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Interim Chemical Review Committee
Fifth session
Geneva, 2 – 6 February 2004
Item 5(a) on the provision agenda*

INCLUSION OF CHEMICALS IN THE INTERIM PRIOR INFORMED CONSENT PROCEDURE - SUPPORTING DOCUMENTATION

Endosulfan

Note from the Secretariat

1. Annexed to this note is the documentation provided by Jordan in support of their notification of final regulatory action on endosulfan.

List of Documentation Annexed to UNEP/FAO/PIC/ICRC5/10/Add.1

Supporting documentation on endosulfan from Jordan:

English Translations to First Regulatory Action Against Endosulfan from Jordan
Focused Summary – Endosulfan
Study of the extent of pollution of Jordanian environment as a result of pesticide use
JMPR Report – Endosulfan 1998
Excerpt of the Pesticide Manual, 10th edition - endosulfan

Translations of document No. 3a

Re: First Regulatory action against endosulfan

Session 271 of the Agricultural Pesticide committee

Date 25/7/1991

Excerpts of the minutes related to the control actions against endosulfan

The committee of pesticides met on Thursday the 25th /7/1991 under the chair ship of the head of protection division (Dr. Hani Hadadain) and the membership of:

1\ Dr. Yousif Alshoraigi
2\ Dr. Eisa Abanba
3\ Eng. Khalid Musfat
4\ Eng. Said Magad
5\ Eng. Khalaf Alogla
6\ Eng. Mahmoud Altibish
7\ Dr. Mazin Akkawi

The following issues were discussed:

Paragraph No1 was not relevant and therefore was not translated.

Paragraph No. 2; Study of the insecticide Thiodan presented by the Eastern Company and intended to be imported from Germany.

The committee decided to refuse registration because of the following reasons;

- High toxicity to humans and environment and to its persistence in the environment as it contains chlorinated hydrocarbons;
- No need by agricultural sector because of availability of other alternatives;
- Long safety period for vegetables.

The committee decided;

To stop granting any new import license for formulations containing this active ingredient and cancellation of registered product after the expiry of their license. All companies must be aware by the committee decision.

The Eng. Mahmoud Altibish refused the committee decision for the reasons indicated later (decision item No. 2).

Other issues discussed in this meeting were not translated as they were not relevant.

Signature of attendants:

:

Translations of document No. 3b
Re: Second Regulatory action against endosulfan
Session 325 of the Agricultural Pesticide committee
Date 4/5/1994
Excerpts of the minutes related to the control actions against endosulfan

The committee of pesticides met on Wednesday the 4th /5/1994 under the chair ship of Eng. Mazin Alhafawna, the director of Agric. Protection and the membership of:
1\ Dr. Mazin Akkawi
2\ Eng. Mahmoud Altibish
3\ Eng. Ahmed Alhafawna
4\ Eng. Khalaf Alogla
5\ Dr. Eisa Abanba
6\ Eng. Khalid Musfat
7\ Eng. Said Magad

The following issues were discussed:
Agenda items No. 1 – 5 were not relevant and therefore were not translated.
Agenda item No. 6;
The committee listened to reports (The assumed report??? was translated as document No. 5) from representatives of ministry of health and the Jordanian university about re-registration of the insecticide Thiodan which contain the active substance endosulfan.

- The committee refused the re-registration as this product (Thiodan) contains chlorinated hydrocarbon substance and some scientific research confirm that it may cause cancer as well as it was found in underground water.

Other issues discussed in this meeting were not translated as they were not relevant.

Signature of attendants:
- Head of Agric. Quarantine Division, Eng. Said Magad

- Chair of the committee, Eng. Mazin Alhafawna
- Representative of faculty of agric., Jordanian University, Dr. Mazin Akkawi

(refuse agenda item No. 6 regarding the insecticide endosulfan as it is still approved by EPA and we rely on registration on certificates from EPA.).

- Head of analytical center of pesticides, Eng. Ahmed Alhafawna.
- Representative of ministry of environment, Eng. Khalaf Alogla

(Refuse agenda item No. 5, not relevant to endosulfan and therefore not translated).

- Representative of agrochemical merchants, Eng. Mahmoud Altibish.

(Refuse agenda item No. 6 and I accept its registration because it was registered by US-EPA).

- Representative of ministry of health, Dr. Eisa Abanba

(Refuse agenda item No. 6 as we still do not have any thing which oppose the registration of the above mentioned pesticide if it is used according to recommended directions)

- Representative of national center for research, Eng. Khalid Musfat

(Refuse agenda item No. 5, not relevant to endosulfan and therefore not translated).

Focused Summary- Endosulfan

1\ INTRODUCTION:

This section should provide a brief statement / summary of the final regulatory actions and the reasons for the action taken (e.g. occupational health concerns, environmental concerns). Could include:

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

There are two regulatory actions (1991 and 1994).

Events for 1991 action:

A company called Alshargia applied for registration of imported Thiodan (from Germany). The control action was taken in the in the session No. 271 of the agricultural pesticide committee dated 25/7/1991.

Event for 1994 action:

The committee listened to reports (The report was study of the extent of pollution of Jordanian environment as a result of pesticide use, document No. 5) from representatives of ministry of health and the Jordanian university about re-registration of the insecticide Thiodan which contain an active substance endosulfan. The control action was taken in the in the session No. 325 of the agricultural pesticide committee dated 4/5/1994.

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

The banning of endosulffan would reduce the hazards to human health (high toxicity, and long safety period) and environment (persistent, decrease pollution of ground water and soil).

© An overview of the regulatory system of the notifying country if relevant;

Pesticides were used to be regulated by the law of Agriculture No. 20 for the year 1973, through a multi-stake holder committee called the Agricultural Pesticides Committee. Recently the law was amended to the Interim Law of Agriculture No. 44 for the year 2002. According to this law a national multi-stake holder committee called Pesticides Registration Committee is formed and responsible for registration, re-registration and cancellation of registration of pesticides within the Hashemite Kingdom of Jordan. The pesticide division within the ministry of agriculture is responsible for approval of label while the provinces had the authority of granting license for retailers as well as inspection of any miss-use or off law activities.

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

There are two regulatory actions (refusal of registration) against this product in Jordan.

The first Decision (1991):

It is prohibited to place on the market or use plant products containing endosulfan. The decision at that time was the refusal of registration for import of the formulation Thiodan. This decision further included the stoppage of granting any new import license for formulations containing this active ingredient and cancellation of registered product after the expiry of their license.

The second Decision (1994):
The committee refused the re-registration of this product (Thiodan) as it contains chlorinated hydrocarbon substance and research findings indicated that it can cause cancer and it is found in ground water.

11\ RISK EVALUATION;
This section should provide evidence that a risk evaluation was carried out under the prevailing conditions of the notifying country. It should confirm that criteria Annex 11 (b) are met. May include;

(a) Key finding of a national risk evaluation;

First Decision (1991):

- High toxicity to humans and environment and persistence in the environment as it contains chlorinated hydrocarbons;
- No need by agricultural sector because of availability of other alternatives;
- Long safety period for vegetables (the safety period in Jordan is determined based on information submitted on the label, no national data, source; interveiws).

(Minutes of the meeting did not clearly indicate a national exposure data were generated or considered in making the national risk evaluation)

Second decession (1994):

- Contains chlorinated hydrocarbon substance;
- Cause cancer;
- Found in underground water.

The national data available was study of the extent of pollution of Jordanian environment as a result of pesticide use (see document No. 5). This document reported the presence of endosulfan residues among other pesticides in soil samples analyzed. The relevant recommendation in this document was given below;
The necessity of being more strict on prevention of import of the banned chlorinated pesticides (like endrin and aldrin).

(b) Key data reviews consulted and a brief description;

- pesticide manual
- FAO/WHO

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

There are general data of a monitoring program of pesticide residues in Jordan environment. This national data is study of the extent of pollution of Jordanian environment as a result of pesticide use (see document No. 5).
Summary of actual (or potential) human exposure and or environmental fate.
This document (No. 5) reported the presence of endosulfan residues among other pesticides in soil samples analyzed. The relevant recommendation in this document was given below;
The necessity of being more strict on prevention of import of the banned chlorinated pesticides (like endrin and aldrin)

111\ RISK REDUCTION AND RELEVANCE TO OTHER STATES
This section should provide evidence that the control action is of relevance to other states. Could include information on the followings;

(a) Estimation of quantities of chemicals used or imported/exported at the time of the regulatory action and if possible information on ongoing trade;

The Hashemite Kingdom of Jordan has imported 240 Kg of endosulfan in 1990. Jordan has no information on ongoing trade.

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

The Hashemite Kingdom of Jordan has no information.

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

The product was registered to be used as insecticide.

Focused Summary- Dimefox

I\ INTRODUCTION:

This section should provide a brief statement / summary of the final regulatory actions and the reasons for the action taken (e.g. occupational health concerns, environmental concerns). Could include:

(c) The events that led to the final regulatory action

The committee received information about potential hazards of dimefox to human health and the environment. The action was taken in the 68th session of the agricultural pesticides committee dated 29/10/1980. It was stated that the control action will enter into force on 1/1/1981.

