple juice as processed commodity is needed. Furthermore, wet apple pomace is used as feed item. Based on the new STMR and HR values of 0.13 and 0.38 mg/kg for the raw commodity, the following STMR-P and HR-P values were estimated (values were not rounded as interim results):

Apple juice: STMR-P = 0.0455 mg/kg
Apple wet pomace: STMR-P = 0.273 mg/kg HR-P = 0.798 mg/kg

B.6.12 Proposed EU MRLs and justification for the acceptability of those MRLs (Annex IIA 6.7; Annex IIIA 8.6)

Plant commodities. The re-evaluation of all residue trials received for the current intended uses of azinphos-methyl shows that MRLs of 0.5 and 0.05* mg/kg can be recommended for pome fruit and potatoes.

Animal commodities. Apple wet pomace is the only commodity containing relevant residues which is used as animal feed item. For such a processed commodity, which is likely to originate from a number of farms, the STMR-P (0.273 mg/kg) instead of the HR-P is chosen as the likely highest residue to occur in practice to calculate the animal dietary burden (see Table B.6.12-1).

Table B.6.12-1 Farm animal dietary burden estimation for azinphos-methyl

	% dry matter (DM)	Chicken	Dairy Cattle	Beef Cattle	Pig	Residue (mg/kg)	Intake (mg/kg) dry matter basis			
							Chicken	Dairy Cattle	Beef Cattle	Pig
Body weight		1.9 kg	550 kg	350 kg	75 kg					
Daily maximum feed (DM)		0.12 kg	20 kg	15 kg	3 kg					
Maximum percentage		% DM	% DM	% DM	% DM					
I. Green forage (incl. hay)										
Grasses	20	0	100	100	0	0	0	0	0	0
Alfalfa/clover	20	0	40	40	15	0	0	0	0	0
Forage rape	14	0	0	35	15	0	0	0	0	0
Kale/cabbage	14	5	35	35	15	0	0	0	0	0
Sugar beet (leaves and tops)	16	0	30	30	25	0	0	0	0	0
Silage	20	0	100	100	15	0	0	0	0	0
Fruit pomace (apples)	23	0	10	30	0	0.273	0	0.119	0.356	0
Hay	85	0	100	100	15	0	0	0	0	0
II. Grains										
Grains except maize	86	70	40	80	80	0	0	0	0	0
Maize	86	70	30	30	40	0	0	0	0	0
Bran (wheat and rye)	89	15	20	20	20	0	0	0	0	0
III. Straw (cereals)	86	0	20	50	0	0	0	0	0	0
IV. Pulses	86	30	20	20	40	0	0	0	0	0
V. Root and tubers (e.g. potatoes)	15	20	30	60	60	0	0	0	0	0
Swede/turnip	10	20	30	60	60	0	0	0	0	0
VI. Oil seed (meal, cake)	86	10	30	30	20	0	0	0	0	0
Intake (mg/kg feed)							0	0.119	0.356	0
Intake (mg/kg bw)							0	0.004	0.015	0
Intake (mg/animal)							0	2.380	5.340	0

The dairy cattle feeding study described in the 1996 monograph (Wargo, 1978) was carried out with a dosage of 11, 33 and 77 mg/kg feed. No residues of azinphos-methyl and azinphos-methyl oxygen analogue were found by GLC and HPLC in tissues (<0.01 mg/kg) and milk (<0.001 mg/l, LOQ reported in the study, but not validated).
Based on the new intended uses (status February 2002), the animal dietary burden was estimated for dairy cattle as 0.12 mg/kg feed (0.004 mg/kg bw) and for beef cattle as 0.36 mg/kg feed (0.02 mg/kg bw). The results of the feeding study and the animal dietary burden calculation show that in practice no detectable residues are to be expected in milk and cattle

tissues. The MRL recommendation of the 1996 monograph at the LOQ was confirmed: 0.02* mg/kg for milk and 0.05* mg/kg for cattle meat, meat by-products, fat, others.

The only feed item relevant for poultry is potato. Because of the 'nil residue situation' in potato, in practice no detectable residues are to be expected in eggs and other poultry products. The MRL recommendation of the 1996 monograph at the LOQ was confirmed: 0.05* mg/kg for eggs, poultry meat, meat by-products, fat, others.
The MRL-proposals are summarized in Table B.6.12-2.

Table B.6.12-2 Community MRLs, proposed MRLs, STMR, and HR values arising from the use of azinphos-methyl

Commodity	EC-MRL	MRL proposal, mg/kg	STMR, mg/kg	HR, mg/kg
Pome fruit	0.5	0.5	0.13	0.38
Potatoes	-	0.05*	0	0
Milk	-	0.02*	0	-
Eggs	-	0.05*	0	0
Other products of animal origin	-	0.05*	0	0

B.6.13 Estimates of potential and actual dietary exposure through diet and other means (Annex IIA 6.9; Annex IIIA 8.8)

B.6.13.1 Long-term dietary risk assessment

The dietary risk assessment on a national basis (NTMDI, German model, Table B.6.13-1) and international level (TMDI, WHO, European diet, Table B.6.13-2) is based on an ADI value of 0.005 mg/kg bw for azinphos-methyl. Where an MRL could not be recommended, the LOQ of 0.05 mg/kg (or 0.1 mg/kg for tea and hops) is used in the dietary risk calculation. Based on the recommended MRLs and the LOQs, the NTMDI and the TMDI were calculated as 70% and 35% of the ADI.

Table B.6.13-1 NTMDI calculation (German model)

ADI (mg/kg bw): 0.005					
Mean food consumption (g/d) of a 4-to-6-year-old girl					
Food	raw[1]	processed[2]	whole	MRL (mg/kg)	Intake (mg/kg) bw
1. FRUITS AND TREE NUTS	72.0	89.3	161.3		
Citrus fruit and citrus juice	18.0	26.5	44.5	0.05	0.00016481
Tree nuts	1.4	3.8	5.2	0.05	0.00001926
Pome fruit	13.0	35.6	48.6	0.5	0.00180000
Stone fruit	8.7	10.7	19.4	0.05	0.00007185
Berries and small fruit[3]	9.0	8.6	17.6	0.05	0.00006519
Miscellaneous fruit	21.3	3.5	24.8	0.05	0.00009185
Dry fruits	0.6	0.6	1.2	0.05	0.00000444
2. VEGETABLES	33.0	75.4	108.4	0.05	0.00040148
3. PULSES		1.5	1.5	0.05	0.00000556
4. OIL SEEDS	0.3	11.0	11.3	0.05	0.00004185
5. POTATOES		71.1	71.1	0.05	0.00026333
6. TEA*					
7. HOPS*					
8. CEREALS	0.2	107.8	108.0	0.05	0.00040000
9. SPICES (without ginger)	0.1	0.1	0.2	0.05	0.00000074
10. GINGER		0.2	0.2	0.05	0.00000074
11. TEA LIKE PRODUCTS		0.3	0.3	0.1	0.00000222
12. COCOA BEANS		29.4	29.4	0.1	0.00021778
13. SUGAR BEET		0.3	0.3	0.05	0.00000111
Intake whole (mg/kg bw):	**0.0036**				
Percent of ADI (%):	**71.04**				
Mean food consumption (g/d) of a 36-to-50-year-old woman					
Food	raw[1]	processed[2]	whole	MRL (mg/kg)	Intake (mg/kg) bw
Wine grapes (wine)		97.6	97.6	0.05	0.00008133
Tea		1.1	1.1	0.1	0.00000183
Hops		4.9	4.9	0.1	0.00000817
Coffee beans (raw)		26.5	26.5	0.05	0.00002208
Intake whole (mg/kg bw):	**0.0001**				
Percent of ADI (%):	**2.27**				

Explanations:
1. raw = without any preparation/processing
2. processed = e.g. washed, peeled, cooked, baked, preserves
3. strawberries, cane fruit and other small fruit and berries without wild fruit and wild berries
* Food which is normally not consumed by a 4-to-6-year-old girl

Table B.6.13-2 TMDI calculation (WHO, European diet)

ADI (mg/kg bw): 0.005 Mean food consumption in g/d (WHO European diet (1994))			
Food	Consumption (g/day)	MRL (mg/kg)	Intake (mg/kg bw)
FOOD OF PLANT ORIGIN	1252.1		
1. FRUITS AND TREE NUTS	288.7		
Citrus fruit	49.1	0.05	0.00004092
Tree nuts	4.1	0.05	0.00000342
Pome fruit	51.3	0.5	0.00042750
Stone fruit	23.4	0.05	0.00001950
Berries and small fruit[1]	122.1	0.05	0.00010175
Miscellaneous fruit	38.7	0.05	0.00003225
2. VEGETABLES	339.3	0.05	0.00028275
3. PULSES	9.4	0.05	0.00000783
4. OIL SEEDS	26.0	0.05	0.00002167
5. POTATOES	240.8	0.05	0.00020067
6. TEA	2.3	0.1	0.00000383
7. HOPS[2]	4.9	0.1	0.00000817
8. CEREALS	223.3	0.05	0.00018608
9. SPICES	0.3	0.05	0.00000025
10. GINGER			
11. TEA LIKE PRODUCTS			
12. COCOA BEANS	3.1	0.05	0.00000258
13. SUGAR BEET	106.1	0.05	0.00008842
14. COFFEE BEANS	7.9	0.05	0.00000658
FOOD OF ANIMAL ORIGIN	608.5		0.00000000
Eggs	37.5	0.05	0.00003125
Milk	342.7	0.02	0.00011423
Meat	205.3	0.05	0.00017108
Edible offals	12.6	0.05	0.00001050
Fat	10.4	0.05	0.00000867
Intake whole (mg/kg bw):	**0.00177**		
Percent of ADI (%):	**35.4**		
Explanations:			
1. strawberries, cane fruit and other small fruit and berries without wild fruit and wild berries			
2. value from German food consumption			

The NEDI (German model, Table B.6.13-3) and IEDI (WHO, European diet, Table B.6.13-4) were calculated based on the STMR values for the intended uses. The IEDI/NEDI was estimated at about 2-10% of the ADI. It can be concluded that the long-term intake of residues of azinphos-methyl resulting from uses that have been considered is unlikely to present a public health concern.

Table B.6.13-3 NEDI calculation (German model)

ADI (mg/kg bw): 0.005 Mean food consumption (g/d) of a 4-to-6-year-old girl					
Food	raw[1]	processed[2]	whole	STMR (mg/kg)	Intake (mg/kg) bw
Pome fruit	13.0	35.6	48.6	0.13	0.000468
Potatoes		71.1	71.1	0	0
Intake whole (mg/kg bw):	**0.0005**				
Percent of ADI (%):	**9.36**				
Explanations:					
1. raw = without any preparation/processing					
2. processed = e.g. washed, peeled, cooked, baked, preserves					

Table B.6.13-4 IEDI calculation (WHO, European diet)

ADI (mg/kg bw): 0.005 Mean food consumption in g/d (WHO European diet (1994))			
Food	Consumption (g/day)	STMR (mg/kg)	Intake (mg/kg bw)
FOOD OF PLANT ORIGIN			
Pome fruit	51.3	0.13	0.00011115
Potatoes	240.8	0	0
FOOD OF ANIMAL ORIGIN			
Eggs	37.5	0	0
Milk	342.7	0	0
Meat	205.3	0	0
Edible offals	12.6	0	0
Fat	10.4	0	0
Intake whole (mg/kg bw):	**0.000111**		
Percent of ADI (%):	**2.22**		

B.6.13.2 Short-term dietary risk assessment

The short-term dietary intake was calculated according to the deterministic model described by the 2000 JMPR *(Pesticide residues in food – Report 2000, FAO Plant Protection Paper 163, Rome, 2001)* and is based on the acute reference dose (ARfD) of 0.075 mg/kg bw proposed by the RMS and UK consumption data (see Table B.6.13-5). The NESTI represented 0 – 10 % of the ARfD for adults and 0 – 40 % of the ARfD for toddlers. It can be concluded that the short-term intake of residues of azinphos-methyl, resulting from uses that have been considered, is unlikely to present a public health concern.

Table B.6.13-5 National estimate of short-term intake (NESTI)

ARfD (mg/kg bw): 0.075								
Food	Portion size	Unit weight	Processing factor	Variability factor	HR (mg/kg)	STMR-P (mg/kg)	Intake (mg/kg) bw	Percent of ARfD (%)
Food portion sizes of UK toddlers aged 1.5 to 4.5 years (97.5th percentile)								
Apples, fruit	199	112	1	7	0.38		0.02282621	30.43
Apples, juice	559					0.0455	0.00175410	2.34
Pears	279	150	1	7	0.38		0.03089793	41.20
Potatoes	227	216	1	7	0		0	0
Food portion sizes of UK adults aged 16 to 64 years (97.5th percentile)								
Apples, fruit	308	112	1	7	0.38		0.00531241	7.08
Apples, juice	452					0.0455	0.00029338	0.39
Pears	274	150	1	7	0.38		0.00636405	8.49
Potatoes	684	216	1	7	0		0	0

B.6.15 References relied on

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner
AIIA-6; AIIIA-8	Lenz, C.A.	1995	Azinphos-methyl – Freezer storage stability study in crops. Bayer study no.: GU 131601 Bayer report no.: 106796 GLP Unpublished RIP 2002-225	Y	BAY
AIIA-6; AIIIA-8	Lemke, V.J.	1996	Azinphos-methyl – Freezer storage stability study in crops. Data for 36 months. Bayer study no.: GU 131601 Bayer report no.: 106796-1 GLP Unpublished RIP 2002-311	Y	BAY
AIIA-6.3; AIIIA-8.2	Spiegel, K. Hoffmann, M.	2001	Determination of residues of azinphos-methyl after spray application of Gusathion 25 WP to apple trees in Germany, Great Britain and France. RA-2136/99 (0563-99, 0564-99, 0565-99, 0566-99) GLP Unpublished RIP2002-142	Y	BAY
AIIA-6.3; AIIIA-8.2	Spiegel, K. Hoffmann, M.	2001	Determination of residues of azinphos-methyl after spray application of Gusathion 25 WP to apple trees in Spain, France, Italy and Greece. RA-2135/99 (0559-99, 0560-99, 0561-99, 0562-99) GLP Unpublished RIP2002-143	Y	BAY
AIIA-6.3; AIIIA-8.2	Sur, R. Hoffmann, M.	2001	Determination of residues of azinphos-methyl after spray application of Gusathion 25 WP to apple trees in Germany, Belgium and France . RA-2136/00 (0085-00, 0388-00, 0389-00, 0390-00) GLP Unpublished RIP2002-144	Y	BAY

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protectiou claimed Y/N	Owner
AIIA-6.3; AIIIA-8.2	Sur, R.	2001	Determination of residues of Gusathion 25 WP (a.s. azinphos-methyl) following spray application on apple and pear in the field in Greece, Spain, Italy and France. RA-2135/00 (0072-00, 0391-00, 0392-00, 0393-00) GLP Unpublished RIP2002-145	Y	BAY

B.9 Ecotoxicology

B.9.2 Effects on aquatic organisms (Annex IIA 8.2; Annex III 10.2)

B.9.2.1 Acute toxicity

Study title:
Hommen, U.(2000): **Calculation of a HC5 for the acute toxicity of azinphos-methyl to freshwater fish.** Bayer AG, Final Report from 2000-09-01.