(d) Significance of the regulatory action, e.g. one use or many uses, level or degree of exposure;

The banning of dimefox would reduce the hazards to human health and the environment as it is highly toxic and persistent in the environment.

© An overview of the regulatory system of the notifying country if relevant;

Pesticides were used to be regulated by the law of Agriculture No. 20 for the year 1973, through a multi-stake holder committee called the Agricultural Pesticides Committee. Recently the law was amended to the Interim Law of Agriculture No. 44 for the year 2002. According to this law a national multi-stake holder committee called Pesticides Registration Committee is formed and responsible for registration, re-registration and cancellation of registration of pesticides within the Hashemite Kingdom of Jordan. The pesticide division within the ministry of agriculture is responsible for approval of label while the provinces had the authority of granting license for retailers as well as inspection of any miss-use or off law activities.

(e) Scope of the regulatory action-precise description of the chemicals subject to the regulatory action;

It is prohibited to place on the market or use plant products containing dimefox. The decision at that time was against the registered formulations (Pestox 50 EC). This decision was interpreted to include all formulations containing dimefox.

II\ RISK EVALUATION;

Study of the extent of pollution of Jordanian environment as a result of pesticide use

Final Report

Prepared by;
Ministry of Agriculture / Department of Plant Protection
Jordanian University / Center for Consultation, Technical Service and Studies
Royal Scientific Society / Center of Industrial Chemistry
Royal Scientific Society / Center of Environmental Research

1992-1993

Presented to:
Ministry of Municipal, Rural and Environmental Affairs

Chapter Six
Summary, Conclusions and Recommendations

Summary:

6-1-1 Agricultural Soil:

1\ Residues of the pesticide (DDT) were the most found residues in soil samples collected from Ala Qwar area, irrespective of whether being collected from bare area or underneath greenhouses (plastic houses). Sometimes it was found in samples from high land (Wadi Shoaib, Wadi Kafranga, Albaqaa, and Sail Alzargaa). The concentration of this pesticide and its derivatives was higher in surface levels compared to deeper levels. The difference in concentration between various areas is due to the use of DDT in mosquito control as control campaigns were more intensive in Ala Qwar area but their effects can reach to the valleys connected to it. Also DDT is found as impurity in the pesticide dicofol, a commonly used pesticide in Jordan.

2\ Residues of other pesticides were also found at variable frequencies in the soil samples analyzed. They include Chlorinated pesticides (endrin, dieldrin, endosulfan, lindane, chlorothalonil, dicofol, tetradifon, bromopropylate), phosphorous pesticides (of which, dimethoate, pyrazophos, methidathion, azinphos, chlorpyriphops, phosphamidon), carbamate pesticides (carbofuran, carbaryl, oxamyl, pirimicarb), pyrethroid pesticides (cypermethrin, deltamethrin) and nitrogenous pesticides (hexaconazol, penconazol, oxadiazon, oxadixyl, procymidone, vinclozolin).

3\ In most cases the concentration of residues was higher in surface soil samples analyzed compared to deeper levels. The concentration underneath used greenhouses was higher compared to unused ones. This was mainly attributed to the continuation in the use of these pesticides in agricultural activities which increases their concentration in surface levels upon each use and to their ability to decompose in soil which result in gradual decrease of their concentration upon stoppage of use or increase in depth.

4\ On the other hand, residues of three pesticides were also found (procymidone, oxadixyl, vinclozolin) in soil samples from deeper levels, some times at concentrations comparable to their concentration in surface levels. Also concentration does not decrease underneath unused greenhouses. This was mainly attributed to the persistence of these pesticides in soil which may cause infiltration of these pesticides (vinclozolin, oxadixyl) to deeper levels with irrigation water because of their relatively higher solubility in water. The penetration of the pesticide (procymidone) during soil tillage and preparation for planting due to its higher association with soil particles. The presence of the residues of these pesticides was attributed to their intensive use in agriculture (which increases their concentration in surface levels specifically in the cases of repeated use).

5\ The level of pollution in the analyzed soil samples from underneath greenhouses was higher than in bare areas, in Ala Qwar was higher than in high lands and this regarding the number of found (detected) pesticides and their concentration. Also some agricultural pesticides of higher environmental persistence (oxadixyl, procymidone, vinclozolin) were found in a way which looks like absolute in Ala

Qwar area or underneath greenhouses. While some of the highly persistence phosphorous pesticides were found (azinphos, pyrazophos, methidathion) in Ala Qwar at a frequency higher than in high lands.

6-1-2 Water:

1\ Sample of exhaust water from absorptive pit of a pesticide factory in Al Dileel area was found to contain higher concentration of some pesticides. But most of these pesticides were not found in the Al Dileel runoff water.

2\ Water samples coming from the stations of sewage water treatments at Alkharba Alsamra, Irbid, Jarash, and Alsalat were found to contain lindane and its isomers (a-HCH, b-HCH, d-HCH) (generally known as ΣHCH compounds) at concentration approaching (330 ng/liter), and this is close to their level in sewage water in other parts of the world. However these samples did not contain other chlorinated compounds. It was not possible to identify nitrogenous or phosphorous pesticides because of the high interference but there are some indications that Alkharba Alsamra station share relatively higher concentration of the pesticide (dimethoate) and a lower concentration of a number other pesticides (mostly phosphorous).

3\ No pesticide residues were found in the valley water of Alyabis and Alwala. While some were found in water samples from the northern part of Ala Qwar (Alarab dams and Sharhabeel, King Abdalla canal, and agricultural water drainage) at a frequency and concentration generally lower than in water samples from the southern area of Ala Qwar (Alkafreen dams and Shoaib valley, the canal, and agricultural water drainage). The high pollution of the southern part of King Abdalla canal was partially attributed to its mixing with King Tilal Dam at Deer Ala, as dam water normally contains some pesticides at concentration higher than their counterparts in canal water. The agricultural water drainage in the wide lake of the dam was considered as one source of pesticide residues in dam water. While there is some indication that some part of it (e.g. dimethoate) may result from treated water coming out of Alkharba Alsamra station. While control operation in the area surrounding the station led to the presence of the pesticide (cypermethrin) in the sites near to the station only.

4\ The pesticides (lindane, dimethoate, ethoprophos) were generally the most found in surface water samples from Ala Qwar and high lands. In addition to these, samples from Ala Qwar area repeatedly contain residues of the pesticides (oxadixyl and DDT). This is in consistency with the intensive use of these pesticides (specifically oxadixyl, DDT and dimethoate) and their high persistence in the environment (DDT, lindane, oxadixyl) or high solubility in water ((ethoprophos, oxadixyl, dimethoate).

5\ None of the pesticides studied was detected in drinking or underground well water samples from Alazrak area, Amman, Alzargaa, Albagora- Aladasia and Wadi Rajab. While some of these residues were found in some wells in the southern areas of Ala Qwar. This was attributed to the high permeability of the soil in these areas.

6\ The pesticides concentrations in most surface and underground water samples examined were lower than the maximum residue level in European specifications for drinking water which is considered the most conservative in this area. (100 ng/liter for a single pesticide or 500 ng/liter for total pesticides in the sample). It was notice that about 13 samples contain pesticide exceeding the single pesticide level. These were

collected from Ala Qwar area (six samples of agricultural drainage water, one water sample from Shoaib valley and six samples from King Abdalla canal).Only one water sample (from Shoaib valley) contain total pesticide concentration exceeding the collective pesticides level. While in the Swamp area of King Tilal Dam, both the singular and collective levels were exceeded in many cases especially in the main stream of Alzargaa runoff.

6-1-3 Dam sediments:
No chlorinated pesticides residues were found in the analyzed samples from Sharhabeel dam sediments, while DDE was continuously found alone or in combination with DDD in the sediments of other dams (Dams of King Tilal, Alarab valley, Shoaib valley, Alkafireen). The concentrations were very small and range between (0.2-5.6 ng/Kg).

6-1-4 Local fishes:

Samples of three types of fishes (Misht, Shabot, and Balbot) from three different places (King Tilal dam, farms in Ala Qwar, farm in ALazrak) were collected. The fishes from the dam and Alqor were found to contain (DDD and DDE). Also the fishes from Alqor were found to contain (DDT) while fishes from the dam and one sample from Alazrak fishes were found to contain the pesticide (lindane) and its isomers. The concentration in the Misht fishes was generally lower than that in Shabot and Balbot. Generally the concentration detected was lower or similar to its counterparts in other parts of the world, and far lower than the maximum level in many of the developed countries in fish products.

Conclusions:
1\ The presence of pesticide residues in the local environment was attributed to three different sources; spraying for public health protection, spraying for agricultural purposes and sewage water drainage.