Objektive:
For a refined risk assessment of possible effects of azinphos-methyl on freshwater fish species a sensitive distribution can be calculated to allow the estimation of a concentration protecting 95% of the species (HC5 = Hazardous Concentration for 5 % of species). Because only the acute toxicity of azinphos-methyl is under consideration here, this evaluation restricts to the acute toxicity on freshwater fish only.

Data used for calculation:
The US-EPA data base of aquatic toxicity data, Aquire (http://www.epa.gov/ecotox/), was searched for records concerning effects of azinphos-methyl (trade name in the US: Guthion, CAS No. 86500) on freshwater animals. Only records with complete documentation were considered. 94 records were found for fish. Possible duplications per species were identified if mean concentrations and test duration were equal. Four possible duplicates were excluded from the analysis. In addition, data from the open literature not included in the Aquire database were used, too. Seven additional 96 h LC50 values were found including two additional fish species.

Method for HC5-calculation:
The HC5 was calculated according the following formula:

$$\log(HC5) = \bar{x} - k_p s$$

where $\bar{x}$ and s are mean and standard deviation of the log transformed toxicity data and k is a factor depending on the number of data, the protection level (e. g. 95% for the HC5) and the confidence level (e. g. 50% for the median estimation of the HC5). HC5 values were calculated for acute toxicity, in particular for test duration of 24, 48 and 96 hours. If more than one record was available for a species, the geometric mean of the reported concentrations was used for further analysis.

Result:
The HC5 according Aldenberg & Jaworska (2000) declined with test duration from 2 µg as/l for 24 hours down to 0.535 µg as/l for 96 hours. Exposure time and sample size (number of species) suggest that **the 96 h-HC5 of 0.535 µg as/L** based on 21 species represents both, the most reliable and the safest HC5 estimation for the acute toxicity of azinphos-methyl to freshwater fish.

Comment of the RMS:
Because the original data used for the probabilistic approach of HC5 calculation were not submitted an evaluation of validity and plausibility of the values is not possible. In addition to that other fish toxicity data (available in the UBA data base) are relevant for risk assessment, too but not considered in the HC5 calculation. For this reason the result of the HC5 calculation is not accepted.

B.9.2.2 Chronic toxicity

Study title:
Carlisle, J. C. (1985): Toxicity of Azinphos-methyl (®Guthion Technical) to Rainbow Trout Early Life Stages. Mobay Chemical Corporation, Study No. 84-666-02)

Guideline:
Not reported

GLP compliance:
A statement of compliance that the study was conducted in accordance with Good Laboratory Standards and an quality assurance statement for periodically inspections by the Quality Assurance Unit are available.

Material and methods:
The study reported is the second of two studies. The first study was not used completely because of technical problems call its validity into question. In that study on test day 18 and 19 a diluter malfunction caused delivery of pure stock solution to the test vessels for up to 16 hours. This event was followed by much higher mortality in the replicate "A" of the second and third lowest concentrations (0.31 and 0.58 µg as/L) during the following three days. Consequently the study was repeated.

The 47 d ELS-test was conducted between September 1984 and October 1985. The test materials was azinphos-methyl (Guthion technical) with a content of active substance of 87.3%. The test vessels consisted of 20-liter stainless steel tanks Fifty fertilised eggs from *Oncorhynchus mykiss* were used for each of two replicates per test concentration. The nominal concentrations were 0.25, 0.5, 1, 2 and 4 µg as/L. The water temperature was maintained between 9.4 to 14.2 °C. Temperature and dissolved oxygen content of the test solutions were measured daily. Dead fish were removed and counted as well as hatched fry and swimups daily. On test termination all survivors in each chamber were sacrificed and weighted to the nearest milligram. The concentrations of active substance in the test solutions was determined by liquid chromatographic analysis. The percent hatch, survival to the swim up stage, survival to term, final mean weight and bio mass in each concentration were compared with that in the control group using analysis of variance.

Results:
The mean measured concentrations ranged from 94 to 154% of nominal concentrations. The results are given on basis of mean measured concentrations. There was no concentration-related effect on hatching or survival to swim up stage. Final survival was significantly less than control survival in the 1.14 to 4.75 µg as/L exposure groups. Therefore the highest no-effect concentration (NOEL) for survival was 0.47 µg as/l. Final mean fish weight and bio mass (as product of survival and mean weight) were reduced in the 0.29 to 4.75 µg as/L exposure groups. Only one replicate of the 0.29 µg as/L group was statistically separable from the controls, and the 13 % difference in that group is considered a marginal or threshold effect. In summary, the predicted EC10 for bio mass was 0.29 µg as/L.

Comment of the RMS:
Because of technical problems in the first test and appearance of effects in the lowest concentration group resulting in no definitive NOEC in the second study, both studies are assessed to be invalid. Summarising the results of both studies the 47 d - NOEC of 0.18 µg as/L from the first study seems to be plausible and the value is in the same order as valid ELS-Test with a 85 d - NOEC of 0.23 µg as/L (see Volume 3, Annex B.8.2.2-1 of the monograph).

B.9.2.3 Micro- or Mesocosm study

Study title:
Dorgerloh, M. and Sommer, H. (2001): **Gusathion M WP 25 – Indoor Microcosm (Water/Sediment) with Rainbow Trout (*Oncorhynchus mykiss*) Simulating Multiple Applications.** Bayer AG, Report No.: DOM 20081

Guidelines:
This indoor microcosm study was specially designed and the basic technical procedures followed as much as possible the internationally accepted guidelines Directive 92/69/EEC, C1 (1992) and OECD No. 203 (rev. 1992).

GLP compliance:
Yes (certified laboratory)

Material and methods:
The test was conducted between January 10 and March 02 in 2001. The test material was "Gusathion M WP 25" with a content of active substance of 24.2% azinphos-methyl. Young rainbow (*Oncorhynchus mykiss*) with a mean body wet weight of 1.57 g (range 0.6 to 2.6 g) were exposed under static conditions at water temperature of 13.2 to 14.6 °C to simulate repeated applications via spray drift. Aqueous solutions of "Gusathion M WP 25" were sprayed three times in weekly intervals directly on the surface of the indoor microcosm aquaria (total volume: 100 L, water column about 34 cm, sediment layer about 2 cm) in weekly intervals. Four concentrations levels with the following nominal concentrations (µg as/l) after each application were tested (A: 0.44, 0.44, 0.5; B: 0.88, 0.88, 1.0; C: 1.76, 1.76, 2.0 and D: 3.52, 3.52, 4.0). The 21 d test was implemented with 20 fish per test level and one replicate. The test included a water/sediment control and a water only control. Fish were fed daily with 24 h old larvae of *Artemia salina*. The test aquaria were aerated to avoid oxygen depletion below 60 % of the saturation value. Water quality parameters (pH, dissolved oxygen concentration and temperature) were measured daily, concentrations of nitrate and nitrite were determined weekly throughout the test. Observations regarding mortality and any adverse sublethal effects were made 4 – 6 hours after each pray application an then daily. Analytical determination of the active substance concentration were made in each newly prepared stock solution. Water samples were taken from the aquaria 6 hours and 7 days after each of the three applications.

Results:
Water quality parameters as criteria for the validity of the test were within the appropriate range according recommendations for fish testing (O_2-saturation: 72 to 90 %; pH: 6.7 to 7.4; temperature: 13.2 to 14.6 °C; nitrate: <1 mg/L; nitrite: <0.05 to 0.17 mg/L). Analytical determinations of the active substance in all freshly prepared stock solutions resulted in recovery rates of 78.5 to 111 % of nominal. The measured active substance concentrations in

the test water taken from the aquaria before and after each application were in accordance with the predicted concentrations in relation to the fate of the substance in water. In the both control groups neither adverse effects nor mortality occurred. No sublethal and lethal effects were observed during the exposure time of 21 d in the lowest test level A. In test level B no lethal effects were observed during the test duration, but sublethal effects were observed starting on day 13, six days after the second application. Based on this observations and taken into account the residue before last treatment (0.144 µg as/L) and the higher application rate (0.5 µg as/L) at the last treatment a **21d NOEC of 0.64 µg as/L** for three applications and a LOEC of 1.32 µg as/L were derived on basis of maximum initial concentration immediately after the last application.

Comment of the RMS:
With respect to the short half-live of azinphos-methyl in water/sediment system RMS do not follow Notifiers original interpretation in the report of a NOEC of 1.38 µg as/L as a cumulative nominal concentration of three applications.

Study title:
Heimbach, F.; Hendel, B. & Sommer, H. (2001): **Biological effects and fate of Azinphos-methyl WP 25 in outdoor microcosm ponds.** Bayer AG, Report No.: HBF/Bt 03. (WAT2001-227)

GLP compliance:
This study was conducted in compliance with the Principles of Good Laboratory Practice (Chemikaliengesetz, dated July 25, 1994, current version of Anhang 1 and the current OECD Principles of Good Laboratory Practice (GLP). The test facilities have been inspected and certified as working in compliance with the Principles of Good Laboratory Practice by the competent authorities (Institute for Environmental Biology: File IV C 4 – 31.11.60.03, 4th March 1999, and Institute for Metabolism and Residue Analysis: File IV C 4 – 31.11.62.03, 4th March 1999).

Material and methods:
The objective of this study was to investigate the ecological effects of Azinphos-methyl WP 25 (Fl.-No. F995120410, TOX-No. 05301-00) in outdoor microcosms serving as an aquatic model ecosystem for lentic freshwater ecosystem with different trophic levels, and the fate of the test substance in the test system. The twelve test tanks (6 m^3 water, 1m water depth) used in tbis study are especially designed systems which allow the establishment of almost identical conditions at the start of a study. The bottoms of the artificial tanks were covered with natural sediment (approximately 10 cm in height) seven months prior to study start. The water was composed of local ground water. Natural communities developed spontaneously from seeds and roots of aquatic plants as well as from air borne and naturally transferred stages of planktonic, benthic and filamentous algae organisms during the months before study start. In General, the artificial ponds are representative of a small standing water. The test substance was applied during the early growing season in April, May and June 2000 three times at an interval of 20 days onto the water surface of nine test ponds. The treatment levels were 0.10, 0.32, 1.0, 3.2 and 10 µg as/L per application (2 replicates 0.10 to 3.2 µg as/L, 1 replicate for 10 µg as/L). Three further tanks were used as untreated controls. The microcosms were investigated for a period of 14 days before and 103 days after first treatment. Several times during the study period water and sediment samples were taken and analysed to investigate the concentration of the test substance in water and sediment. Further parameters studied were the taxonomic composition of zooplankton, macroinvertebrates and

emergence of insects. The physico-chemical water parameters and the content of chlorophyll-a and phaeophytin of phytoplankton were also evaluated. The coverage of the sediment with macrophytes and filamentous algae were estimated. Two diumal cycles of oxygen concentration, water temperature and pH were recorded during the study.

Results:
An average of 86.5 % of the total applied amount of azinphos-methyl was analysed in the water four hours after the first application, confirming nominal concentrations. 4 hours after the second and the third application 94.9 % and 109.2 % were analysed, respectively.The active ingredient disappeared after all applications constantly. Even at the highest treatment of 10 µg as/L less than 1 % of the substance was detected 21 days after the third application.

The average half-life of azinphos-methyl in water for the test concentrations of 0.32 to 10 µg as/L was 4.9 days (minimum: 1.8 days, maximum: 7.8 days). During the whole time of the study the amount of azinphos-methyl in the sediment was below the limit of quantification or even below the limit of detection, respectively.

In general, direct and indirect effects of the application of "Azinphos-methyl WP 25" to the chemical and physical parameters of the pond water as well as phytoplankton have not been observed at any test concentration. The values determined for all physico-chemical parameters in this study are located in the range of natural ponds.

During the last weeks of the study some differences were observed between the individual basins. These fluctuations are caused by an increasing difference in the composition of the biocoenosis of the test ponds over time. It has been shown in previous experiments that the biocoenosis of individual ponds can be considered as quite similar for about 10 weeks after separation of the ponds; thereafter, the ponds diverge more and more from one another. In conclusion, the minor differences in the final part of the study reported here cannot be considered to be an effect caused by the test substance.

The biological data showed some effects on a small group of organisms only. No effects on the coverage of the ponds and the biomass of macrophytes and filamentous algae were observed at any treatment level.

Table B.9.2-1:

	Test concentration [µg as/L]				
ASS SAMPLES	0.1	0.32	1.0	3.2	10.0
Total numbers					
Oligochaeta					
Mollusca					
Hirudinea					
Nematocera (Larvae of Chironomidae)					
Ephemeroptera (Baetidae)					
Shannon-Weaver Index (Diversity)					
Evenness (Diversity)					
Stander's Index (Similarity) *)					
Principal Response Curves *)					
Benthic Macroinvertebrates					
Benthos: total numbers					
Oligochaeta (Tubificidae)					
Nematocera (Chironomidae)					
Mollusca					
Hirudinea					
Shannon-Weaver Index (Diversity)					
Evenness (Diversity)					
Stander's Index (Similarity)					
Principal Response Curves					
Emerged Insects					
Emergence: total numbers					
Nematocera					
Chironomidae					
Cricotopus spec.					
Micropsectra spec.					
Chaoboridae					
Ephemeroptera (*Cloeon* spec.)					
Trichoptera					
Shannon-Weaver Index (Diversity)					
Evenness Index (Diversity)					
Stander's Index (Similarity)					
Principal Response Curves					
Zooplankton					
Rotatoria					
Keratella cochlearis		+		+	
Keratella quadrata				+	
Polyarthra spec.					
Synchaeta spec.					
Phyllopoda					
Daphnia longispina					
Acroperus harpae					
Chydorus sphaericus					
Simocephalus vetulus					
Copepoda					
Nauplius		+			
Calanoida					
Cyclopoda					
Ostracoda					
Shannon-Weaver Index (Diversity)					
Evenness (Diversity)					
Stander's Index (Similarity)					
Principal Response Curves					
Community-NOEC		X			
lowest species-NOEC		X			

\+ Increase in numbers

	No effect
	Weak and/or short effects with recovery
	Medium effects with recovery
	Distinct effects with recovery
	Distinct effects without recovery until end of study

Data of the artificial substrate samplers (ASS) evaluated showed no effects, neither at species nor at community level, up to the highest test concentration of 10 µg as/L. Also no reaction to the test substance applied could be found for the benthic macroinvertebrates.