2\The spraying for public health protection purposes in the area surrounding Alkharba Alsamra station led to the presence of residues of the pesticide (cypermethrin) in Aldileel runoff water. But the effect is limited as the pesticide is quickly adsorbed to runoff sediments. While in Ala Qwar area the control campaigns use DDT, more persistent in the environment, which results in the presence of its residues in all components of Ala Qwar environment, including the agricultural soil, dam sediments, fishes and sometimes the agricultural drainage water. But the concentrations found were generally low, may be because of the special conditions dominants in Ala Qwar (high temperature, high rate of bright sunlight and intensive irrigation) which may assist in reducing the concentrations, by evaporation or by chemical or biological degradation. On the other hand there is no important source of pollution with the pesticide (DDT) in the high lands except in some valleys connected to Ala Qwar like Shoaib valley, Kafranga and Alzargaa runoff.

3\ Spraying for agricultural purposes include the use of great number pesticides belonging to different classes including the banned chlorinated pesticides (aldrin, endrin, dieldrin).This has been reflected by the presence of great number of these pesticides in samples from soil and surface water of low and high lands. It was noticed that there was preference in Ala Qwar areas and underneath greenhouses for

the use of pesticides of high persistence in the environment (like oxadixyl, procymidone, vinclozolin, pyrazophos, azinphos, methidathion). This may sometimes cause these pesticides to reach deeper layers in the soil, and to the persistence of its residues for longer period.

4\ The water coming from sewage water treatments caused an increase in the concentration of the pesticide (lindane) and its isomers in the receiving surface water. There are some indications that specifically Alkharba Alsamra station caused an increase in the concentration of dimethoate basically and other pesticides at lower degree. This may be due to the fact that the station has been greatly overloaded. Residues of the pesticide (lindane) were found in the water and fishes of King Tilal dam which receive the water coming from Alkharba Alsamra station.

5\ The effects of the above mentioned sources did not reach the underground water except in southern areas of Ala Qwar where the soil is highly permeable and underground wells in these areas were used extensively for irrigation purposes. These limited effects may also be attributed to the nature of the pesticides used in Jordan generally and in Ala Qwar specifically where insecticides and fungicides dominates and herbicides were less frequently used. However in other parts of the world herbicides (highly water soluble) were usually more dominants and these compounds are usually appear in underground water of these areas.

6\ The low concentration of most pesticides studied, in most areas, can be mostly attributed to the climatic and environmental factors dominant locally and also to the types of pesticides used in Jordan which are environmentally degradable in most cases. Clear exceptions were some chlorinated, nitrogenous and phosphorous pesticides which are relatively more persistent in the environment. With regard to places the clear exception was King Tilal dam, extension of Alzargaa runoff from Alkharba Alsamra station to the dam, where high concentration of some pesticides was clearly found in water samples sometimes.

Recommendations:

1\ The necessity of being more strict on prevention of import of the banned chlorinated pesticides (like endrin and aldrin).

2\ To study the possibility of banning the use of some pesticides which has high environmental persistence, high water solubility or both. This must be based on field studies for evaluation of the ability of these pesticides to degrade under local conditions generally, and Ala Qwar environment specifically.

3\ More strict monitoring of King Abdalla canal water for the purpose of its protection from contamination with pesticides residues as great portion of the canal water is used for drinking purposes after treatment in station Zee.

4\ To study possible alternative to DDT for public health protection purposes.

5\ To be assure about the purity of dicofol specially its contents of DDT impurities.

6\ The necessity of evaluation of factors affecting the residues of the pesticide (DDT) under local environment causing the decline of its residues in Ala Qwar specifically compared to the application rates there, and this may need exclusive field and Laboratory studies.

7\ To study and evaluate the extent of pollution in Ala Qwar environment with the compound (ETU, ethylene thiourea) which is produced from the degradation of dithiocarbamate pesticides, which are the most commonly used pesticides in Jordan.

8\ It is recommended to execute a monitoring program including collection of surface and underground water samples from selected positions in the high and low (Ala Qwar) lands, specially in king Tilal dam area where preparation are currently going on for extending Alkharba Alsamra station for the purposes of upgrading its capacity.

9\ To increase the awareness among farmers about expected hazards (long term) of pesticides misuse.

10\ The necessity of encouraging the activities which reduce the pollution of underground water in Ala Qwar like restricting banana cultivation to impermeable soils and prevention of irrigation within 24 hours after spraying pesticides.

ENDOSULFAN JMPR 1998

First draft prepared by
D.B. McGregor
International Agency for Research on Cancer
Lyon, France

Explanation

Endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzo-dioxathiepin-3-oxide), an insecticide, has been evaluated toxicologically on several occasions by previous Joint Meetings (Annex 1, references 2, 4, 8, 10, 38, 44, and 56), the latest being the 1989 JMPR (Annex 1, reference 56), when an ADI of 0-0.006 mg/kg bw was established. Endosulfan was reviewed by the present Meeting within the Periodic Review Programme of the Codex Committee on Pesticide Residues. In this evaluation, full use was made of the review of endosulfan prepared by the Australian National Registration Authority, the entire version of which may be obtained at http://www.dpie.gov.au/nra/prsendo.html. This monograph summarizes the new data and relevant data from the previous monographs and monograph

addenda on endoslufan (Annex 1, references 4, 9, 11, 39, and 58)

Evaluation for acceptable daily intake

1. Biochemical aspects

(a) Absorption, distribution, and excretion

When radiolabelled endosulfan was administered to mice as a single dose of 4 mg/kg bw by gavage, a single dose of 4.7 mg/kg bw in the diet, or a 21-day administration of 2.4 mg/kg bw per day in the diet, most of the radiolabel was recovered from the faeces. Within three weeks after cessation of treatment, a total of 87-100% of the administered dose was recovered, with little difference with dosing regime. While biliary excretion was not studied, the percent of chemical absorbed after oral dosing would appear to have been moderate to high. Three weeks after the final administration, the residues in tissues were greater in animals fed the compound for 21 days, the higher concentrations being found in the liver (about 2 ppm) and spleen (about 1.4 ppm) at this time. Little residue was found in the kidneys and fat, even after repeated administration, and there was no accumulation of radiolabelled endosulfan residues (Christ & Kellner, 1968).

Single oral doses of 0.3 mg endosulfan and its two isomers administered to male Balb/c mice were not completely absorbed from the gastrointestinal tract but were excreted with the metabolites endosulfan sulfate and diol in the faeces. Only the diol metabolite was excreted via the urine; the sulfate metabolite was the only form of endosulfan found in tissues, with relatively large amounts in liver, small intestine, and visceral fat and trace amounts in muscle and kidney. When endosulfan was fed to Balb/c mice in the diet at a concentration of 10 ppm for up to 49 days, the sulfate metabolite was detected in the liver and visceral fat of all animals. Both isomers and the sulfate and diol metabolites of endosulfan were detected in the faeces, while the only endosulfan product detected in the urine of these animals in this early study was the diol metabolite. After a single dose of up to 0.3 mg ^{14}C-labelled endosulfan to Balb/c mice, about 65% of the radiolabel was recovered; the faeces accounted for the highest concentrations, followed (in rank order) by visceral fat > urine > small intestine > kidney > brain > expired carbon dioxide > blood (Deema et al., 1966).

At the end of a 24-month study in which NMRI mice were given diets containing 0, 2, 6, or 18 ppm technical-grade endosulfan (see Donaubauer, 1988), the concentrations of endosulfan and its main metabolites endosulfan hydroxyether, sulfate, lactone, and diol were measured in the liver and kidneys. No endosulfan was detected in either the liver or the kidney. In mice given 18 ppm endosulfan, the concentrations of the hydroxyether, lactone, and diol metabolites were at or below the level of detection (0.02 ppm), while the endosulfan sulfate concentrations were 0.1-0.2 ppm in kidney and 0.7-1.1 ppm in liver. The tissue concentrations of endosulfan sulfate in mice at 2,

6, and 18 ppm, respectively, were: kidney, 0.2-0.4 ppm, 0.04 ppm, and

0.1-0.2 ppm; and liver, 0.06-0.07 ppm, 0.12-0.45 ppm, and 0.7-1.1 ppm (Leist, 1989a).

After oral or intravenous administration of ^{14}C-endosulfan to male and female Wistar rats at a dose of 2 or 0.5 mg/kg bw, respectively, > 80% (intravenous) or 90% (oral) of the dose was eliminated in the urine and faeces within seven days; elimination was essentially complete within the first 1-2 days. The half-lives for urinary and faecal elimination for males and females were biphasic, with an earlier half-life of 6-14 h and a later half-life of 33-67.5 h. Elimination in urine of the intravenous and oral doses, respectively, accounted for 11 and 13% of the dose in males and 2 and 24% of the dose in females; the corresponding figures for elimination in faeces were 65 and 82% in males and 60 and 72% in females. The highest tissue concentrations were found in the kidneys (1.8 ppm), liver (0.23 ppm in males; 0.48 ppm in females), and retroperitoneal fat (0.16 ppm in females). The concentrations of residues were < 0.1 ppm in all other tissues examined. The absorption of endosulfan was estimated to be 60-70% on the basis of a comparison of areas under the curve after intravenous and oral administration and about 90% on the basis of a comparison of elimination of radiolabel administered by the two routes (Table 1; Kellner & Eckert, 1983; Stumf & Lehr, 1993).