Data on emerged organisms displayed weak to medium effects only at the highest test concentration of 10 µg as/L for several species: Ephemeroptera and Trichoptera have been proofed to be less sensitive than Chironomidae and Chaoboridae. In any case there was a full recovery till study termination even at the highest test concentration.
At the community level (Diversity and Similarity Indices, Principal Response Curves) weak to medium effects only have been evaluated for the highest test concentration with a full recovery before the end of the study. The Principal Response Curves indicated an effect also for the test concentration of 3.2 µg as/L, also with full recovery.

For zooplankton Phyllopoda have been shown to be the most sensitive group of organisms in this study. There was no effect up to a test concentration of 0.32 µg as/L, but at 1.0 µg as/L *Daphnia longispina* was affected by the test substance. A full recovery could be demonstrated at the end of the study at this test concentration. At the test level of 3.2 µg as/L Phyllopoda were affected more with a distinct effect on *Daphnia longispina* showing no recovery. All other Phyllopoda recovered. At the highest test concentration effects expanded to other taxonomic groups: also some rotatoria and copepoda were influenced, definitely showing a full recovery till study termination.

Obviously there was a strong response of the zooplankton community level-indices, reflecting weak to distinct effects for the highest test concentration. Also at the test concentration of 3.2 µg as/L the Stander's Index and the Principal Response Curve showed distinct effects without recovery. The effects at 1.0 µg as/L have been proofed to be medium and with a full recovery of the Principal Response Curve. No effects have been observed at the lower test concentrations.

Conclusion:
An overall DT_{50} of 4.9 days was observed for azinphos-methyl in water of the microcosms. No effect (NOECcommunity), neither on biocoenosis nor single species, have been identified up to a concentration of **0.32** µg as/L. At 1.0 µg as/L slight effects on Phyllopda, i.e. *Daphnia longispina*, have been observed. Apparently these effected populations recovered completely during the study. At higher test concentrations of 3.2 µg as/L the effects on *Daphnia longispina* were distinct without a full recovery until study termination. In the highest test concentration also other organism groups, i.e. Rotatoria and Copepoda have been affected by the test substance. Thus, taking into account all data on single species as well the community parameters, the ecological acceptable concentration (EAC) is stated to be **1.0 µg as/L** due to a full recovery of all organism groups at this test concentration.

Comment of the RMS:
The study is accepted and evaluated to be valid. The RMS do not agree with the conclusion of the Notifier that a full recovery of *Daphnia longispina* occurred at 1 µg as/L. Because the control values were not reached only a tendency of recovery can be stated. For this reason the EAC is not used for risk assessment.

B.9.2.4 Exposure and risk assessment for aquatic organism

Study title:
Heimbach, F. (2001): **Studies for a Refined Risk Assessment of Azinphos-methyl to Earthworms, Aquatic Invertebrates and Fish.** Unpublished report from 9th March 2001.

Risk assessment:

The base set data show a high acute toxicity of azinphos-methyl to fish with a steep dose-response curve, and chronic endpoints are in the same range as acute ones.

Assessment factors, which are used on standard acute and chronic studies, consider the ACR (acute chronic relationship) for acute studies, differences in species sensitivity, multiple applications, and natural exposure conditions. Since the degradation of azinphos-methyl is not pH-dependent under natural conditions, laboratory studies already reflect the worst case exposure scenario.

The assessment factor for azinphos-methyl based on the two higher tier microcosm studies (Fish: NOEC 0.64 µg as/L; Invertebrates: NOEC 0.32 µg as/L, EAC 1.0 µg as/L) should be adjusted accordingly. Since fish might be considered with more care than other organisms as invertebrates and algae, an assessment factor of two based on the fish microcosm NOEC seems advisable for a final conclusion following the HARAP recommendations. This means, an environmental concentration of 0.3 ppb can be considered as a maximum initial PEC to avoid unacceptable effects on fish populations under natural conditions.

Comments of the RMS:

Higher tier monospecies indoor microcosm study demonstrate that adult fish under more realistic exposure conditions are very sensitivewith a nominal 21 d - NOEC of 0.64 µg as/L for repeated applications. This value is supported by a provisionally calculation of a 96 h - HC5 = 0.535 µg as/L on basis of toxicity data for 21 species submitted by the notifier. However, these calculation cannot be validated because of missing data.

The refined risk assessment is based on the nominal $NOEC_{community}$ of 0.32 µg as/L derived from a mesocosm study with invertebrates and the $NOEC_{water/sediment}$ of 0.64 µg as/L from a prolonged fish test with sediment. In addition to that the risk for early life stages with the NOEC from an ELS (0.18 g/l) needs to be considered, because some sensitive endpoints were not integrated in the prolonged fish test. An accumulation of the active substance in water phase is not expected because dissipation time in outdoor microcosm study was short with a DT_{50} of 4.9 d and multiple applications in indoor water/sediment toxicity study with fish did not result in concentrations higher than initial. However, accumulation in sediment cannot be excluded.

Taking the findings altogether into account, the RMS agrees with the conclusion of the Notifier that the nominal $NOEC_{community}$ of 0.32 µg as/L from multispecies outdoor microcosm can considered as an acceptable concentration for the protection of aquatic organisms.

TER calculations according to the actual Guidance Paper for Aquatic Ecotoxicology (Sanco/3268/2001, 1 October 2001) on basis of 90th drift percentile indicate an unacceptable high risk for aquatic organism by the higher intended application rates in orchards and

assuming only single applications with TER-values below 1 even at the highest distance of 50 m to surface water (tables B.9.2-2 – B.9.2-5). In potatoes TER -values of > 1 were reached for the use-rates intended when considering risk-mitigation measures like buffer-zones to surface-water (tables B.9.2-6 – B.9.2-9).

Tables B.9.2-2 – B.9.2-9: TER calculation in different crops and application rates according to the actual Guidance Paper for Aquatic Ecotoxicology (Sanco/3268/2001, 1 October 2001)

Table B.9.2-2:

Distance (m)	Drift rate (%)	$PEC_{init.}$ (µg as/L)	TER Invertebrates: $NOEC_{community}$ = 0.32 µg as/L	TER Fish: 21 d $NOEC_{water/sediment}$ = 0.64 µg as/L
0	100.000	250.000	0.0	0.0
3	15.730	39.325	0.0	0.0
5	8.410	21.025	0.0	0.0
10	3.600	9.000	0.0	0.1
15	1.810	4.525	0.1	0.1
20	1.090	2.725	0.1	0.2
30	0.540	1.350	0.2	0.5
40	0.320	0.800	0.4	0.8
50	0.220	0.550	0.6	1.2

Table B.9.2-3:

Distance (m)	Drift rate (%)	$PEC_{init.}$ (µg as/L)	TER Invertebrates: $NOEC_{community}$ = 0.32 µg as/L	TER Fish: 21 d $NOEC_{water/sediment}$ = 0.64 µg as/L
0	100.000	200,000	0,0	0,0
3	15.730	31,460	0,0	0,0
5	8.410	16,820	0,0	0,0
10	3.600	7,200	0,0	0,1
15	1.810	3,620	0,1	0,2
20	1.090	2,180	0,1	0,3
30	0.540	1,080	0,3	0,6
40	0.320	0,640	0,5	1,0
50	0.220	0,440	0,7	1,5

Table B.9.2-4:

Distance (m)	Drift rate (%)	$PEC_{init.}$ (µg as/L)	TER Invertebrates: $NOEC_{community}$ = 0.32 µg as/L	TER Fish: 21 d $NOEC_{water/sediment}$ = 0.64 µg as/L
0	100.000	166,667	0,0	0,0
3	15.730	26,217	0,0	0,0
5	8.410	14,017	0,0	0,0
10	3.600	6,000	0,1	0,1
15	1.810	3,017	0,1	0,2
20	1.090	1,817	0,2	0,4
30	0.540	0,900	0,4	0,7
40	0.320	0,533	0,6	1,2
50	0.220	0,367	0,9	1,7

Table B.9.2-5:

Distance (m)	Drift rate (%)	$PEC_{init.}$ (µg as/L)	TER Invertebrates: $NOEC_{community}$ = 0.32 µg as/L	TER Fish: 21 d $NOEC_{water/sediment}$ = 0.64 µg as/L
0	100.000	146.667	0.0	0.0
3	15.730	23.071	0.0	0.0
5	8.410	12.335	0.0	0.1
10	3.600	5.280	0.1	0.1
15	1.810	2.655	0.1	0.2
20	1.090	1.599	0.2	0.4
30	0.540	0.792	0.4	0.8
40	0.320	0.469	0.7	1.4
50	0.220	0.323	1.0	2.0

Table B.9.2-6:

Distance (m)	Drift rate (%)	$PEC_{init.}$ (µg as/L)	TER Invertebrates: $NOEC_{community}$ = 0.32 µg as/L	TER Fish: 21 d $NOEC_{water/sediment}$ = 0.64 µg as/L
0	100,000	166,667	0,0	0,0
1	2,770	4,617	0,1	0,1
5	0,570	0,950	0,3	0,7
10	0,290	0,483	0,7	1,3
15	0,200	0,333	1,0	1,9
20	0,150	0,250	1,3	2,6
30	0,100	0,167	1,9	3,8
40	0,070	0,117	2,7	5,5
50	0,060	0,100	3,2	6,4

Table B.9.2-7:

Distance (m)	Drift rate (%)	$PEC_{init.}$ (µg as/L)	TER Invertebrates: $NOEC_{community}$ = 0.32 µg as/L	TER Fish: 21 d $NOEC_{water/sediment}$ = 0.64 µg as/L
0	100,000	133,333	0,0	0,0
1	2,770	3,693	0,1	0,2
5	0,570	0,760	0,4	0,8
10	0,290	0,387	0,8	1,7
15	0,200	0,267	1,2	2,4
20	0,150	0,200	1,6	3,2
30	0,100	0,133	2,4	4,8
40	0,070	0,093	3,4	6,9
50	0,060	0,080	4,0	8,0

Table B.9.2-8:

Distance (m)	Drift rate (%)	$PEC_{init.}$ (µg as/L)	TER Invertebrates: $NOEC_{community}$ = 0.32 µg as/L	TER Fish: 21 d $NOEC_{water/sediment}$ = 0.64 µg as/L
0	100,000	80,000	0,0	0,0
1	2,770	2,216	0,1	0,3
5	0,570	0,456	0,7	1,4
10	0,290	0,232	1,4	2,8
15	0,200	0,160	2,0	4,0
20	0,150	0,120	2,7	5,3
30	0,100	0,080	4,0	8,0
40	0,070	0,056	5,7	11,4
50	0,060	0,048	6,7	13,3

Table B.9.2-9:

Distance (m)	Drift rate (%)	$PEC_{init.}$ (µg as/L)	TER Invertebrates: $NOEC_{community}$ = 0.32 µg as/L	TER Fish: 21 d $NOEC_{water/sediment}$ = 0.64 µg as/L
0	100,000	40,000	0,0	0,0
1	2,770	1,108	0,3	0,6
5	0,570	0,228	1,4	2,8
10	0,290	0,116	2,8	5,5
15	0,200	0,080	4,0	8,0
20	0,150	0,060	5,3	10,7
30	0,100	0,040	8,0	16,0
40	0,070	0,028	11,4	22,9
50	0,060	0,024	13,3	26,7

B.9.2.5 References relied on

Annex Point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner
AIIA-8.2.1	Hommen,U.	2000	Final Report Calculation of a HC_5 for the acute toxicity of azinphos-methyl to freshwater fish unpublished WAT2001-225	Y	BAY
AIIIA-10.2	Carlisle, J. C.	1985	Toxicity of Azinphos-methyl (®Guthion Technical) to Rainbow Trout Early Life Stages. Mobay Chemical Corporation, Study No. 84-666-02) unpublished WAT2001-297	N	BAY
AIIIA-10.2.2	Dorgerloh,M. and Sommer,H.	2001	Gusathion M WP 25 - Indoor microcosm (water/sediment) with rainbow trout (Oncorhynchus mykiss) sumulating multiple applications GLP, unpublished WAT2001-226	Y	BAY
AIIIA-10.2.2	Heimbach,F.; Hendel,B. and Sommer, H.	2001	Biological effects and fate of Azinphos-methyl WP 25 in outdoor microcosm ponds GLP, unpublished WAT2001-227	Y	BAY
AIIA-8.2, AIIIA-10.2	Heimbach, F.	2001	Studies for a Refined Risk Assessment of azinphos-methyl to Earthworms, Aquatic Invertebrates and Fish unpublished WAT2001-239	Y	BAY
	Anonym	2000	Bekanntmachung des Verzeichnisses risikomindernder Anwendungsbedingungen für Nichtzielorganismen. Bundesanzeiger Nr. 100 vom 27.04.2000, S. 9878		
	Anonym	2000	Bekanntmachung über die Abtrifteckwerte, die bei der Prüfung und Zulassung von Pflanzenschutzmitteln herangezogen werden. Bundesanzeiger Nr. 100 vom 08.05.2000, S. 9879 – 9881		

B.9.5 Effects on other arthropod species (Annex IIA 8.3.2, Annex IIIA 10.5.1)

B.9.5.1 Extended laboratory tests and semi-field tests

The following additional studies and statements were submitted by the notifier

PARASITOIDS

Title: Effects of Gusathion M WP 25 on the parasitoid Aphidius rhopalosiphi (Hymenoptera, Aphidiidae) - extended laboratory test - dose response test -
Author: Moll, M. (1999)
BBA-Ref.-No.: ANA2000-393
Test substance: Formulation Gusathion M WP 25
Azinphos-methyl 23.9 %
Guideline: Aphidius (Mead-Briggs 1992)
Test species: *Aphidius rhopalosiphi*
Developmental stage: Imagines
Substrate: Natural substrate (apple leaves, 2D)
Exposure route: dried residue
Exposure duration: 48 h
Results:

Appl. rate	Mortality	Sublethal effects
4 g WP 25/ha	31 %	22 % (Parasitation capacity)
10 WP 25g/ha	86 %	
20 WP 25g/ha	94 %	
40 WP 25g/ha	100 %	
80 WP 25g/ha	100 %	
LR_{50} 1.2 g as/ha		

Validity: positive
GLP compliance: positive

Title: Effects of Gusathion M WP 25 on the parasitoid Aphidius rhopalosiphi (Hymenoptera, Aphidiidae) - extended laboratory study
Author: Moll, M. (1998)
BBA-Ref.-No.: ANA2000-389
Test substance: Formulation Gusathion M WP 25
Azinphos-methyl 25 %
Guideline: Aphidius (Mead-Briggs 1997)
Test species: *Aphidius rhopalosiphi*
Developmental stage: Imagines

Substrate: Natural substrate (Apple leaves 2-D application, and trees 3-D application)
Exposure route: dried residues
Exposure duration: 24 h
Results:

Appl. rate	Mortality	Sublethal effects
2 g WP 25/ha (2-D)	0 %	27 % (Parasitation capacity)
4 g WP 25/ha (2-D)	0 %	36 % (Parasitation capacity)
10 g WP 25/ha (2-D)	30 %	
24 g WP 25/ha (2-D)	85 %	
48 g WP 25/ha (3-D)	52 %	
48 x 3 g WP 25/ha (3-D)	100 %	
4800 g WP 25/ha x 3	100 (3d-6 weeks after 3rd application)	
4800 g WP 25/ha x 3	70 (10 weeks after 3rd app.)	
4800 g WP 25/ha x 3	3 (13 weeks after 3rd app.)	15 %(Parasitation capacity)

Remarks: 1. Test - Extended laboratory test: Application on leaves for treatments 2 to 24 g/ha (2-D) in the lab. Treatments of 48 g/ha were conducted in the field (3-D).
2. Test -Persistencetest: 3 applications to apple trees (3-D), findings after 3d, 7d, 2 weeks, 3 weeks, 6 weeks after 3rd application (100 %) and 10 weeks and 13 weeks after 3rd application.