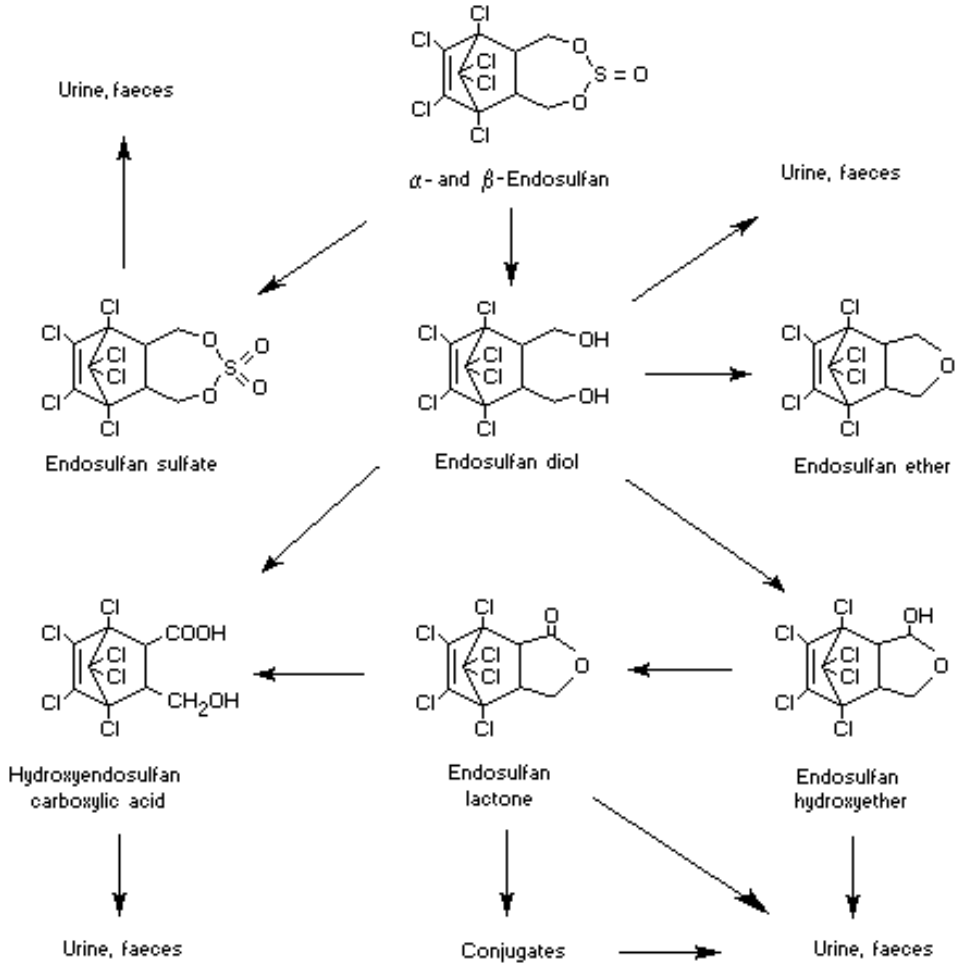
^{14}C-Endosulfan (alpha or ß isomer) was rapidly excreted by female rats after a single oral dose of 2 mg/kg bw or administration in the diet at a concentration of 5 ppm. After a single oral dose, > 85% was excreted within 120 h (> 70% after 48 h), mainly in the faeces and to a lesser extent in the urine. After dietary administration for 14 days, followed by a 14-day recovery period, > 72% of the administered dose was recovered. Biliary excretion of radiolabel in male rats given 1.2 mg/kg bw as a single dose approached 50% for the alpha isomer and 30% for the ß isomer over 48 h. There appeared to be little enterohepatic circulation. The tissue concentrations of residues were generally highest in the kidneys and liver and lower in other tissues, including fat. At the end of the 14-day recovery period, residues were confined to the kidneys and to a lesser extent the liver, with half-lives of about seven days in kidneys and three days in liver. By far the largest proportion of the radiolabel administered was metabolized to highly polar products, most of which could not be extracted from faeces (28%) or tissues (71%). Of the extractable fraction, unidentifiable polar metabolites constituted 6.2% in faeces and 13% in urine. The apolar metabolites of endosulfan identified in faeces and urine were the diol, the lactone, the alpha-hydroxyether, and the sulfate. The metabolites occurred at similar concentrations, ranging from 3.4 to 9.1% of the radiolabel in the urine of rats given single doses and from 2.4 to 4.2% in the urine of rats given endosulfan in the diet. The three apolar metabolites (endosulfan diol, hydroxyether, and lactone) accounted for 7.5% of the single dose and 3.2% of the dietary dose in the urine, 9% of the administered dose in bile, and 21% in faeces. No accumulation in fatty tissues was found (Dorough et al., 1978). A metabolic scheme is

presented in Figure 1.

Table 1. Pharmacokinetics of ^{14}C-endosulfan in rats after oral and intravenous administration

Pharmacokinetic parameter	Oral (2 mg/kg bw)	Intravenous (0.5 mg/kg bw)
T_{max}	Males, 3-8 h; females, 18 h	Males and females, 5 min
C_{max}	Males, 0.25 ± 0.06 µg/ml; females, 0.18 ± 0.05 µg/ml	Males and females, 0.18 ± 0.04 µg/ml
Elimination half-life	Males (biphasic), 8 h , 110 h	Males (triphasic), 0.77 h, 12.5 h, 157 h
	Females (monophasic), 75 h	Females (biphasic), 1.2 h, 47 h
Faecal excretion	Males, 82%; females, 72%	Males, 66%; females, 59%
Urinary excretion	Males, 12%; females, 22%	Males, 13%; females, 24%
Urinary excretion half-life	Males (biphasic), 6.2 h, 67.5 h	Males (biphasic), 7.5 h, 60 h
	Females (biphasic), 5.6 h, 33 h	Females (biphasic), 7.6 h, 42 h
Faecal excretion half-life	Males (biphasic), 7.7 h, 34 h	Males (biphasic), 8.6 h, 34.5 h
	Females (biphasic), 11.4 h, 30 h	Females (biphasic), 13.6 h, 40 h

Figure 1. Mammalian metabolism and excretion of endosulfan



Most endosulfan metabolites are polar and are yet to be identified.

Groups of 24 male Sprague-Dawley rats received dermal applications of [5a,9a^{14}C]-endosulfan at a dose of 0.1, 0.76, or 10 mg/kg bw, without washing. Four animals from each group were killed at 0.5, 1, 2, 4, 10, and 24 h, and radiolabel was measured in the collected excreta and various organs and tissues, including the application site after washing with soapy water. No skin irritation was seen at the application site. Adsorption onto the skin was essentially complete within 0.5 h at all doses and accounted for 63-80% of the applied dose. Movement away from the application site was slow, with 73, 73, and 89%, respectively, of the adsorbed doses remaining at the application site after 24 h. By 10 h, each group had excreted less than 1% of the applied dose. By 24 h, the excretion was 11, 10, and 4% of the applied dose in the groups given 0.1, 0.76, and 10 mg/kg bw, respectively (Craine, 1986).

Groups of 16 female Sprague-Dawley rats received dermal applications of [5a,9a^{14}C]-endosulfan at 0.09, 0.98, or 11 mg/kg bw,

and the site was washed with soapy water after 10 h. Four animals from each group were killed at 24, 48, 72, and 168 h, and radiolabel was measured in the collected excreta and various organs and tissues, including the application site. No skin irritation was seen at the application site, and there were no signs of systemic toxicity. Recovery of radiolabel was 84-115%. The amount of the applied dose that was removed by washing was 28, 47, and 69%, respectively, at the three doses. After 168 h, 63, 87, and 65%, respectively, of the dose that was not removed by washing was either adsorbed on to the skin or had penetrated and been distributed and excreted; an average of 2.4-3.3% of the adhering dose remained at the application site. Excretion was maximal between 24 and 48 h, faeces accounting for about two-thirds of the label. The total residues at 168 h represented 2.5, 2.3, and 1.3% of the applied dose (about 3.5, 4.3, and 4.2% of the adhering dose) at the doses of 0.09, 0.98, and 11 mg/kg bw, respectively, and were present mainly in liver and kidney (Craine, 1988).

At the end of a 24-month study in which Sprague-Dawley rats were given diets containing 0, 3, 7.5, 15, or 75 ppm technical-grade endosulfan (see Ruckman et al., 1989), the concentrations of endosulfan and its known metabolites, endosulfan hydroxyether, sulfate, lactone, and diol, were measured in the liver and kidneys. As no quantifiable residues were detected in organs of rats at 15 ppm, the organs of animals at 7.5 and 3 ppm were not investigated further. Neither alpha- nor ß-endosulfan, the substances administered in the diet, or any of the metabolites was quantifiable in either organ, with the exception of endosulfan sulfate in animals at 75 ppm, which was found at concentrations of 0.2-0.4 mg/kg in liver. No residues were observed in the kidneys of this group (Dorn & Werner, 1989; Leist, 1989a).

Twelve lactating goats were given endosulfan (purity unspecified) in gelatine capsules at a dose of 1 mg/kg bw per day for 28 days. The tissue concentrations of residues were generally low, the highest being detected on the first day after cessation of treatment, with

0.29 ppm in kidney, 0.2 ppm in the gastrointestinal tract, and 0.12 ppm in liver. The concentrations in the kidney were increased one week after treatment, reaching 0.49 ppm on day 8, but no residues were detected 21 days after treatment ceased. Endosulfan residues did not accumulate in the fat; the concentrations reached 0.06 ppm on day 1 after the end of treatment, but none were detected by day 8 after treatment (Indraningsih, et al., 1993).

Groups of three lactating Holstein cows were given diets containing 0, 0.3, 3, or 30 ppm ^{14}C-endosulfan for 30 days. Between days 7 and 29, the average concentrations in milk were 3.4, 40, and 462 ppb endosulfan equivalents in the three groups. After the dosing period, the loss of radiolabel from milk (measured in one cow per group) was 81% within seven days in the cow given 0.3 ppm and 96% within 14 days in cows given 3 and 30 ppm (Bowman, 1959). The blood concentrations rose during the first 21 days, to 0.15 and 1.97 ppm at

the doses of 3 and 30 ppm, respectively, but were always below the detection level (0.06 ppm) in the groups at 0.3 ppm. The concentrations of residues found at the three doses were: liver, 0.35, 2.45, and 25.3 ppm; kidney, 0.05, 0.35, and 6.29 ppm; and omental fat, 0.07, 0.71, and 7.08 ppm (Keller, 1959a).

Two lactating East Friesian sheep were given a single oral dose of 0.3 mg/kg bw ^{14}C-endosulfan and were killed after 40 days. The radiolabel was maximal in blood after 24 h, when it was equivalent to 0.07 µg/ml. The total radiolabel eliminated in milk over 17 days was 0.37% and 1.82% of the dose in the two sheep, respectively. Radiolabel was excreted mainly via the urine (41%) and faeces (50%). About half of the 50% in faeces was unmetabolized endosulfan. Fat, kidney, and liver of the sheep contained 0.02-0.03 µg/g endosulfan; all of the remaining tissues had considerably lower concentrations. The total radiolabel found in organs and tissues accounted for < 1% of the administered dose (Gorbach et al., 1965).

In pigs fed endosulfan at 2 ppm in their diets for up to 81 days, the compound was detected in fatty tissue at concentrations of 0.07, 0.09, and 0.04 ppm after 27, 54, and 81 days of treatment, much less than the residues seen after administration of 7 ppm DDT: 8.3, 9.1, and 9.7 ppm after 27, 54, and 81 days treatment, respectively. Liver and muscle contained about 15-fold less DDT residues than fat. Thus, while much less endosulfan was found in fatty tissues, it does not appear to bioaccumulate as does DDT (Maier-Bode, 1966).

The systemic absorption of endosulfan over 96 h after dermal administration to two rhesus monkeys of single doses of 2.2-3 mg/kg bw of an aqueous suspension of ^{14}C-endosulfan (purity, 94.6%) for 10 h was 22% of the administered dose. An additional 11% remained in the skin, 10.5% was found in the carcass, and 4.3 and 3.7% of the administered dose was excreted in faeces and urine, respectively; however, as only 50% of the administered dose was recovered, the figures calculated for absorption may not be accurate indications of the extent of dermal absorption of endosulfan. A plateau was reached in blood and plasma concentrations at 36 h, and there may have been no

significant additional dermal absorption after that time. Concentrations in the liver, kidneys, and fat were highest (0.48, 0.083, and 0.23 ppm, respectively), while there were negligible concentrations in the brain (Lachmann, 1987).

In a study of the penetration of endosulfan through rat and human skin *in vitro,* radiolabelled endosulfan formulated as an emulsifiable concentrate containing 353 g/L endosulfan, which had been diluted to concentrations of 0.4-4 mg/ml in water, was applied at nominal doses of 0.01, 0.1, and 1 mg/cm^2 to rat and human skin mounted in dermal penetration cells. The rate of penetration was, on average, 4.3 times greater through rat skin than that of humans. The percentage of the applied dose varied with the concentration: 61% of the lowest dose applied to human skin and 96% of that applied to rat skin penetrated, and 20% of the highest dose applied to human skin and

40% of that applied to rat skin penetrated. When the skin was washed 10 h after application, the amount of endosulfan that penetrated decreased to 4% in the human and 9% in the rat skin. Endosulfan that passed through human skin was metabolized or degraded to a greater extent than that which passed through rat skin (Noctor & John, 1995).

2. Toxicological studies

(a) Acute toxicity

The LD_{50} of endosulfan varies widely depending on the route of administration, species, vehicle, and sex of the animal (see Table 2). Certain other reports are available, but it has been argued that most of them are not acceptable by current standards (Bremmer & Leist, 1998). Female rats are clearly more sensitive than male rats, and, on the basis of a single study, this sex difference appears to apply to mice also. Endosulfan is generally highly toxic after oral and inhalation exposure. The lowest oral LD_{50} value is 9.6 mg/kg bw in female Sprague-Dawley rats.

The isomers of endosulfan also show high acute toxicity after oral administration. The clinical signs of poisoning include piloerection, salivation, hyperactivity, respiratory distress, diarrhoea, tremors, hunching, and convulsions. Like endosulfan, its metabolites were more or less toxic according to the vehicle used and the species exposed. In general, the toxicity of the lactone and sulfate metabolites was similar to or less than that of the parent compound, while the hydroether, ether, and, in particular, the diol were far less toxic. The clinical signs of poisoning were similar to those induced by the parent compound and included piloerection, salivation, hyperactivity, respiratory distress, diarrhoea, tremors, hunching, and convulsions.

Phenobarbital was an effective therapeutic measure against an absolute lethal dose of endosulfan in rats, reducing the clinical signs of poisoning and the mortality rate. Diazepam was not effective (Ebert & Weigand, 1984).

Table 2. Acute toxicity of technical-grade endosulfan and its isomers and metabolites

Species	Strain	Sex	Route	Vehicle	LD_{50} (mg/kg bw)	Reference
Technical-grade endosulfan						
Rat	Sprague-Dawley	M	Oral	25% in food	2	

800	Bracha (1977)					
Rat	Sprague-Dawley	F	Oral	25% in food		
45	Bracha (1977)					
Rat	CD	M/F	Oral	Maize oil		
43	Lightowler & Gardner (1978)					
Rat	Haffkine	M	Oral			
110	Bhide & Naik (1984a)					
Rat	Haffkine	F	Oral			
15	Bhide & Naik (1984a)					
Rat	Holtzman	M	Oral	Corn oil		
87	Elsea (1958)					
Rat	Wistar	M	Oral	2% starch		100-
160	Diehl & Leist (1988a)					
Rat	Wistar	F	Oral	2% starch		
23	Diehl & Leist (1988a)					
Rat	Sprague-Dawley	M	Oral	5% CMC		
40	Reno (1975)					
Rat	Sprague-Dawley	F	Oral	5% CMC		
9.6	Reno (1975)					
Mouse	Kasauli	M	Oral	Tween 80		
35	Bhide & Naik (1984b)					
Mouse	Kasauli	F	Oral	Tween 80		
14	Bhide & Naik (1984b)					
Dog	Mongrel	M/F	Oral	Gelatin capsule		
77	Nogami (1970)					
Rat	HoeWISKf	M	Dermal[a]			> 4
000	Diehl & Leist (1988b)					
Rat	HoeWISKf	F	Dermal[a]			
500	Diehl & Leist (1988b)					
Rabbit	NWS	M	Dermal[a]			
290	Bhide & Naik (1984c)					
Rabbit	New Zealand	M	Dermal[a]			500-
1 000	Bracha (1977)					
Rabbit	New Zealand	F	Dermal[a]			1
000-2 000	Bracha (1977)					
Rat	Sprague-Dawley	M/F	Inhalation			>
21 000	Bracha (1977)					
						mg/
m^3 for 1 h						
Rat	Wistar	M	Inhalation	Ethanol + PEG		35
mg/m^3	Hollander & Weigand (1983)					
						for
4 h						
Rat	Wistar	F	Inhalation	Ethanol + PEG		13
mg/m^3	Hollander & Weigand (1983)					
						for
4 h						

Table 2. (continued)

Species	Strain	Sex	Route	Vehicle	LD_{50} (mg/kg bw)	Reference
Endosulfan alpha isomer						
Rat			Oral		76	Goebel et al. (1982)
Mouse	Albino	F	Oral	Tween 80	11	Dorough et al. (1978)
Endosulfan ß isomer						
Rat			Oral		240	Goebel et al. (1982)
Mouse		F	Oral	Tween 80	36	Dorough et al. (1978)
Endosulfan sulfate						
Mouse	Albino	F	Oral	Tween 80	8	Dorough et al. (1978)
Rat	Wistar	F	Oral	Starch suspension	76	Hollander & Kramer (1975a)
Rat	Wistar	M	Oral		570	Ehling & Leist (1991a)
Rat	Wistar	F	Oral		40	Ehling & Leist (1991a)
Dog	Beagle	M	Oral	Starch suspension	15	Hollander & Kramer (1975b)
Rat	Wistar	M	Dermal		2 700	Ehling & Leist (1991b)
Rat		F	Dermal		280	Ehling & Leist (1991b)
Endosulfan diol						
Mouse	Albino	F	Oral	Tween 80	> 2 000	Dorough et al. (1978)
Rat		F	Oral	Starch suspension	> 1 500	Hollander & Kramer (1975c)
Rat	Albino	F	Oral		> 15 000	Weigand (1982a)
Rat	Wistar	M/F	Oral		> 5 000	Ehling & Leist (1991c)
Mouse	Albino	F	Oral	Tween 80	120	Dorough et al. (1978)
Rat		F	Oral	Starch suspension	1 750	Hollander & Kramer (1975d)
Rat	Wistar	M/F	Dermal		> 2	