Validity: positive
GLP compliance: positive

Title: Toxicity of Azinphos-methyl WP 25 to the parasitic wasp, Aphidius rhopalosiphi (Hymenoptera: Braconidae), in a semi-field test
Author: Baxter, I. (2000)
BBA-Ref.-No.: ANA2001-195
Test substance: Formulation Azinphos-methyl WP 25
Azinphos-methyl 25 %
Guideline: -
Species: *Aphidius rhopalosiphi*
Developmental stage: Adults
Substrate: Natural substrate (Gerstensämlinge mti Fruktose
Exposure route: deposit
Exposure duration: 72 h

Results:

* Number of applications:	1
Field rate per application:	20 g WP 25/ha
Final check:	2(A) %
Final check:	0(+34P) %
* Number of applications:	1
Dosage per application:	40 g WP 25/ha
Final check:	0(A) %
Final check:	0(+10 P) %
* Number of applications:	1
Dosage per application:	200 g WP 25/ha
Final check:	7(A) %
Final check:	10(P) %

Remarks: Three application rates of Azinphos-methyl WP 25 (24.2 % azinphos-methyl; 5, 10, 50 g as/ha) were tested under semi-field conditions on treated barley plants (one- to two-leaf growth stage, 10 to 15 cm tall, sowing density based on rate of 175 kg seed/ha) using max. 48 h old adult *Aphidius rhopalosiphi* (10 females into each of 4 replicates per treatment for fecundity assessments; 40 wasps of mixed sex ratio into each of 4 replicates per treatment for activity assessments). Plant were maintained outdoors under UV-permeable rain protection. Bioassays commenced within 1 h of application. Tap water was used as control treatment and dimethoate 40 (0.85 L/ha) as a toxic reference treatment. During the test the temperature was between 33 and 38°C and the humidity around 25 -- 30 %. Endpoints were parasitation efficiency (P = numbers of aphid mummies after 24 and 48 hrs) and activity (A = numbers of wasps trapped on yellow sticky traps after 24 and 48 hrs). The study was conducted under GLP and is considered not to be valid because of the unfavourable test conditions.

Findings: There was not statistical significant difference in the number of trapped wasps between the control and the treated replicates. However, the mortality of wasps was not registrated in the test. In addition to that, the fecundity of the control and the treated wasps was only 1.45 mummies/female. In laboratory studies a minimum number of mummies/female of 5 is demanded as criteria for validity. Taking this into account, the possibility of determining statistically significant effects and a reliable NOEC is to low.
Validity: negative
GLP compliance: positive

Title: Effects of Azinphos-methyl WP 25 on adults of the parasitoid Trichogramma cacoeciae Marchal (Hymenoptera, Trichogrammatidae) - extended laboratory study -
Author: Moll, M. (2001)
BBA-Ref.-No.: ANA2001-196
Test substance: Formulation Azinphos-methyl WP 25
Azinphos-methyl 25 %
Guideline: Trichogramma (Hassan et al. 2000)
Test species: *Trichogramma cacoeciae*

Developmental stage: Imagines
Substrate: Natural substrate (Apple leaves, 2-D application)
Exposure route: dried residues
Exposure duration: 7 d
Results:

Appl. rate	Mortality	Overall effects
1.65 g WP 25/ha	-	0 % (Parasitation capacity)
4.13 g WP 25/ha	-	7 % (Parasitation capacity)
10.3 g WP 25/ha	-	18 % (Parasitation capacity)
25.8 g WP 25/ha	-	27 % (Parasitation capacity)
129 g WP 25/ha	-	80 % (Parasitation capacity)

Remarks: Azinphos-methyl WP 25 (24.2 % azinphos-methyl) was tested under extended laboratory conditions using app. 24 h old adult *Trichogramma cacoeciae* (38 to 162 wasps per cage; 4 replicates per treatment). Wasps were exposed to dried spray deposits of 0.4 to 31.25 g as/ha (diluted in 200 L water/ha) on treated apple leaves (singularly treated using a laboratory spraying equipment). Deionized water was used as control treatment and Perfekthion EC (6 mL/ha) as a toxic reference treatment. The duration of the test was 11 d (7 d exposure + 4 d incubation of host eggs). Endpoint was parasitation efficiency. The toxic standard caused 76.2 % reduction compared to the control. The study was conducted under GLP and is considered valid.
Findings: Effects on parasitation efficiency showed a consistent dose-response-relationship and the ER_{50} was calculated 11.0 g as/ha (CL 95 %: 5.2 to 48.5 g as/ha).
Validity: positive
GLP compliance: positive

Plant dwelling Predators

Title: Effects of Gusathion M WP 25 on the ladybird beetle Coccinella septempunctata L. (Coleoptera, Coccinellidae) - extended laboratory study -
Author: Moll, M. (1998)
BBA-Ref.-No.: ANA2000-390
Test substance: Formulation Gusathion M WP 25
Azinphos-methyl 25 %
Guideline: Coccinella (Pinsdorf 1989)
Test species: *Coccinella septempunctata*
Developmental stage: Larvae
Substrate: Natural substrate (apple trees, 3-D application)
Exposure route: dried residues
Exposure duration: 10 d

Results:

Appl. rate	Mortality	Sublethal effects
4800 g WP 25/ha	100 %	
1440 g WP 25/ha	100 %	
480 g WP 25/ha	100 %	
48 g WP 25/ha	100 %	
48 x3 g WP 25/ha	90*	
4800 g WP 25/ha x 3	100 %	
4800 g WP 25/ha x 3	9 %	73 % (Fertility)

Remarks: 3 applications were made at a time interval of 6 - 9 days. –
* 90 % mortality was not corrected due to high control mortality of 70 %.
Validity: positive
Inspite high control mortality after the 1st and 3rd application the findings are considered valid, because the 10 weeks value after the 3rd application is considered acceptable for the control (23,3 %).
GLP compliance: positive

notifier's Statement
SCHMUCK, R. (2001). The refined risk assessment for Azinphos-methyl proposed by the notifier is mainly supported by a number of publications which indicate that under field conditions the effects caused by Azinphos-methyl on populations of hymenopteran species are considerably lower than observed under laboratory conditions and indicated by deterministic risk assessment models currently used for *Aphidius rhopalosiphi*. In order to verify these assumptions the notifier conducted two studies which are reported above in order to address i) differences in sensitivity between species and ii) differences in sensitivity of *Aphidius rhopalosiphi* tested under more natural exposure conditions compared to former laboratory tests.
Conclusions:
i) The ER_{50} for *Trichogramma cacoeciae* was considerably higher than the LR_{50} for *Aphidius rhopalosiphi* (11 g as/ha compared to 1.2 g as/ha).
ii) ii) Under more realistic exposure conditions the ER_{50} for *Aphidius rhopalosiphi* must be considerably higher than 50 g as/ha. According to the notifier both findings support the view that under field conditions there is no unacceptable risk on the population level for sensitive species such as hymenopteran parasitoids. In order to protect non-target arthropods the notifier proposed buffer zones and spray drift reducing application technique.

Comment of the RMS:
Findings reported by the notifier are not conclusive at all. The RMS does not follow notifiers conclusions. As the semi-field test using *Aphidius rhopalosiphi* is not valide, the LR_{50} as determined for *Aphidius rhopalosiphi* in the extended laboratory study is relevant for the risk assessment. In addition to that, the recovery of harmed populations and a recolonisation of treated crops by sensitive species is still questionable.

B.9.5.2 Risk assessment

According to the list of supported uses submittedby the notifier (dated 22.02.2002) maximum rates for field crops (potato) are 0.12 to 0.5 kg as/ha per single application with a total maximum of 2 applications. Field rates for orchards (pears and apples) reach from 0.44 to 0.75 kg as/ha per single application with a maximum number of 3 applications per year. Relevant for risk assessment is the LR_{50} of 1.2 g a.s./ha as determined in the extended laboratory test using *Aphidius rhopalosiphi*.

Table B.9.5-1: Field crops

Max. Number of Treatments and Application rate: 1 - (2) x 120 g as/ha
Scenario: Potato (90th Percentile)

Distance (m)	Drift rate (%)	PEC1) (g as/ha)	TER
0	100	120	0.01
1	2.8	3.4	0.4
5	0.6	0.7	1.8

1) PEC for 1 application, drift values according to Ganzelmeier et al. 2000

Max. Number of Treatments and Application rate: 1 - (2) x 240 g as/ha
Scenario: Potato (90th Percentile)

Distance (m)	Drift rate (%)	PEC1) (g as/ha)	TER
0	100	240	0.005
1	2.8	6.8	0.2
5	0.6	1.4	0.9

1) PEC for 1 application, drift values according to Ganzelmeier et al. 2000

Max. Number of Treatments and Application rate: 1 - (2) x 400 g as/ha
Scenario: Potato (90th Percentile)

Distance (m)	Drift rate (%)	PEC[1] (g as/ha)	TER
0	100	400	0.003
1	2.8	11.2	0.1
5	0.6	2.4	0.5

According to the TER-calculations, the risk for non-target arthropods within and outside the treated fields is unacceptable high.

Table B.9.5-2: Orchards

Max. Number of Treatments and Application rate: 1 - (3) x 500 g as/ha
Scenario: Orchards (90th Percentile, late)

Distance (m)	Drift rate (%)	PEC[1] (g as/ha)	TER
0	100	500	0.002
3	15.7	79	0.02
5	8.4	42	0.03

1) PEC for 1 application, drift values according to Ganzelmeier et al. 2000

Max. Number of Treatments and Application rate: 1 - (3) x 600 g as/ha
Scenario: Orchards (90th Percentile, late)

Distance (m)	Drift rate (%)	PEC[1] (g as/ha)	TER
0	100	600	0.002
3	15.7	94	0.01
5	8.4	50	0.02

Max. Number of Treatments and Application rate: 1 - (3) x 750 g as/ha
Scenario: Orchards (90th Percentile, late)

Distance (m)	Drift rate (%)	PEC[1] (g as/ha)	TER
0	100	750.0	0.002
3	15.7	118	0.01
5	8.4	63	0.02

According to the TER-calculations, the risk for non-target arthropods within and outside the treated fields is unacceptable high.

B.9.5.3 References relied on

Annex Point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner
AIIA-8.3.2, AIIIA-10.5	Baxter, I.	2000	Toxicity of Azinphos-methyl WP 25 to the parasitic wasp, Aphidius rhopalosiphi (Hymenoptera: Braconidae), in a semi-field test GLP, unpublished ANA2001-195	Y	BAY
AIIA-8.3.2, AIIIA-10.5	Moll, M.	2001	Effects of Azinphos-methyl WP 25 on adults of the parasitoid Trichogramma cacoeciae Marchal (Hymenoptera, Trichogrammatidae) - extended laboratory study GLP, unpublished ANA2001-196	Y	BAY

AIIA-8.3.2, AIIIA-10.5	Moll, M.	1998	Effects of Gusathion M WP 25 on the parasitoid Aphidius rhopalosiphi (Hymenoptera, Aphidiidae) - extended laboratory study GLP, unpublished ANA2000-389	Y	BAY
AIIA-8.3.2, AIIIA-10.5	Moll, M.	1998	Effects of Gusathion M WP 25 on the ladybird beetle Coccinella septempunctata L. (Coleoptera, Coccinellidae) - extended laboratory study – GLP, unpublished ANA2000-390	Y	BAY
AIIA-8.3.2, AIIIA-10.5	Moll, M.	1999	Effects of Gusathion M WP 25 on the parasitoid Aphidius rhopalosiphi (Hymenoptera, Aphidiidae) - extended laboratory test - dose response test – GLP, unpublished ANA2000-393	Y	BAY
AIIA-8.3.2, AIIIA-10.5	Schmuck, R.	2001	Refined Risk assessment for Azinphos-methyl and Non-target Arthropod Species unpublished ANA2001-197	Y	BAY

B.9.6 Effects on earthworms (Annex IIA 8.4, Annex IIIA 10.6.1)

B.9.6.1 Field study

Title:
Effects of Azinphos-methyl WP 25 on the earthworm biocoenosis of a grassland area in northern Italy one month and six month after application (Vighi and Heimbach, ARW2001-36, Vighi and Heimbach, ARW 2002-18).

Report-No.: HBF/RgF 55 and HBF/RgF 56

Guideline: ISO 11268-3, 1999 and BBA VI, 2-3, 1994

GLP: yes

Years of study: 2000 and 2001

Study site:
Grassland site at Brugherio, near Milano (Italy). The site was not treated with chemical fertilisers or pesticides for about 10 years. The area had a size of 11 x 140 m. Irrigation was performed from the end of May to mid-September 2000 in about weekly intervals (except when there was sufficient rainfall) by overflowing an irrigation channel adjacent to the test field. The first irrigation took place in the night following to the second application of azinphos-methyl. After the third application the field was irrigated one week after the application. No information about the amount of water used for irrigation is given. The site was mown 6 times in 2000. The cuttings were partly removed to avoid a thick layer of grass on the soil surface.

The study was designed in a randomised block design. Each plot (= replicate) was 100 m^2 (10 x 10 m) in size and was replicated four times. The pre-treatment earthworm density was checked on 14 April 2000. The mean density was about 200 individuals/m^2.

Soil:
sandy loam, pH 5.65, organic carbon content 2.4 %

Vegetation:
Poa pratensis (approximate coverage 50 %), *Lolium perenne* (30 %), *Arrenatherum elatior* (10 %), *Bromus mollis* (10 %), *Anthoxanthum odoratum* (5 %), *Trifolium pratense* (20 %), *Plantago lanceolata* (5 %), *Rumex* sp..

Application:
Azinphos-methyl WP 25 (trade name Gusathion) was applied 3 times at 1 kg as/ha with 20 days intervals. The first application was done on may 5, the second on may 25 (with an irrigation in the following night) and the third application on June 14 (the next irrigation was done on June 21). Benomyl 50 WP was applied as reference substance at 4 kg as/ha once on may 5.

Sampling:
Four samples per plot and sampling date were taken using the formaldehyde-extraction method (10 L of 0.2 % formaldehyde solution). A 30 minutes extraction time was chosen.
The sampling dates were one month after the last application on 14 July, about 4.5 month after the last application on 3 November and about 11 month after the first application on 6 April 2001. Directly after treatment the soil surface was checked for dead and alive earthworms.