000 Ehling & Leist (1991d)

Table 2. (continued)

Species	Strain	Sex	Route	Vehicle	LD_{50} (mg/kg bw)	Reference
Endosulfan ether						
Mouse	Albino	F	Oral	Tween 80	270	Dorough et al. (1978)
Rat		F	Oral	Starch suspension	> 15 000	Hollander & Kramer (1975c)
Rat	Albino	F	Oral		> 15 000	Weigand (1982b)
Endosulfan hydroxyether						
Mouse	Albino	F	Oral	Tween 80	120	Dorough et al. (1978)
Rat		F	Oral	Starch suspension	1 750	Hollander & Kramer (1975d)
Endosulfan lactone						
Mouse	Albino)	F	Oral	Tween 80	120	Dorough et al. (1978)
Rat	Wistar	F	Oral	Starch suspension	290	Hollander & Kramer (1975e)
Rat	Wistar	M	Oral	Starch suspension	160	Hollander & Kramer (1975f)
Rat		M/F	Oral	Sesame oil	100/120	Kramer & Weigand (1971)

M, male; F, female; CMC, carboxy methyl cellulose; PEG, polyethylene glycol
[a] 24 h on intact skin

The dermal irritancy of technical-grade endosulfan (purity, 98.6%) was tested in three New Zealand white rabbits by clipping the hair from a dorsal area of about 25 cm^2 and 24 h later applying 500 mg endosulfan moistened with deionized water on a 6.25-cm^2 cellulose patch, which was then covered with a semi-occlusive bandage. Exposure was for 4 h, after which the test material was removed with warm tap-water. The exposed area was examined 0.5-1, 24, 48, and 72 h after removal of the patch. On the basis of the evaluation system defined by EEC guideline B.4 ('Acute toxicity skin irritation' of Directive

92/69/EEC), the overall mean scores for dermal irritation were 0 for both erythema and eschar formation and oedema formation. No signs of systemic toxicity were observed (Bremmer, 1997a).

The ocular irritancy of technical-grade endosulfan (purity, 98.6%) was tested in three New Zealand white rabbits by applying 100 mg to the conjunctival sac of one eye of each rabbit; the other, untreated eye served as the control. The eyes were exposed for 24 h, after which the endosulfan was washed out, and the eyes were examined for ocular lesions 1, 24, 48, and 72 h later. On the basis of the evaluation system defined by EEC guideline B.5 ('Acute toxicity eye irritation' of Directive 92/69/EEC), the overall mean scores for irritation were 0.66 for redness of conjunctiva, 0 for chemosis of conjunctiva, 0 for opacity of cornea, and 0.11 for irritation of the iris. No signs of systemic toxicity were observed. Endosulfan was not irritating to the eye (Bremmer, 1997b).

The cutaneous allergenic potential of endosulfan (purity, 98.6%) was examined in 20 treated and 10 control male albino guinea-pigs. The maximal tolerated concentration of endosulfan suitable for the induction phase of the main study and a suitable non-irritating concentration of topically applied endosulfan were identified for the challenge application in a preliminary study. For intradermal induction, injection of 0.1 ml of a 0.5% solution in corn oil emulsified 1:1 (v:v) with Freund's complete adjuvant was selected. One week after these injections, a 6-cm^2 patch of filter paper saturated with about 0.3 ml of a 50% solution of endosulfan in corn oil was applied to the shaved skin of each guinea-pig, and the area was occluded with aluminium foil secured by impermeable adhesive tape, which was left in position for 48 h. Irritation was assessed 24 and 48 h later. On test day 22, the guinea-pigs were challenged with the non-irritating 50% endosulfan in corn oil applied as during the induction phase. The dressing was left in position for 24 h. The application sites were assessed for erythema and oedema 24 and 48 h later. On the basis of the evaluation system defined by EEC guideline B.6 ('Acute toxicity -- Skin sensitization' of Directive 92/69/EEC), none of the treated guinea-pigs developed skin reactions. Endosulfan was therefore considered to be non-sensitizing for guinea-pig skin (Arcelin, 1996).

(b) Short-term toxicity

Mice

Groups of 10 male and 10 female Hoe:NMRKf mice were fed diets containing endosulfan at a concentration of 0 or 18 ppm for six weeks, equal to 0 or 3.7 mg/kg bw per day for males and 0 or 4.6 mg/kg bw per day for females, to determine whether 18 ppm was the NOAEL in this strain, as it was found to be in CD-1 mice in a three-month study (Barnard et al., 1984). There were no clinical signs attributable to treatment. The mean food intake of mice receiving endosulfan was slightly decreased in males and slightly increased in females. Mean body-weight gain was reduced in treated males during the second half

of the experiment but was slightly increased in females throughout the experiment. Two treated females died, on days 28 and 38; the causes were not established, but no histological changes were found in the mouse that was not autolysed. The absolute and relative weights of the liver of males and females at 18 ppm were higher than those of controls and statistically significantly so in females (17 and 12%, respectively). A NOAEL was not identified, as an increase in liver weights was seen in females at the only dose tested (Donaubauer et al., 1985).

Groups of 20 CD-1 mice of each sex were fed diets containing endosulfan (purity, 97.2%) at a concentration of 0, 2, 6, 18, or 54 ppm for three months, equal to 0, 0.24, 0.74, 2.1, and 7.3 mg/kg bw per day for males and 0, 0.27, 0.8, 2.4, and 7.5 for females. Clinical signs attributable to treatment, consisting of convulsions and salivation, were seen in one male and one female at the high dose. There was a marked treatment-related decrease in the survival rate (about 50%) of male and female mice at the high dose; the mean food intake of these animals was significantly reduced for the first two weeks of the study, and the mean body-weight gain of males at the high dose was reduced during the first week of treatment. A significant reduction in neutrophil count was observed in males at 54 ppm at week 6 (72%, $p < 0.01$), and a lower count (62%), which was not statistically significant, was also observed at week 13. The blood glucose concentration was reduced by about 11% in females at all doses ($p < 0.01$) at week 6, and the serum lipid concentration was increased by 15% in females at 54 ppm at week 13. The NOAEL was 18 ppm, equivalent to 2.1 mg/kg bw per day, on the basis of convulsions and salivation, decreased survival, and increased serum lipid concentrations at 7.3 mg/kg bw per day (Barnard et al., 1984).

Groups of 10 four-week-old ddY mice of each sex were fed diets containing technical-grade endosulfan (purity, 91.4%) at concentrations of 0, 10, 30, 100, or 300 ppm, equal to 0, 1.2, 4.1, 15, and 42 mg/kg bw per day in males and 0, 1.4, 4.7, 14, and 42 mg/kg bw per day in females, for 12 months. There were no apparent treatment-related clinical signs or deaths. Males at the high dose showed a small (1%) but significant decrease in mean corpuscular volume, and transient, non-dose-related increases in haemoglobin (11%), haematocrit (4%), and eosinophil counts (33%) were seen in

males at 30 ppm. A significant decrease in serum aspartate aminotransferase activity was seen in males at 100 and 300 ppm and a decrease in bilirubin concentration in males at the high dose. The only change in organ weights was a dose-related increase in the relative weight of the adrenals in females, which was about 30% and statistically significant at 300 ppm. There were no treatment-related changes on gross pathological examination; the histopathological effects consisted of dose-related granulomatous changes in the liver and lymph nodes. In the liver, granuloma, giant-cell infiltration, and/or large histiocytic cells filled with brown pigment were found in treated mice; these effects were significant at 100 and 300 ppm. In the lymph nodes, giant-cell infiltration and/or reticuloendothelial

cell proliferation were found at the same doses. Testicular atrophy was seen erratically, with incidences of 30, 70, 33, 50, and 80% in the five groups, respectively, but was not considered to be related to treatment. The NOAEL was 30 ppm, equal to 4.1 mg/kg bw per day, on the basis of histological findings in the liver and lymphatic system (Arai et al., 1981).

Rats

Groups of male albino rats were given endosulfan in peanut oil by gavage at a dose of 0 or 11 mg/kg bw per day for 30 days. In addition, the possible interaction between endosulfan and the chemosterilant, metepa, was investigated in groups of rats receiving either metepa alone at 30 mg/kg bw per day for 30 days or in combination with endosulfan at 11 mg/kg bw per day. There were three deaths in the endosulfan-treated group. Endosulfan alone had no significant effect on body weights, organ weights, blood chemistry, or histopathological appearance. No potentiation of the toxicity of metepa was seen (Nath et al., 1978).