Earthworm species:
Lumbricus terrestris, Lumbricus castaneus, Lumbricus rubellus, Aporrectodea caliginosa, Aporrectodea rosea, Allolobophora chlorotica, Allolobophora georgii, Octolasion tyrtaeum, Eisenia fetida (one ind.)

Results:

Soil surface search:
4 and 7 days after the first application some dead or alive earthworms were observed on the azinphos-methyl and the benomyl-treated plots. Whereas in the control plots in total 2 dead and 3 alive earthworms were found, in the benomyl plots 12 dead and 12 alive earthworms, and in the azinphos-methyl plot 8 dead and 5 alive earthworms were observed. After the second and the third application no surface activity in the control and the azinphos-methyl was observed. In the benomyl plots this was not checked, because the treatment was only done once on May 5.

Formaldehyde extraction:One month after treatment the overall earthworm density and extraction efficiency were low (mean earthworm density 37/m^2). The extraction efficiency was between 0 and 33 %. In general the earthworms on the site were uneven distributed depending on the plot.
Because of the low densities and the timing of the sampling, the results of this sampling date are not evaluated.

In autumn 2000 the mean density was 138 earthworms/m^2. The extraction efficiency was between 64 and 84 %. In spring 2001 the mean density was 75 earthworms/m^2 and the extraction efficiency was in the range of 38 up to 76 %.

Table B.9.6-1 Effects of azinphos-methyl and benomyl on earthworms numbers and biomasssix and eleven month after treatment in % compared to control (control = 100 %) (Vighi and Heimbach 2001, ARW2001-36)

	Azinph.-meth. (0 w, 1 kg a.s./ha) one month Abund. (% of contr.)	Azinph.-meth. (0 w, 1 kg a.s./ha) one month Fresh wt (% of contr.)	Ben. (0 w, 4 kg a.s./ha) one month Abund. (% of contr.)	Ben. (0 w, 4 kg a.s./ha) one month Fresh wt (% of contr.)	Azinph.-meth. (0 w, 1 kg a.s./ha) 11 mth Abund. (% of contr.)	Azinph.-meth. (0 w, 1 kg a.s./ha) 11 mth Fresh wt (% of contr.)	Ben. (0 w, 4 kg a.s./ha) 11 mth Abund. (% of contr.)	Ben. (0 w, 4 kg a.s./ha) 11 mth Fresh wt (% of contr.)
Overall abundance	89	101	73 *	92	107	133	70 *	96
Overall juveniles	79	91	64 *	77	79	80	42*	43*
Overall adults	95	102	81	95	145 *	168 *	109	131
Juvenile epilobous	76	84	68	83	79	89	44	50
Juvenile *Lumbricus terrestris*	/	/	/	/	nf	nf	nf	nf
Juvenile *Lumbricus castaneus*	71	76	29	27	nf	nf	nf	nf
(Other) juvenile tanylobous	100	97	41 *	54 *	75	52	19*	19*
Adult *Lumbricus terrestris*	83	106	17 *	16	317	330	233	212
Adult *Lumbricus castaneus*	107	124	53 *	31 *	173	171	80	87
Adult *Lumbricus rubellus*	100	231	200	697	nf	nf	nf	nf
Adult *Aporrectod. caliginosa*	95	101	86	104	137	145	107	121
Adult *Aporrectod. rosea*	83	100	100	104	nf	nf	100	159

Adult*Allolobophora chlorotica*	200	768	233	753	136	181	114	172
Adult *Allolobophora georgii*	nf	nf	20	14	100	114	nf	nf
Adult *Octolasion tyrtaeum*	100	115	13 *	15 *	200	101	167	114

* sign. p < 0.05; t-test/Mann-Whitney U-test
nf = not found
/ impossible to calculate as no earthworms of this species found in cootrol

Table B.9.6-2: Ratio of juveniles to adults six and eleven month after treatment

	6 month		12 month	
	Abundance	Biomass	Abundance	Biomass
Control	0.8	0.25	1.69	0.79
Azinphos-methyl	0.6	0.17	0.97 *	0.41 *
Benomyl	0.51	0.16	0.53 *	0.21 *

* sign. p < 0.05; t-test/Mann-Whitney U-test

B.9.6.2 Risk Assessment

The effects of benomyl on earthworms after the first application demonstrate that the reference substance has worked. The conditions of the first application indicate that the test substance reached the soil and has been done in a phase of earthworm activity (effects of benomyl, surface activity after the application). For the other two applications (May 25 and June 14) this may not have been the case due to the summer season conditions. As benomyl has not been applied in these dates again, and as there was an irrigation in the night after the second application on May 25 with an unknown amount of water, there is uncertainty about the conditions of this application.
The sampling in June is not taken into account because of a low extraction efficiency of 0-33 %. Therefore two sampling dates 6 and 11 month after application are available.

The results from the evaluation about six month after application show that there are slight, no significant reductions in some species, especially in the juveniles. There were some species with a low density (e.g. *Allolobophora chlorotica, A. georgii* and *A. rosea* with in total 3 to 6 individuals in 16 samples in the control). For these species a risk assessment cannot be done. Eleven month after application there was an increase compared to control especially in the adult number of some species, e.g. *Lumbricus terrestris, Lumbricus castaneus* and *Aporrectodea caliginosa*. Concerning juveniles there was still a non-significant decline of about 20 % compared to control. Expressed as the ratio of juveniles to adults this fact leads to a significant difference of the juvenile/adult ratio of azinphos-methyl and benomyl in comparison to the control 11 month after application.

The previous field test according to Heimbach, 1988; ARW 95-00091 was done with application rates of 2 x 1.5 kg as/ha and 2 x 6 kg.as/ha. The present field test was done with 3 x 1 kg as/ha, so the overall amount for the lower rate is comparable for both tests. Nevertheless, there is an uncertainty about the exposure conditions for at least one application (irrigation in the night after application on May 25 with an unknown amount of water) in the new test. Therefore, it is not possible to do a risk assessment for earthworms for 3 kg as/ha from the results available. As a long-term risk for 2 kg as/ha cannot be excluded from the results given, it is concluded that on the data base available 2 kg as/ha reaching the soil surface might lead to a risk for earthworm populations in the field. As this amount is not part of the intended uses, no additional risk management is considered necessary. For every rate above 2 kg as/ha a proper risk assessment is needed.

B.9.6.3 References relied on

Annex Point/ reference number	Author(s)	Year	Title Source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner
AIIIA-10.6.1.3	Heimbach, F.	1988	Influence of Gusathion M 200 EC on the earthworm fauna of a grassland area, GLP, unpublished ARW9500091	Y	BAY
AIIIA-10.6.1.3	Vighi, M., Heimbach, F.	2001	Effects of azinphos-methyl WP 25 on the earthworm biocoenosis of a grassland area in northern Italy one month and six month after application, GLP, unpublished ARW2001-36	Y	BAY
AIIIA-10.6.1.3	Vighi, M., Heimbach, F.	2001	Effects of azinphos-methyl WP 25 on the earthworm biocoenosis of a grassland area in northern Italy one month, six month and one year after application, GLP, unpublished ARW2002-18	Y	BAY

The Norwegian Agricultural Inspection Service –Pesticides Section

Information on Resolutions following recommendations from the Council for Pesticides (Council Meeting 20 September 2002)

Case 33/02 Gusathion – azinphos-methyl (Bayer CropScience Agro), application for renewal of approval, J. no. 200200430

Resolution

Gusathion is being phased out. This is due to the fact that Azinphos-methyl's ecotoxicological properties are extremely worrying and the substance has been found during monitoring, despite the fact that its use is limited in the monitored areas. Norway is obliged to reduce the use of this substance under the terms of the North Sea Agreement. The Norwegian Agricultural Inspection Service is aware that Gusathion has great agronomical significance, and therefore grants a renewed approval for the product for 1 year for existing areas of use. The sale and use of the product must be discontinued in accordance with the usual rules. All use of Gusathion is prohibited after 31.12.2005.

Area of Application

To be used against certain gnawing and sucking insects in pome fruits, stone fruits, cultivated blueberries, strawberries on open land, brassicas, and ornamentals in greenhouses and on open land.

The product must not be used on crops or weeds in bloom or when bees are actively foraging (0400 to 2300 if the temperature is above 10^{o}C, or 0600 to 2200 if the temperature is not above 10^{o}C).

Preharvest interval

Cultivated Blueberries: Before blooming or after harvesting
Other edible crops: 21 days

Tax Class

4

Hazard classification

T; Toxic
Bee symbol + sun *(amended)*
R23/25 Toxic by inhalation and if swallowed *(amended*)
R43 May cause sensitisation by skin contact
S24 Avoid contact with skin
S23 Do not breathe spray
S45 In case of accident or if you feel unwell seek medical advice immediately (show the label where possible)
(S1): Keep locked up

(S2): Keep out of the reach of children
Very toxic to aquatic organisms. Must not be used less than 30 metres from water bearing ditches, streams, dams or larger occurrences of water (amended)

Toxic to earthworms *(enhanced*)
Toxic to bees and other pollinating insects, do not apply on crops or weeds in bloom or when bees are actively foraging. *(amended)*
Harmful to non-target arthropods
Very toxic to birds (*enhanced*)
Use suitable protective clothing (see precautions)
Dispose of empty packaging safely (see waste disposal)

Jordforsk

MEMO

Use and Findings of the azinphos-methyl insecticide in the JOVÅ programme.

To: **Kristin Espeseth, The Norwegian Agricultural Inspection Service**
From: **Gro Hege Ludvigsen, Jordforsk(The Norwegian Soil Research Institute)**
Olav Lode, Planteforsk (The Norwegian Crop Research institute)
Date: **4 September 2002**

Please find attached a summary of use and findings of azinphos-methyl. The summary is based on the results reported by the Norwegian Agricultural Environmental Monitoring Programme (JOVÅ)(Ludvigsen & Lode, 2002) and a new compilation of the results from 2002.

As regards the columns listing the number of decares sprayed every year and the dose used, the following method has been applied: The area sprayed is the actual area which has received chemical spray, once or more frequently. The dose used is the total dose which the area has received throughout the year. In some cases this dose has been given by repeated spraying (this applies especially to fungicides), but certain herbicides or insecticides may also have been used twice a year.

Table1. Use and Findings of Azinphos-Methyl

Field Location/ Type of test-ing: R=Random Sample C=Composite Sample	Year	Decare sprayed - surface area.	% sprayed of the total agricul-tural surface area	Dose used gramm e/decar e	Consu mption kg active ingredi ient	No of findings and no of samples taken	Max concent ration µg/l	Averag e concent ration	Sprayed in week no(s)	Found week no	Sample taken week nos
Streams and Rivers											
Heiabekken	1996	20	0.6	128	2.6	0 out of 22	-	-	25	-	16→50
Råde	1997	30	1	38	1.1	0 out of 19	-	-	24	-	14→50
Østfold	1998	35	1	86	3.0	0 out of 23	-	-	25, 29	-	8→50
	1999	6	<1	153	0.9	0 out of 19	-	-	22	-	8→48
R	2000	58	2	123	7.2	0 out of 18	-	-	21→23	-	17→50
	2001	57	2	110	6.3	0 out of 18	-	-	22→24	-	16→50
Vasshaglona	1996	8	2	51	0.4	1 out of 15	0.34	-	19	36	15→50
Grimstad	1998	8	2	32	0.3	0 out of 18	-	-	29	-	8→50
AustAgder	2000	22	6	51	1.1	0 out of 17	-	-	19→32	-	1→45
C	2001	50	13	82	4.1	0 out of 19	-	-	19→29	-	15→51
	2002	-	-	-	-	1 out of 8	0.55	-	-	29	17→29
Skas-Heigre-Canal Ro-galand C	1999	-	-	-	-	1 out of 15	0.06	-	-	28	16→42
River Lier Elverhøy Buskerud R	1998	-	-	-	-	2 out of 14	0.64	0.39	-	24, 34	14→46
Ditches											
Heiabekken G3	2002	-	-	-	-	1	0.15	-	-	22	22, 24

Scope of Analysis

Azinphos-methyl is analysed by the Norwegian Crop Research Institute's pesticide laboratory and is included in method M03. This substance has been in the analysis spectrum from 1996 – 2002. The limit of detection in 1996-1998 was 0.1 µg/l, and 0.05µg/l in 1999- 2002. This substance has therefore been tested for in approximately 1050 samples of stream water during the period 1995-2002. In order to save space, table 1 gives the results for those years when the substance has either been used or found. The 2001 farming data from all fields is not yet ready. The possibility that azinphos-methyl may have been used in more areas than Vasshaglona and Heiabekken can, therefore, not be excluded. The substance has also also been tested for in other surveys (ditch water, ground water etc) carried out during the period 1996-2002.

In Vasshaglona (Grimstad, Aust-Agder) and Skas-Heigre Canal (Rogaland) the samples were taken as composite samples over 14 days. In Heiabekken Råde (Østfold) and the River Lier (Buskerud) random sampling was used.

Precipitation

There has not been time to collect and analyse precipitation data connected to these findings, due to the short notice given for updating the data by the Norwegian Agricultural Inspection Service.

Demonstrations

There are few findings of azinphos-methyl. Azinphos-methyl has been detected 4 times in the rivers/streams. One finding with 0.34 µg/l of azinphos-methyl was first made in Vasshaglona in Septem-

ber 1996, a long time after the substance had been sprayed. Azinphos-methyl was detected twice in June and August 1998 in the River Lier (Elverhøy in the middle of the Lier valley) with the highest concentration in August of 0.64 µg/l. Given the water flow of the River Lier, this must be described as a relatively high concentration. In June 1998 0.14 µg/l were found in the River Lier.

A relatively low concentration of azinphos-methyl was found in Skas-Heigre Canal in 1999 (0.06 µg/l). There have been no detections of azinphos-methyl in either 2000 or 2001.

Azinphos-methyl was also found in a test in Vasshaglona in the middle of July 2002. The finding was 0.55 µg/l.

In addition to the above mentioned findings, azinphos-methyl was also found in 2002. This discovery was made as part of a special survey of Heiabekken in connection with the TRACER research project. Samples are taken from open and closed ditches during precipitation. 0.15 µg/l azinphos-methyl was found at the end of May in a ditch outlet, which flows out into Heiabekken in the middle of the catchment area. The samples were taken following rainfall which led to drainage from both surface and closed ditches. The total water flow in the stream was, however, relatively low. Moreover, data has not yet been collected on the use of pesticides in the catchment area in 2002. Further interpretations of this must therefore wait.

Development in use

There is relatively limited use of azinphos-methyl in the JOVÅ area. Out of the six catchment areas which record use of pesticides, only Vasshaglona and Heiabekken have recorded use. There have been no detections of the substance in Heiabekken and in most years there have been no detections in Vasshaglona.

Assessment of the Results

Azinphos-methyl is considered to have low mobility and its solubility in water is not particularly high (20-30 ppm at 20°C). The halflife in soil is demonstrated to take several weeks in field trials, while pure degradation tests in the laboratory have shown a halflife of one to two weeks. The degradation of this substance occurs mainly abiotically (oxidisation, hydrolysis, demethylation). Azinphos-methyl is extremely poisonous to fish.

Three out of six findings have been made in the most intensive spraying season, while two findings have been made later in the autumn (August and September). There have, however, been few findings of the substance and it is therefore difficult to interpret clear links between the use and finding of this material. It is therefore difficult to say whether the findings in August and September came a long time after the spraying, or whether the spraying occurred shortly before these findings.

The limit for environmental damage is 0.01 µg/l. All findings exceed this limit. With a detection limit of 0.05 µg/l, we will not trace all the findings which are potentially damaging to the environment.

Litterature

Ludvigsen & Lode 2002. Jordsmonnsovervåking i Norge (Soil Surveillance in Norway). Pesticides 2000. Jordforsk report 6/02 s 46.

WHO/PCS/92.52

Pesticide residues in food - 1991

Joint FAO/WHO Meeting on
Pesticide Residues

EVALUATIONS 1991

PART II - TOXICOLOGY

IPCS

International Programme on Chemical Safety

WORLD
HEALTH
ORGANIZATION

AZINPHOS-METHYL

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EXPLANATION

Azinphos-methyl was evaluated for acceptable daily intake by previous Joint Meetings in 1965, 1968 and 1973 (Annex I, 3, 12, 22). An ADI of 0 - 0.0025 mg/kg bw was established at the last evaluation. Since that time additional information has become available and the results of studies submitted to the present meeting are summarized in this monograph addendum.

EVALUATION FOR ACCEPTABLE DAILY INTAKE

BIOLOGICAL DATA

Biochemical aspects

Absorption, distribution and excretion

The pharmacokinetic behaviour of carbonyl-^{14}C-labelled azinphos-methyl was investigated in rats. The material was almost completely absorbed from the digestive tract, and irrespective of dose and route of administration, 60 to 70% was eliminated in the urine and 25 to 35% in the faeces within 48 hours. Less than 0.1% of the administered activity was eliminated with the respiratory air within 24 hours of dosing, and in rats with biliary fistulas around 30% of the intravenously administered activity was eliminated in the bile within 24 hours of dosing. Two days after dosing the total activity content in the animal (excluding digestive tract) was less than 5% of the administered dose; by 4 days this had declined to 2% and by 16 days to 1%. Six hours after dosing, the highest concentrations of radioactivity were found in the organs of elimination (liver and kidney) with relatively high concentrations found in blood. The activity concentrations decayed rapidly in all organs up to 2 days post dosing, but thereafter the activity was more slowly eliminated. At 16 days after dosing the highest concentration was found in the erythrocytes. *In vitro* studies, in which whole blood was incubated with labelled parent compound, did not show any accumulation of radioactivity in the blood constituents (Patzschke *et al.*, 1976).

Dimethylthiophosphate (DMTP), one of the primary metabolites of azinphos-methyl, was detected in the urine of rats following dermal application of azinphos-methyl. A strong correlation was found between the amount of pesticide applied and urinary DMTP levels (Franklin *et al.*, 1982).

Urinary DMTP levels were measured in workers applying azinphos-methyl using orchard air-blast equipment and these data were used to estimate exposure to azinphos-methyl. These estimates were compared to exposure estimates derived by chemical analysis of patches attached to the protective clothing. It was concluded in by the author that urinary metabolite data provide a more reliable and accurate estimate of exposure than patch data (Franklin *et al.*, 1986).

A sample of radiolabelled ^{14}C azinphos methyl was applied to the forearm of an unspecified number of human subjects and the urinary excretion of radiolabel was quantified. Data obtained after intravenous dosing was used to correct the skin penetration data for incomplete urinary recovery. Using these data it was estimated that dermal penetration approximated 16% of the applied dose (Feldmann & Maibach, 1974).

The dermal penetration of azinphos-methyl through ventral forearm skin in man was around 16% of the applied dose over a 24 hour exposure period. This absorption increased by a factor of around 3.5 if the application site was occluded and increased by a factor of around 3.8 when damaged skin was compared to intact skin (Webster & Maibach, 1985).

The pharmacokinetic behaviour of benzazimide was investigated in rats using the ring-labelled ^{14}C-compound. After oral administration the ^{14}C- activity was almost completely absorbed from the gastrointestinal tract. Elimination of the activity took place quickly, 24 hours after administration only 1.3% of the amount administered was present in the animal not including the gastrointestinal tract. More than 99% of the amount administered was eliminated within 48 hours (54 to 66% in the urine and 33 to 45% via the faeces) (Weber *et al.*, 1980).

Biotransformation

The metabolism of azinphos-methyl was investigated by administration of ring-UL-^{14}C azinphos-methyl to male and female Sprague-Dawley rats. The metabolic pathway of azinphos-methyl in rats is proposed as detailed in Figure 1. Upon absorption, azinphos-methyl is rapidly metabolized by mixed function oxidases and GSH-transferases in the liver and other tissues, which results in the formation of azinphos-methyl oxygen analog, mercaptomethylbenzazimide, glutathionyl methylbenzazimide and desmethyl isoazinphos-methyl. Further hydrolysis, methylation and oxidation of mercaptomethyl-benzazimide forms benzazimide, methylthiomethylbenzazimide and its corresponding oxidised metabolites. Hydrolysis of glutathionyl methyl-benzazimide may result in the formation of cysteinylmethyl-benzazimide. Subsequent oxidation of cysteinyl-methylbenzazimide forms its corresponding sulfoxide and sulfone (Kao, 1988).

The rate of disappearance of azinphos-methyl effected by a hepatic oxidative desulfurating system and a demethylating system was investigated in liver homogenates from four different species (rat, guinea pig, chicken and monkey). Azinphos-methyl was metabolized by both systems and homogenates from all species were uniformly active (Rao & McKinley, 1969).

Effects on enzymes and other biochemical parameters

The acute oral toxicity of azinphos-methyl, dissolved in propylene glycol, was investigated in groups of female mice and the effect of the oxime antidote, toxogonin (80 mg/kg bw intraperitoneally, 15 minutes prior to oral dosing), was determined. Antidote treatment reduced the toxicity of azinphos-methyl by increasing the LD_{50} by a factor of 2 (Sterri *et al.*, 1979).

Figure 1. Metabolic Pathway of Azinphos-methyl in Rats

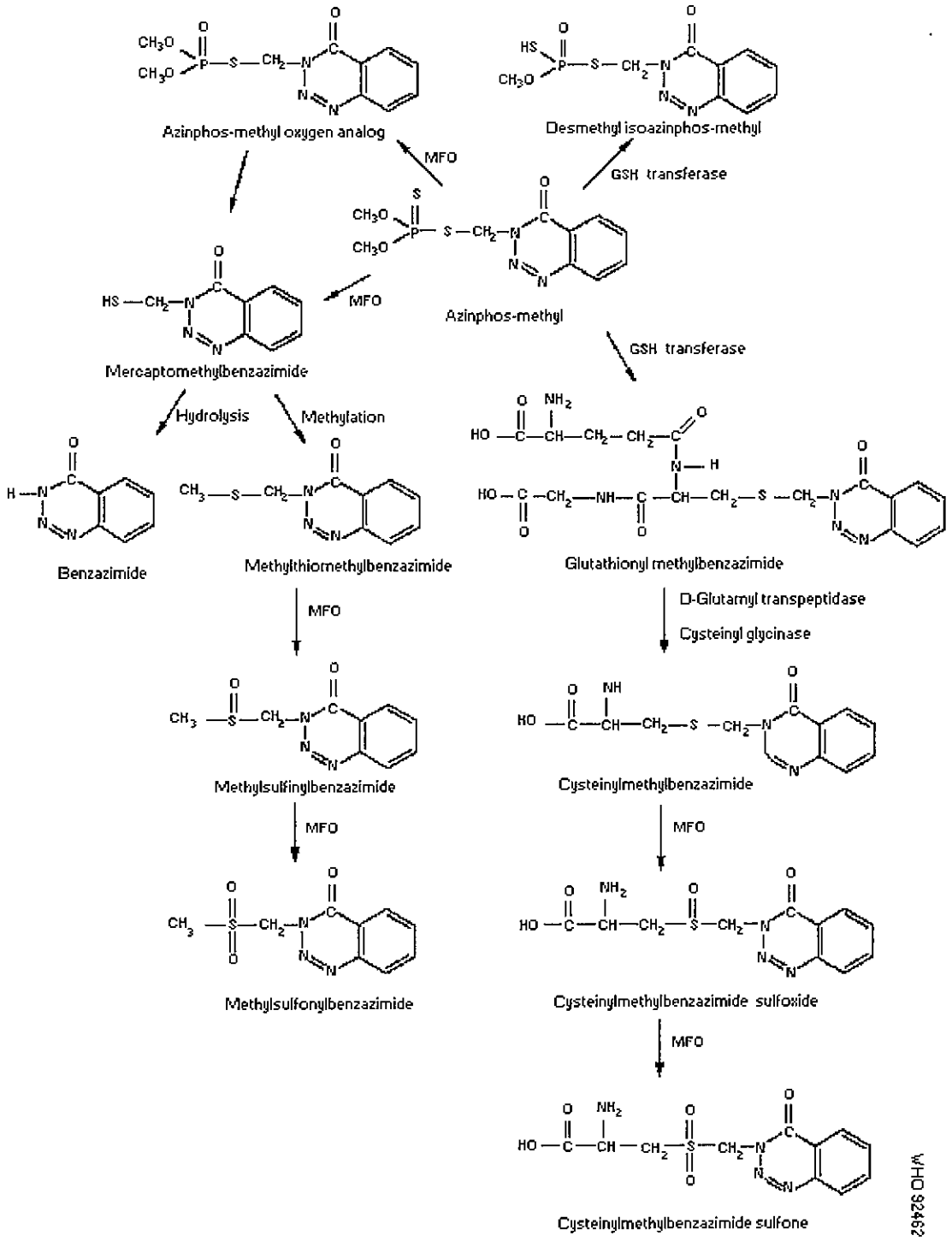

Table 1. Results of acute toxicity tests with azinphos-methyl and related materials

Test material	Route	Species	Vehicle	$LD_{5(}$
Azinphos methyl	Oral	Rat	DMSO	m 5 f 6

Benzazimide	Oral	Rat	DMSO	m 4 f 2
Methyl benzazimide	Oral	Rat	DMSO	m 3 f 3
Azinphos methyl	Oral	Rat	Cremophor EL	m 2
Azinphos methyl	Oral	Rat	Cremophor EL	m 9 m 1
Azinphos methyl	Oral	Rat	Cremophor EL	m 6 m 1
	Dermal	Rat	Cremophor EL	m 2 f 1
Azinphos methyl	Oral	Rat	Cremophor EL	m 7
Azinphos methyl	Oral	Rat	CMC (fasted)	m 1 f 1
			(non-fasted)	m 1 f 1

Table 1 (contd).

Test material	Route	Species	Vehicle	LD_{50}
Benzazimide	Oral	Rat	CMC (fasted)	m 5 f 3
			(non-fasted)	m 5 f 4
Methyl benzazimide	Oral	Rat	CMC (fasted)	m 4 f 3
			(non-fasted)	m 5 f 4
Azinphos methyl	Oral	Rat	Cremophor EL	m 4 f 4
	Dermal	Rat	Cremophor EL	m 2
	Oral	Dog	Cremophor EL	m >
Azinphos methyl	Oral	Rat	Methylene chloride/ Tween/80 Gum Arabic	m 2 f 2
	Dermal	Rat	Acetone/Ethanol/ Peanut Oil	f 9
Benzazimide	Dermal	Rabbit	Tap water	m > f >
Azinphos methyl	4 hr inhal	Rat	PEG/ETOH	m 1 f 1
Benzazimide	4 hr inhal	Rat	None	m > (mg

Table 1 (contd).

Test material	Route	Species	Vehicle	LD_{50}
Azinphos methyl	1 hr inhal	Rat	PEG/ETOH	m 3 f 3
Azinphos methyl	Oral	Rat	Cremophor EL	m 1
Azinphos methyl	Oral	Rat	Cremophor EL	m 1
Methamidophos	Oral	Rat	Cremophor EL	m 3
Azinphos methyl + Methamidophos	Oral	Rat	Cremophor EL	m 1
Azinphos methyl	Oral	Rat	Cremophor EL	m 9
Propoxur	Oral	Rat	Cremophor EL	m 3
Azinphos methyl + Propoxur	Oral	Rat	Cremophor EL	m 2
Azinphos methyl	Oral	Rat	Cremophor EL	m 9
Azinphos ethyl	Oral	Rat	Cremophor EL	m 1
Azinphos methyl + Azinphos ethyl	Oral	Rat	Cremophor EL	m 1

* sex not specified.
m male
f female

Toxicological studies

Acute Toxicity Studies

Results of acute toxicity tests with azinphos-methyl and related materials are listed in Table 1.

Short-term studies

Rats

A 12-week inhalation study has been described in the published literature. Groups of 10 male and 10 female Wistar rats were exposed in inhalation chambers to mean aerosol concentrations in the air of 0 (control), 0.195, 1.24 and 4.72 mg/m3 azinphos methyl, for 6 hours daily, 5 days per week, for 12 weeks. At the high dose level body weight gain was depressed in males and erythrocyte cholinesterase activity was inhibited in males and females. There was no other evidence of any reaction to treatment. The NOAEL was 1.24 mg/m^3 based on reduced weight gain in males at the high dose level (Kimmerle, 1976).

Rabbits

In a dermal toxicity study in rabbits, azinphos-methyl was applied for 6 hours per day, to the shaved dorsal and lateral skin, at

dose levels of 0 (control), 2, or 20 mg/kg bw, for 5 days per week for 3 weeks. Each group consisted of 6 males and 6 females, with the skin left intact in 3 animals of each sex and abraded in the others. Investigation of cholinesterase activity revealed a marginal (approximately 30%) depression of erythrocyte activity, compared to controls, in males and females treated with 20 mg/kg. Cholinesterase activity in plasma and brain, and erythrocyte activity at 2 mg/kg, remained undisturbed by treatment. There was no difference between the groups with intact and abraded skin, and all other investigations (clinical signs, measurement of food intake and weight gain, further clinical chemistry, haematology and urinalysis and pathological investigations including limited histopathology) revealed no treatment-related changes. The NOAEL was 20 mg/kg bw, since only erythrocyte cholinesterase activity was affected at this dose, the highest dose used, with no effect on cholinesterase activity in brain (Flucke & Schilde, 1980).