Groups of 100 male SPF Wistar rats were fed diets containing technical-grade endosulfan (purity, 97.9%) at concentrations of 360 or 720 ppm, equal to 34 and 68 mg/kg bw per day for four weeks. Twenty control rats received diet alone. Fifty treated and 10 control animals were maintained for an additional four-week withdrawal period. The objective of the experiment was to determine the toxicological significance of yellow, granular deposits observed in the proximal convoluted renal tubules in a 13-week and a two-generation feeding study. One rat in each treated group died, with no signs of poisoning. The behaviour and general condition of the rats and their food and water consumption were unaffected by treatment, and body-weight gains were comparable in treated and control groups. Enlargement of the liver was observed in rats at 360 and 720 ppm at the end of the treatment period, and the kidney and brain weights were significantly elevated in those at 720 ppm; these treatment-related changes in organ weights had, however, disappeared by the end of the withdrawal period. The kidneys of treated rats were darkly discoloured, but their appearance had returned to normal by the end of the withdrawal period. Histopathological examination by light and electron microscopy showed granular pigmentation and larger, more numerous lysosomes in renal proximal tubule cells after treatment; these changes had decreased by

the end of the withdrawal period, and no lysosomal changes were found in brain or liver. Analysis of residues showed that storage of alpha-endosulfan was dose-dependent, temporary, and confined to the kidney, where it was detected as endosulfan sulfate and endosulfan lactone. The amount of ß-endosulfan was 230 times less than that of alpha-endosulfan. The concentrations of the sulfate and lactone metabolites were many times lower in liver, and only traces of endosulfan remained in the kidney at the end of the withdrawal period (Leist & Mayer, 1987).

Groups of 15 male and 15 female Wistar rats were exposed by

inhalation to technical-grade endosulfan (purity, 97.2%) at a concentration of 0, 0.5, 1, or 2 mg/m^3 air for 6 h/day, five days per week for 29 days, for a total of 21 exposures. One male rat at the high dose was emaciated, had a pale skin, and adopted a high-legged position; no other clinical signs were seen in the treated animals. No neurological disturbances, opacity of the refractive media, impairment of dental growth, or changes in the oral mucosa were seen. The body-weight gain of males at the high dose tended to be depressed from day 20 of exposure until day 29, the end of the recovery period, but no other changes in body-weight gain or food consumption were seen. Non-dose-related increases in erythrocyte and haemoglobin concentrations were seen at the end of exposure, but not at day 29; these concentrations were reported to be within normal ranges for this strain of rat. Apart from a transient, non-dose-related increase in creatinine concentration and a decrease in serum aspartate aminotransferase activity in females at the high dose, no treatment-related change in blood chemistry was noted, and no histological changes were seen in any of the rats (Hollander et al., 1984)

Technical-grade endosulfan (purity, 97.2%) was applied in a solution in sesame oil to the shaved skin of the nape of groups of six Wistar rats of each sex 21 times over 30 days, for 6 h/day on five days per week under an occlusive bandage. Males were given a dose of 0, 12, 48, 96, or 190 mg/kg bw per day, and females received 0, 3, 6, 12, or 48 mg/kg bw per day. In males, deaths and clinical signs of poisoning consisting of tremors, tonoclonic convulsions, and/or hypersalivation were observed only at the highest dose. In females, clinical signs of poisoning were observed at $\geq$ 2 mg/kg bw per day; deaths occurred mainly in the group receiving 48 mg/kg bw per day, although single animals died on day 18 after receiving 3, 6, or 12 mg/kg bw per day, none having shown signs of poisoning. Serum cholinesterase activity was 33% lower in males at 192 mg/kg bw per day, whereas the activities of the erythrocyte and brain enzymes were reduced by 12 and 7%, respectively. In females at 48 mg/kg bw per day, serum cholinesterase activity was 21% lower than in controls, and there were no effects in the activities of erythrocyte and brain enzymes. The NOAEL was 6 mg/kg bw per day (Ebert et al., 1985a,b).

Technical-grade endosulfan (purity, 97.2%) as a solution in sesame oil was applied to the shaved skin of the nape of groups of six male and six female Wistar rats 21 times over 30 days, for 6-h periods under an occlusive bandage, at doses of 0, 1, 3, 9, 27, or 81 (males only) mg/kg bw per day. No signs of toxicity were observed in males or females at 1 and 3 mg/kg bw per day, but two males at 9 mg/kg bw per day died, one on day 5 with no previous signs of poisoning and the other on day 8 after piloerection, hypersalivation, blood-encrusted nose, stagger, and dyspnoea. There were no deaths among males at 27 mg/kg bw per day, but three at 81 mg/kg bw per day died. None of the females at 9 mg/kg bw per day died and no clinical signs of toxicity were observed, but five females at 27 mg/kg bw per day died between days 2 and 6 with no previous clinical signs of toxicity. Microscopic changes were seen in the livers of animals at $\geq$ 9 mg/kg bw per day,

consisting of enlargement of parenchymal cells in the periphery and loss of cytoplasmic basoplilia. Serum cholinesterase activity was statistically significantly reduced by treatment, by 70-80% in males at doses of 9-81 mg/kg bw per day but in females by only about 40% at 9 mg/kg bw per day. Brain acetylcholinesterase activity was statistically significantly reduced in males at 9 (21%), 27 (28%), and 81 (24%) mg/kg bw per day and in all treated females by 13-18%. The responses were not dose-related, and the observations in females are probably not biologically significant. Since a later study did not demonstrate a direct inhibitory effect of endosulfan on rat brain acetylcholinesterase activity *in vitro,* the reduced brain enzyme activity found in this study was difficult to interpret. It is also noted that serum enzyme activity was inhibited only at much higher doses in the study of Ebert et al. (1985a,b). The two males at 9 mg/kg bw per day that died had reduced or immature testes and/or sex organs, and the livers of these animals has accentuated lobular markings. The authors reasoned that these effects resulted from a non-substance-related developmental disturbance already present before treatment. No mechanism was proposed for these effects, which were not seen at higher doses. The NOAEL was 3 mg/kg bw per day on the basis of inhibition of serum and brain cholinesterase activity and microscopic changes in the liver (Ebert et al., 1985b,c).

Endosulfan (purity not stated) as a solution in acetone was applied daily to the shaved abdominal skin of groups of 24 albino rats of each sex for 30 days at doses of 0, 19, 38, or 63 mg/kg bw per day for males and 0, 10, 20, or 32 mg/kg bw per day for females. There were no deaths. All doses produced hyperexcitability, tremor, dyspnoea, and salivation, which disappeared after one week. No significant changes in organ:body weight ratios occurred, and there were no treatment-associated effects on histological, haematological, or blood chemical parameters. Liver alanine and aspartate aminotransferase activities were reduced in animals of each sex at the lowest dose, but there were no further reductions with increasing dose. Liver alkaline phosphatase and lactate dehydrogenase activities were increased in females but not in males, again with no increase with dose. Cholinesterase activities were not measured. A NOAEL was not identified (Dikshith et al., 1988).

Groups of 25 CD Sprague-Dawley rats of each sex were fed diets containing technical-grade endosulfan (purity, 97.9%) at concentrations of 0, 10, 30, 60, or 360 ppm, equal to 0, 0.64, 1.9, 3.8, and 23 mg/kg bw per day for males and 0, 0.75, 2.3, 4.6, and 27 mg/kg bw per day for females, for three months. Five animals of each sex per group were maintained for an additional four-week recovery period. Three females died, one each at 0, 60, and 360 ppm. Slight but statistically significant, dose-related reductions in erythrocyte counts and haemoglobin concentrations were seen in males at $\geq$ 30 ppm and in females at $\geq$ 60 ppm, but were within the reported normal range for this strain and age of rat (Leist & Bremmer, 1998); increased mean corpuscular volume was also seen at these doses. Females at 360 ppm had statistically significant decreases in plasma and erythrocyte cholinesterase activities (measured by the Ellman

method) at week 12 (by 41 and 12%, respectively), while increased brain acetylcholinesterase activity was observed at 60 and 360 ppm (19 and 20%, respectively). In males at 360 ppm, urinalysis showed a number of reversible changes, including increased urine volume and urinary protein concentrations and decreased specific gravity. Gross examination revealed enlargement of the liver in males at 360 ppm and of the kidneys at 60 and 360 ppm; increases in the absolute weights of the liver (18%), kidney (29%), and epididymides (8%) were seen in males and of the liver (21%) and kidneys (10%) in females. The kidney weights remained significantly elevated in male rats at 360 ppm (15%, $p < 0.01$) at the end of the withdrawal period.