Dogs

In a 52 week toxicity study in beagle dogs, azinphos-methyl (purity 91.9%) was administered, via the diet, to four groups, each of 4 males and 4 females, at dietary levels of 0 (control), 5, 25 or 125 ppm. Clinical signs of reaction to treatment were confined to a higher incidence of diarrhoea in dogs receiving 125 ppm. Two males receiving 125 ppm failed to gain weight during the course of the study, but food intake remained unaffected by treatment. Haematology and urinalysis revealed no indication of any reaction to treatment. Clinical biochemistry tests revealed a depression of cholinesterase activity in plasma and erythrocytes at 25 and 125 ppm and in brain at termination at 125 ppm. There was also a very slight increase, compared to controls, in liver cytochrome P450 and N-demethylase activity at the high dose and a reduction in albumin levels. Pathological investigations (macroscopic examination, organ weight analysis and histopathology) revealed no evidence of any reaction to treatment with azinphos-methyl. The NOAEL was 25 ppm (equal to 0.74 mg/kg bw/day) based on reduced weight gain and inhibition of acetylcholinesterase activity in brain (Allen, *et al.*, 1990).

Long-term/carcinogenicity studies

Mice

A bioassay of azinphos-methyl (purity 90% from manufacturing specification) for possible carcinogenicity was conducted by the NCI. The experiment involved administering the test material, in the feed, to Osborne-Mendel rats and B6C3F1 mice. Groups of 50 rats of each sex were treated for 80 weeks, then observed for 34 or 35 weeks. Males received time weighted average doses of 78 or 156 ppm, females received 62.5 or 125 ppm. Matched controls consisted of 10 untreated rats of each sex; pooled controls consisted of matched controls combined with 95 male and 95 female untreated rats from similar bioassays of 10 other chemicals. The mouse study was of similar design; groups of 50 mice were treated for 80 weeks, then observed for 12 or 13 weeks, males received 31.3 or 62.5 ppm and females 62.5 or 125 ppm, matched controls consisted of 10 males and 10 females, pooled controls 130 males and 120 females. Typical signs of organophosphate intoxication (hyperactivity, tremors and dyspnoea) were observed in a few animals of both species. Weight gain in treated males and high dose females of both species was lower than in control animals. In rats there was some evidence of decreased survival at the high dose compared to controls but this was not seen in mice. In both sexes, at all doses, survival to termination was adequate for assessment of effects on late appearing tumours. The report concluded that in rats the incidence of tumours of the pancreatic islets, and of follicular

cells in the thyroid in males suggested, but did not clearly implicate, azinphos-methyl as a carcinogen in these animals. There was no similar evidence in female rats and in mice of each sex there

was no increased incidence of tumours that could be related to the administration of azinphos-methyl (National Cancer Institute, 1978).

In a carcinogenicity study of azinphos-methyl (purity 88.6%) in mice, groups of 50 male and 50 female CD-1 mice received dietary levels of 0 (control), 5, 20, or 40/80 ppm for two years. (The study was initially started with 80 ppm as the high dietary level, but this was reduced to 40 ppm after one week, due to severe reaction to treatment, including mortality, at 80 ppm). Following the reduction in the high dietary level, there were no clinical signs of reaction to treatment and mortality remained unaffected by treatment. Weight gain and food intake remained unaffected by treatment at dietary levels up to and including 40 ppm. Haematological investigations revealed no indication of any reaction to treatment. Measurement of acetylcholinesterase activity revealed that at 5 ppm, activities in plasma, erythrocyte and brain remained comparable with control values. At 20 and 40 ppm there was a dose-related inhibition of cholinesterase activity in plasma and erythrocytes. A similar effect was noted in brain, except that males were only affected at 40 ppm, while females exhibited a depression of brain cholinesterase activity at 20 and 40 ppm. Pathological investigations revealed no evidence of any reaction to treatment, in particular there was no evidence of any carcinogenic effect of azinphos-methyl. The NOAEL was 5 ppm (equal to 0.88 mg/kg bw/day) based on inhibition of cholinesterase in plasma, erythrocytes and brain at 20 ppm (Hayes, 1985).

Rats

In a combined long-term toxicity and carcinogenicity study in rats, groups of 60 male and 60 female Wistar rats received azinphos-methyl (purity 87.2%) in the diet at levels of 0 (Control), 5, 15 or 45 ppm. From each group, 10 rats per sex were killed after 12 months, while all survivors were killed after 24 months continuous treatment. There were no clinical signs of reaction to treatment and survival was unaffected by azinphos-methyl. Weight gain of high dose males was slightly less than controls but growth in other groups was not affected by treatment. Clinical biochemistry (apart from acetylcholinesterase investigations), haematology and urinalysis tests revealed no indication of any reaction to treatment. Determinations of acetylcholinesterase activities in erythrocytes, plasma and brain revealed a marked inhibition, compared to controls, in males and females from the high dose group (erythrocytes, plasma and brain) and a less marked effect at 15 ppm (males: erythrocytes, females: erythrocytes and plasma). Acetylcholinesterase activity in brain from rats treated at 15 ppm and in erythrocytes, plasma and brain from rats treated at 5 ppm, remained unaffected by treatment with azinphos-methyl. Pathological examinations (including gross examination, organ weight analysis and histological examination of tissues) revealed no evidence of any reaction to treatment; in particular there was no evidence of any carcinogenic effect of azinphos-methyl. The NOAEL was 15 ppm (equal to 0.86 mg/kg bw/day)

based on effects on body weight gain and brain acetylcholinesterase (Schmidt, 1987).

Reproduction studies

In a two generation (two litters per generation) reproduction study in rats azinphos-methyl (purity 87.2%) was administered to groups of 12 male and 24 female Wistar rats at dietary levels of 0

(control), 5, 15 or 45 ppm. At 15 and 45 ppm there was a decrease in fertility of F_0 rats and the total number of delivered pups. At 45 ppm there was an increased mortality of dams in the F_0 generation and reduced pup viability during lactation. As a consequence of these effects only 5 females were available for mating in the F_{1b} generation. During mating of the F_{1b} generation, fertility was again adversely affected at 15 ppm but not to as great an extent as it was during the F_0 generation. At all stages of the study, there was no evidence of treatment induced malformations and food intake remained unaffected. Clinical signs of reaction to treatment, including cholinergic signs, were seen at the high dose and weight gain was adversely affected at 15 and 45 ppm. The NOAEL was 5 ppm, equal to 0.48 mg/kg bw/day, based on the adverse effects on fertility and body weight gain seen at 15 and 45 ppm (Eiben & Janda, 1987).

A further study was conducted in order to investigate the effects on reproductive performance noted in the study described above. The objectives of this further, one generation study were to investigate whether the slight effect on fertility at 15 ppm could be confirmed, and, if reproducible, to determine whether the effect was attributable to treatment of the male or the female and to determine if reproductive effects were associated with treatment-induced inhibition of cholinesterase activity. Azinphos-methyl (purity 92.0%) was administered to groups of 18 male and 46 female Wistar rats, at dietary levels of 0 (control), 5, 15, or 45 ppm. Treated males and females were paired, and dams allowed to rear litters to weaning. Additional treated males were paired with untreated females. At 15 ppm, when males and females were treated, the viability index was reduced, largely confirming the results of the previous study. However, after treatment of male parental animals only, reproductive parameters remained unaffected, even at 45 ppm. Investigations of cholinesterase activity in parental animals revealed a depression in activity in plasma and erythrocytes at all dose levels, and a depression in activity in brain at 45 ppm in males and at 15 and 45 ppm in females. At 45 ppm, brain cholinesterase activity in pups was also depressed. The NOAEL was 5 ppm, equal to 0.43 mg/kg bw/day, based on the adverse effects on fertility and depression of brain cholinesterase activity seen at 15 ppm (Holzum, 1990).

Special studies on delayed neurotoxicity

In a published report of experiments designed to investigate the potential relationship between delayed neurotoxicity and copper concentration in the serum of hens, it was reported that azinphos-methyl failed to produce neurotoxic symptoms after either single or repeated doses (Kimmerle & Loser, 1974).

In an acute delayed neurotoxicity test, azinphos-methyl (purity 85%)was administered twice, at the unprotected LD_{50} dose level of 330 mg/kg to a group of 30 white leghorn hens, with an interval of 21 days between doses. Groups of untreated control, vehicle control and positive control (TOCP 600 mg/kg bw) animals, each composed of 10 animals, were also included. Atropine was used for symptomatic treatment after dosing. A total of 11 hens treated with azinphos-methyl survived until termination. These animals appeared normal during the last 12 or 13 days of the study, but exhibited varying degrees of impaired locomotor activity soon after dosing. Histopathological examinations indicated that azinphos-methyl did not increase the incidence or severity of lesions in the nerve tissue compared to untreated and vehicle controls. Investigations of neuropathy target esterase activity were not included in the study (Glaza, 1988).

Special studies on embryo/fetoxicity

Mice and rats

The effects of azinphos-methyl on development in rats and mice were investigated in a series of experiments. On the basis of preliminary toxicity studies doses of 0, 1.25, 2.5 and 5.0 mg/kg bw/day were selected for developmental studies in both species, which consisted of two phases. During the first phase, pregnant rats and mice were treated for 10 days, starting on gestational day 6. During the second phase, pregnant rats were treated from day 6 of gestation to day 21 post partum. In the first phase, maternal toxicity was seen only in rats receiving the high dose. When dams and fetuses were examined (day 18 of gestation for mice, day 20 for rats) there was no dose-related increase in anomalies or malformation in rats or mice. In the second phase, dams in the high-dose group were more sensitive to azinphos-methyl in the latter stages of gestation and signs of anticholinesterase intoxication, including mortality, were observed. As a result, only one litter (out of 13) survived to weaning in this group. It was concluded that azinphos-methyl had little primary effect on development in rats and mice (Short, et al, 1978; Short, *et al.*, 1980).

Rats

In a teratology study in rats, groups of 33 inseminated dams received azinphos-methyl (purity 87.7%) from day 6 to day 15 of gestation (day of insemination = day 0), at dose levels of 0 (control), 0.5, 1.0 or 2.0 mg/kg bw/day. From each group, 5 dams were killed on day 16 of gestation and the remaining dams on day 20. On day 16 of gestation, cholinesterase activities in plasma, erythrocytes and brain were depressed, compared to controls, in dams at the high dose only (fetal tissues were not examined). By day 20 of gestation there was indication of recovery in cholinesterase activity in all previously affected tissues and fetal brain cholinesterase activity was comparable with control values. Azinphos-methyl did not affect any maternal reproductive parameters and there was no indication of treatment-related embryotoxicity, fetotoxicity or teratogenicity at any dose level. The NOAEL for maternal toxicity was 1.0 mg/kg bw/day, based on the inhibition of brain cholinesterase activity seen on day 16 of gestation (Kowalski *et al.*, 1987).

Rabbits

In a teratology study in rabbits, groups of 11 or 12 pregnant animals received daily oral doses of azinphos-methyl (purity 92.4%) from day 6 to day 18 of gestation (day of insemination = day 0) at levels of 0 (Control), 0.3, 1.0 or 3.0 mg/kg bw/day. Caesarean section was carried out on day 29 of gestation. Azinphos-methyl induced no evidence of maternal toxicity at any dose level and there were no detectable effects on embryonic nor fetal development (Machemer, 1975).

In a further teratology study in rabbits, groups of 20 inseminated does received daily oral doses of azinphos-methyl (purity 87.7%) from day 6 to day 18 of gestation (day of insemination = day 0) at levels of 0 (Control), 1, 2.5 or 6 mg/kg bw/day. Ataxia in 4 high dose does and tremors in 2 of these same animals represented clinical signs of reaction to treatment.

Plasma and erythrocyte cholinesterase activity, on day 19 of gestation, was depressed compared to controls at the mid and high dose. By day 28 of gestation there was clear evidence of recovery in plasma and erythrocyte cholinesterase activity, although activity in

brain was depressed, compared to controls, at the high dose. Azinphos-methyl did not affect any maternal reproductive parameters and there was no evidence of any treatment-related effect on embryotoxicity, fetotoxicity or teratogenicity at any dose level. The no observable adverse effect level for maternal toxicity was 2.5 mg/kg bw/day, based on the inhibition of brain cholinesterase activity seen on day 28 of gestation (Clemens *et al.*, 1988).

Special studies on genotoxicity

In *Salmonella*/microsome point mutation tests, azinphos-methyl (purity >88.8%) showed no evidence of mutagenic activity which could be classified as positive results in the tests. In one test there was reproducible evidence of a slight dose-dependent increase in revertant frequency in one test strain, but the increase was less than 2-fold (Herbold, 1978; Herbold, 1988; Lawlor, 1987).

Azinphos-methyl (purity 91.1%) exhibited no mutagenic activity in a reverse mutation test with *Saccharomyces cerevisiae* (Hoorn, 1983).

In Chinese hamster ovary cells *in vitro,* azinphos-methyl induced chromosomal anomalies in a dose related fashion. Most commonly observed were chromatid breaks and exchanges. In a test with human lymphocytes *in vitro,* (purity 91.9%) there were no chromosomal aberrations induced in the absence of S-9 mix but clear, treatment-related variations were noted when azinphos-methyl was tested in the presence of S-9 mix at cytotoxic concentrations. In an investigation of ability to induce sister chromatid exchanges in Chinese hamster V79 cells *in vitro,* azinphos-methyl was shown not to increase the frequency of sister chromatid exchange, but did induce some cell cycle delay (Alam, 1974; Herbold, 1986; Chen 1982 *et al.*, 1982a,b).

The potential of azinphos-methyl (purity 91.1%) to cause DNA damage was assessed in Rosenkranz and Leifer's pol test employing two *E. coli* strains which vary in regard to their repair systems for DNA damage. The results showed that azinphos-methyl gave no indication of any effect on DNA damage. In a primary rat hepatocyte unscheduled DNA synthesis assay azinphos-methyl (purity 91.1%) did not induce significant changes in the nuclear labelling of primary rat hepatocytes and it was concluded that azinphos-methyl did not induce DNA damage in this assay (Herbold, 1984; Myhr, 1983).

Mutagenic effects of azinphos-methyl (purity 92.3%) *in vivo* were investigated in a micronucleus test and a dominant lethal test; both in mice. In the micronucleus test two doses of azinphos-methyl (2 x 2.5 or 2 x 5.0 mg/kg bw) were given 24 hours apart and a femoral marrow smear was prepared 6 hours after the second dose; there was no indication of any mutagenic effect. In the dominant lethal study male mice received a single oral dose of 4 mg/kg bw azinphos-methyl and were then mated with untreated females over 12 consecutive periods of 4 days. Fertility remained unaffected and there were no treatment-related differences in implantation parameters (Herbold, 1979a,b).

An effort was made to evaluate the genotoxicity of a variety of pesticides, with the specific objectives of comparing different *in vivo* and *in vitro assays,* examining the spectrum of genetic activity displayed by the selected pesticides and examining the test results in relation to other biological and chemical features of the pesticides. In this research programme azinphos-methyl has been tested in a range of 14 mutagenicity tests, examining point or gene mutations, DNA damage and chromosomal effects. Positive results for azinphos-methyl were seen in only two tests: a forward mutation assay

in mouse lymphoma L5178Y cells (only in the presence of S-9 mix) and a mitotic recombination assay in *Saccharomyces cerevisiae* strain D3. Azinphos-methyl was negative in tests for point/gene mutation and DNA damage in prokaryotes and showed no positive results in tests looking at chromosomal effects (Waters *et al.*, 1982).

Special studies on skin and eye irritation and sensitization

In a skin irritation study employing 6 rabbits, 24 hour exposure to azinphos-methyl at intact and abraded skin sites did not cause any signs of irritation. In an eye irritation study, exposure of the conjunctiva of the eye to azinphos-methyl for 5 minutes (5 rabbits) or 24 hours (3 rabbits) caused no significant reaction (Thyssen & Lorke, 1981).

In a dermal irritation study utilizing 6 New Zealand white rabbits, 0.5 g of benzazimide was moistened with water and kept in contact with the shaved skin for 4 hours. At 30 and 60 minutes and 24, 48 and 72 hours after patch removal there was no evidence of erythema or oedema at the treatment sites. Benzazimide is therefore not a skin irritant in rabbits (Eigenberg, 1987).

The skin sensitizing potential of azinphos-methyl was investigated in guinea pigs, using the Magnusson and Kligman maximization test. The study revealed that azinphos-methyl had a sensitizing effect in 95% of the test animals (Flucke, 1986).

The skin sensitizing potential of azinphos-methyl was investigated in guinea pigs, using the Buehler patch test. By means of a dermal application of a concentration of 25% sensitization was induced in approximately 50% of the test animals (Porter *et al.*, 1987).

In another Buehler patch test dermal application of a concentration of 12.5% induced sensitization in approximately 50% of the test animals when challenged using a 6% concentration, but using a challenge concentration of 0.6% failed to elicit any relevant skin reactions (Heimann, 1987b).

Observations in humans

Employees working in the formulation of azinphos-methyl products have been subjected to regular medical examinations and no general impairment of health has been observed. In one isolated case it was considered probable that contact with azinphos-methyl was the cause of generalized dermatosis in an apparently hypersensitive, very dry skin (Faul, 1981; Miksche, 1981).

Published reports from the pesticide incident monitoring system in the United States of America and additional data from the state of California in the USA have been reviewed. Between 1982 and 1988 a small number of incidents have been reported annually (involving 5-12 persons each year) which have been definitely, probably or possibly associated with azinphos-methyl either alone or in combination with other pesticides. In addition, two incidents occurred in 1987, one involving 26 people, the other involving 32 people. The first involved spray drift in adverse weather conditions. The second involved workers who experienced symptoms including headache, nausea, weakness and vomiting upon entry to a field to pick peaches 3 days after methomyl was applied to the crop and about 6 weeks after an application of azinphos-methyl (US EPA, 1981; Mahler, 1991).

COMMENTS

The toxicokinetics of azinphos-methyl has been investigated following oral administration in rats. It does not accumulate in body tissues.

In a 52-week study in dogs, using dietary concentrations of 0, 5, 25 or 125 ppm the NOAEL was 25 ppm (equal to 0.74 mg/kg bw/day), based on reduced body-weight gain and inhibition of acetylcholinesterase activity in brain at 125 ppm.

Long-term/carcinogenicity studies in rats at dietary concentrations of 0, 5, 15, or 45 ppm and in mice at 0, 5, 20 or 40 ppm showed that azinphos-methyl has no carcinogenic potential in either species. These results clarified earlier equivocal findings in rats in an NCI bioassay. The NOAEL in rats was 15 ppm (equal to 0.86 mg/kg bw/day), based on effects on brain acetylcholinesterase at 45 ppm. In mice the NOAEL was 5 ppm (equal to 0.88 mg/kg bw/day), based on inhibition of cholinesterase in plasma, erythrocytes and brain at 20 ppm.

In a two-generation reproduction study in rats at dietary concentrations of 0, 5, 15 or 45 ppm, fertility and pup viability during lactation were adversely affected, equivocally at 15 ppm and markedly at 45 ppm. The NOAEL was 5 ppm, equal to 0.48 mg/kg bw/day. Teratology studies in rats, mice and rabbits did not indicate teratogenic effects at doses up to 2, 5 and 6 mg/kg bw/day respectively.

The data from genotoxicity studies with azinphos-methyl were conflicting. However, *in vivo* studies were negative, the positive data being confined to some *in vitro* studies. After reviewing the available information it was concluded that it is unlikely that azinphos-methyl is genotoxic to humans.

Acute delayed neurotoxicity tests in hens with azinphos-methyl gave negative results.

The 1973 JMPR reported that daily doses up to and around 0.3 mg/kg bw/day for 30 days in human volunteers had no effect on plasma or erythrocyte cholinesterase activity. New data were not available from occupational exposure or human volunteer studies with azinphos-methyl. A review of the available literature and reports of human poisoning with azinphos-methyl revealed no information relevant to the estimation of the ADI.

Since the critical toxicological end-point was not acetylcholinesterase inhibition, the human data were not appropriate for estimation of the ADI, which was based on the NOAEL in the rat multigeneration study in rats using a 100-fold safety factor.

TOXICOLOGICAL EVALUATION

Level causing no toxicological effect

Mouse:	5 ppm (equal to 0.88 mg/kg bw/day)
Rat:	5 ppm (equal to 0.86 mg/kg bw/day) in a long-term/carcinogenicity study 5 ppm (equal to 0.48 mg/kg bw/day) in a multigeneration study
Dog:	25 ppm (equal to 0.74 mg/kg bw/day)
Human:	0.3 mg/kg bw/day

Estimate of acceptable daily intake for humans

0-0.005 mg/kg bw

Studies which will provide information valuable in the continued evaluation of the compound

Further observations in humans

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See Also:
Toxicological Abbreviations
Azinphos-methyl (ICSC)
Azinphos-methyl (PDS)
Azinphos-Methyl (FAO Meeting Report PL/1965/10/1)
Azinphos-methyl (FAO/PL:1968/M/9/1)
Azinphos-methyl (WHO Pesticide Residues Series 2)
Azinphos-methyl (WHO Pesticide Residues Series 3)
Azinphos-methyl (WHO Pesticide Residues Series 4)

A WORLD COMPENDIUM

The Pesticide Manual

Twelfth Edition

Editor: C D S Tomlin

BRITISH CROP PROTECTION COUNCIL

Compatibility Incompatible with alkaline materials. **Selected tradenames** 'Azinugec E' (Sipcam Phyteurop)

ANALYSIS: **Product** analysis by colorimetric measurement of the phosphorodithioate moiety as a complex (*CIPAC Handbook*, 1970, **1**, 18; FAO Specification (CP/41)). **Residues** determined by glc (D. C. Abbott *et al.*, *Pestic. Sci.*, 1970, 1, 10; M. A. Luke *et al.*, *J. Assoc. Off. Anal. Chem.*, 1981, **64**, 1187).

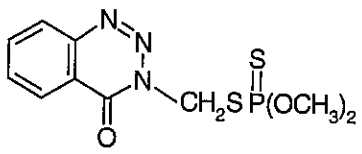
MAMMALIAN TOXICOLOGY: **Reviews** FAO/WHO 20, 21 (see part 2 of the Bibliography). **Oral** Acute oral LD_{50} for rats *c.* 12 mg/kg. **Skin and eye** Acute percutaneous LD_{50} for rats *c.* 500 mg/kg (24 h). Not irritating to skin and eyes of rabbits. **Inhalation** LC_{50} (4 h) for rats *c.* 0.15 mg/l air. **NOEL** (2 y) for rats 2, dogs 0.1, mice 1.4 (all as mg/kg diet), monkeys 0.02 mg/kg b.w. **ADI** (JMPR) No ADI [1973]. **Other** Acute i.p. LD_{50} for rats >7.5 mg/kg. **Toxicity class** WHO (a.i.) Ib; EPA (formulation) I **EC hazard** T+; R28| T; R24| N; R50, R53

ECOTOXICOLOGY: **Birds** Acute oral LD_{50} for Japanese quail 12.5–20 mg/kg. **Fish** LC_{50} (96 h) for golden orfe 0.03, rainbow trout 0.08 mg/l. **Daphnia** LC_{50} (48 h) 0.0002 mg/l. **Bees** Not toxic to bees (depends on application method).

ENVIRONMENTAL FATE: **EHC** 63 (WHO, 1986; a general review of organophosphorus insecticides). **Animals** In mammals, following oral administration, >90% is eliminated in the urine and faeces within 2 days. The major metabolites are the monodesethyl compound and benzazimide. **Plants** In plants, metabolites identified include azinphos-ethyl-oxon, benzazimide, dimethylbenzazimide sulfide and dimethylbenzazimide disulfide. **Soil/Environment** Based on the K_{oc} value and leaching studies, azinphos-ethyl can be classified as a compound with very low mobility. The half-life is several weeks. Metabolites formed in soil under aerobic and anaerobic conditions are: desethyl azinphos-ethyl, sulfonmethylbenzazimid, bis(benzazimidmethyl)ether, methylthiomethylsulfoxide and methylthiomethylsulfone.

47 azinphos-methyl *Insecticide*

organophosphorus



NOMENCLATURE: **Common name** azinphos-methyl (BSI, E-ISO, (*m*) F-ISO); azinphosmethyl (ESA); metiltriazotion * (former exception, USSR)
IUPAC name *S*-(3,4-dihydro-4-oxobenzo[*d*]-[1,2,3]-triazin-3-ylmethyl) *O,O*-dimethyl phosphorodithioate
Chemical Abstracts name *O,O*-dimethyl *S*-[(4-oxo-1,2,3-benzotriazin-3(4*H*)-yl)methyl] phosphorodithioate
CAS RN *[86–50–0]* **EEC no.** 201–676–1 **Development codes** Bayer 17 147; R 1582; E1582
Official codes ENT 23 233; OMS 186

PHYSICAL CHEMISTRY: **Mol. wt.** 317.3 **M.f.** $C_{10}H_{12}N_3O_3PS_2$ **Form** Yellowish crystals. **M.p.** 73 °C **V.p.** 5×10^{-4} mPa (20 °C); 1×10^{-3} mPa (25 °C) K_{ow} logP = 2.96 **Henry** 5.7×10^{-6} Pa m^3 mol^{-1} (20 °C, calc.) **S.g./density** 1.518 (21 °C) **Solubility** In water 28 mg/l (20 °C). In dichloroethane, acetone, acetonitrile, ethyl acetate, dimethyl sulfoxide >250, *n*-heptane 1.2, xylene 170 (all in g/l, 20 °C). **Stability** Rapidly hydrolysed in alkaline and acidic media; DT_{50} (22 °C) 87 d (pH 4), 50 d (pH 7), 4 d (pH 9). Photodegrades on soil surfaces and readily photodegrades in water. Decomposes above 200 °C.

COMMERCIALISATION: **History** Insecticide and acaricide reported by E. E. Ivy *et al.* (*J. Econ. Entomol.*, 1955, **48**, 293). Developed by W. Lorenz and introduced by Bayer AG. **Patents** US 2758115; DE 927270 **Manufacturers** Bayer; General Quimica; Makhteshim-Agan.

APPLICATIONS: **Biochemistry** Cholinesterase inhibitor. **Mode of action** Non-systemic, with contact and stomach action. **Uses** Control of chewing and sucking insects of the orders Coleoptera, Diptera, Homoptera, Hemiptera, and Lepidoptera, on fruit trees (including citrus), vines, strawberries, nuts, vegetables, potatoes, cereals, maize, cotton, ornamentals, beet, soya beans, tobacco, rice, coffee, sugar cane, forestry, and other crops. **Phytotoxicity** Russetting is possible on some fruit varieties with the emulsifiable concentrate formulation. **Formulation types** DP; EC; SC; WP. **Compatibility** Incompatible with alkaline materials. **Selected tradenames** 'Gusathion M' (Bayer); 'Acifon' (General Quimica); 'Azinugec' (Sipcam Phyteurop); 'Cotnion-Methyl' (Makhteshim-Agan)

ANALYSIS: **Product** analysis by lc (*AOAC Methods*, 1995, 989.01, 7.7.02), by hplc (*AOAC Methods*, 1995, 989.01; *CIPAC Handbook*, 1992, **E**, 12) or by i.r. spectrophotometry (*ibid.*, 1995, 980.09; *CIPAC Handbook*, 1985, **1C**, 1970) or by colorimetric measurement of the phosphorodithioate moiety as a complex (*ibid.*, 1970, **1**, 25; FAO Specification CP/4). **Residues** determined by glc (*Analyst (London)*, 1977, **102**, 858; A. Ambrus *et al.*, *J. Assoc. Off. Anal. Chem.*, 1981, **64**, 733; D. H. MacDougall, *Anal. Methods Pestic. Plant Growth Regul.*, 1972, **6**, 397). Methods for the determination of residues are available from Bayer.

MAMMALIAN TOXICOLOGY: **Reviews** FAO/WHO 62, 64 (see part 2 of the Bibliography). **Oral** Acute oral LD_{50} for rats *c.* 9, male guinea pigs 80, mice 11–20, dogs >10 mg/kg. **Skin and eye** Acute percutaneous LD_{50} for rats 150–200 mg/kg (24 h); not a skin irritant; mild eye irritant (rabbits). **Inhalation** LC_{50} (4 h) for rats 0.15 mg/l air (aerosol). **NOEL** (2 y) for rats and mice 5 mg/kg diet; (1 y) for dogs 5 mg/kg diet. **ADI** (JMPR) 0.005 mg/kg b.w. [1991]. **Toxicity class** WHO (a.i.) Ib; EPA (formulation) I **EC hazard** T+; R26/28| T; R24| R43| N; R50, R53

ECOTOXICOLOGY: **Birds** Acute oral LD_{50} for bobwhite quail *c.* 32 mg/kg. Dietary LC_{50} (5 d) for Japanese quail 935 mg/kg diet. **Fish** LC_{50} (96 h) for rainbow trout 0.02, golden orfe 0.12 mg/l. **Daphnia** LC_{50} (48 h) 0.0011 mg/l. **Algae** E_rC_{50} (96 h) for *Scenedesmus* 7.15 mg/l. **Bees** Toxic to bees. **Worms** LC_{50} (14 d) 59 mg/kg. **Other beneficial spp.** Azinphos-methyl is an effective insecticide, therefore an effect on some non-target arthropods cannot be excluded, in particular, where those organisms are directly exposed to the spray treatment.

ENVIRONMENTAL FATE: **EHC** 63 (WHO, 1986; a general review of organophosphorus insecticides). **Animals** In mammals, following oral administration, >95% is eliminated in the urine and faeces within 2 days. The major metabolites are the monodesmethyl compound and benzazimide. **Plants** In plants, major metabolites identified include azinphos-methyl oxon, benzazimide, mercaptomethyl benzazimide and cysteinmethyl benzazimide. **Soil/Environment** Degradation involves oxidation, demethylation, and hydrolysis. Based on the K_{oc} values and leaching studies, azinphos-methyl can be classified as a compound with low mobility. The half-life in soil is several weeks.