Histopathological examination revealed traces of brown pigment in scattered hepatocytes in 25% of male rats and minimal centrilobular enlargement of hepatocytes in 25% of females at 360 ppm. These changes were not observed in rats at the end of the withdrawal period. Yellowish discolouration of renal proximal tubular cells was seen in males at all doses and in females at 30-360 ppm, the degree of pigmentation increasing in a dose-related manner; however, no cell death was associated with this finding. In addition, granular pigmentation was seen in straight portions and occasionally in proximal tubular cells in males at 60 and 360 ppm. The yellow discolouration of the renal tubules in male rats had decreased by the end of the withdrawal period, but trace or minimal pigmentation was still evident. In females at $\geq$ 60 ppm, the traces of pigmentation persisted. Males at 360 ppm also had yellow protein aggregation in the proximal convoluted with intracytoplasmic eosinophilic droplets in the tubules. The increase in incidence and degree of the yellowish discolouration of the proximal tubular cells appeared to be treatment-related, since it did not develop in control rats; however, no adverse effects were reported that might be associated with these findings alone. All rats at 30 ppm showed either trace or minimal discolouration and signs of granular or clumped pigment. Other investigations suggest that the yellow pigmentation is no more than an indication that endosulfan and some of its metabolites are being temporarily stored before urinary excretion, and that it is therefore an indicator of exposure rather than an expression of toxicity. Consequently, this effect was not considered in the evaluation. At doses of 60 and 360 ppm, other treatment-related effects were also

seen when the pigmentation was present, including enlarged kidneys and centrilobular hepatocytes. No treatment-related increase in the incidence of other renal effects was reported in animals at 10 ppm. The NOAEL was thus 10 ppm, equal to 0.64 mg/kg bw per day, on the basis of haematological changes (Barnard et al., 1985).

(c) Long-term studies of toxicity and carcinogenicity

Mice

Groups of 50 six- to seven-week-old B6C3F$_1$ mice of each sex were fed diets containing technical-grade endosulfan (purity, 98.8%) at time-weighted average concentrations of 3.5 or 6.9 ppm for males and 2

or 3.9 ppm for females for 78 weeks. Groups of 20 controls received untreated diet. There were no clear compound-related effects on appearance or behaviour in the treated groups, and the body weights of both males and females were unaffected by treatment. The mortality rate of males at the high dose was increased early in treatment so that, at the end of the experiment, the survival rates were 3/20 controls, 19/50 at the low dose, and 5/50 at the high dose. The mortality rates of female mice were not affected by treatment. No treatment-related clinical signs were recorded, and no treatment-related neoplastic lesions were seen in the females. Owing to the high early mortality rates, no conclusion could be drawn about the carcinogenic potential of endosulfan in males. None of the non-neoplastic changes seen in the kidneys and sex organs of male and female mice could be attributed to treatment. The NOAEL for female mice was 3.9 ppm, equal to 0.58 mg/kg bw per day (US National Cancer Institute, 1978).

Groups of 60 NMRI mice of each sex were fed diets containing technical-grade endosulfan (purity, 97.2%) at concentrations of 0, 2, 6, or 18 ppm, equal to 0.28, 0.84, and 2.5 mg/kg bw per day for males and 0.32, 0.97, and 2.9 mg/kg bw per day for females, for up to 24 months. Ten mice of each sex per dose were killed at 12 and 18 months. The behaviour and general health of the animals were not affected by treatment. The mortality rate of females at 18 ppm was statistically significant decreased at the end of the experiment: control, 45%; 18 ppm, 28% ($p < 0.05$); at week 78, the rates in these two groups of female mice were 82 and 62%. Survival among treated male mice was not statistically different from that of controls. The body weights of males receiving 18 ppm were slightly but significantly lower than those of controls during the first third of the study and remained slightly but not significantly low throughout the remainder of the study. In the other treated groups, there was a tendency to increased body-weight gains, especially in the satellite groups killed at 12 and 18 months. No statistically significant changes were observed in haematological or clinical chemical parameters, and macroscopic examination did not reveal any findings that were related to treatment. No statistically significant changes in organ weights were seen in treated animals at the end of the experiment; however, slight but statistically significant changes in organ weights were observed in animals at 18 ppm at 12 and 18 months, consisting of decreased lung

and ovary weights in females at 12 months and decreased liver weights in males and decreased ovary weights in females at 18 months. Histopathological examination did not reveal any effects that were related to treatment. No increase in the incidence of neoplastic or non-neoplastic lesions was observed. The NOAEL was 6 ppm, equal to 0.84 mg/kg bw per day, on the basis of decreased body weights in males at 24 months and decreased weights of the liver, ovaries, and lung in males and females at 12 and/or 18 months (Donaubauer, 1988, 1989; Hack et al., 1995).

Rats

Groups of 50 male and 50 female Osborne-Mendel rats were fed diets containing technical-grade endosulfan (purity, 98.8%) at time-weighted average doses of 220, 410, or 950 ppm for males and 220 or 400 ppm for females for 78 weeks, with a return to control diets for a further four weeks. Groups of 20 rats of each sex received untreated diet. A highly significant morbidity rate was seen in male rats and, by week 54, 52% of those at the high dose had died. A dose-related reduction in body weight was found in males at all doses. Histopathological examination showed a high incidence of toxic nephropathy (> 90%) in males at the low and high doses and in females, but in none of the controls. Chronic renal inflammation was observed in 40% of control males and 80% of treated males. The toxic nephropathy observed was characterized as degenerative changes in the proximal convoluted tubules at the junction of the cortex and medulla, with associated cloudy swelling, fatty degeneration, and necrosis of the tubular epithelium. Some tubules had hyaline casts, and enlarged, dark-staining regenerative tubular epithelial cells were observed infrequently. In treated males, parathyroid hyperplasia was observed, as were calcium deposits in the stomach, kidney, testis, aorta, and mesenteric artery. A dose-related increase in the incidence of testicular atrophy was seen in treated males, characterized by degeneration and necrosis of the germinal cells lining the seminiferous tubules and multinucleated cells (fusion bodies), resulting in aspermatogenesis. No treatment-related effects were noted in the reproductive organs of female rats. No treatment-related neoplastic lesions were seen in female rats; owing to the high mortality rate in males, no valid conclusion can be drawn about carcinogenicity. A NOAEL was not identified, as treatment-related changes occurred in the kidneys and the testis at all doses (US National Cancer Institute, 1978)

Groups of 50 five-to-six-week-old Sprague-Dawley rats of each sex were fed diets containing endosulfan (purity, 97.1%) at concentrations of 0, 3, 7.5, 15, or 75 ppm, equal to 0, 0.1, 0.3, 0.6, and 2.9 mg/kg bw per day for males and 0, 0.1, 0.4, 0.7, and 3.8 mg/kg bw per day for females, for 104 weeks. Satellite groups of 20 animals of each sex were retained for blood sampling and examination at 104 weeks. Reductions in body weights and body-weight gains were observed in males (group mean, 17% at 104 weeks) and females (group mean, 18% at 104 weeks) at 75 ppm, but no clinical signs of poisoning were seen at any dose. No increase in mortality rates was observed in treated

groups. Increased incidences of enlarged kidneys in females and of aneurysms and enlarged lumbar lymph nodes in males were seen at 75 ppm. Histopathological examination showed that males at 75 ppm had an increased incidence of aneurysm and marked progressive glomerulonephrosis (controls, 20/70; 75 ppm, 30/70). The commonest neoplasms were pituitary tumours in males and females and mammary tumours in females, but the increased incidences did not appear to be related to treatment. The NOAEL was 15 ppm, equal to 0.6 mg/kg bw per day, on the basis of reduced body weights and pathological findings at higher doses (Ruckman et al., 1989; Gopinath & Cannon, 1990; Hack et al., 1995).

Groups of 25 Wistar rats of each sex were fed diets containing technical-grade endosulfan (purity unspecified) at doses of 0, 10, 30, or 100 ppm for up to 104 weeks; groups of five of each sex were killed at 52 weeks. There were no treatment-related clinical signs, and the body weights were unaffected, except for a nonsignificant decrease in the body weights and food consumption of males at the high dose. The survival rate of treated females was reduced, the deaths being associated predominantly with respiratory infections. The weights of the testes of males at 10 ppm were reduced by 7% with respect to controls at 104 weeks ($p < 0.05$), and the kidney weights were significantly ($p < 0.001$) increased (by 16%) in males at the high dose at 104 weeks. Histopathological changes observed in males at the high dose at 104 weeks consisted of enlarged kidneys, mild-to-severe renal tubular dilatation (12/12), mild-to-moderate formation of irregular albuminous casts (10/12), pronounced focal nephritis (7/12), and mild-to-severe degeneration (11/12) of the renal tubular epithelium. At 104 weeks, female rats at the high dose showed minimal degeneration of renal tubules (2/3) and some focal nephritis (1/3). The low survival rate precluded a clear conclusion about the renal changes in female rats. Microscopic alterations in the liver were seen in 50% of males at the high dose at week 104, consisting of focal areas of hydropic cells, which were pale and swollen; the nuclei were surrounded by a clear zone, and a few cells appeared to have eosinophilic cytoplasmic inclusions. Few females at the high dose showed changes in liver cells. A few tumours developed during the experiment, but their occurrence was not dose-related. The NOAEL was 30 ppm, equal to 1.5 mg/kg bw per day, on the basis of effects on the kidney (Keller, 1959c).

Dogs

Groups of six beagle dogs of each sex were fed diets containing technical-grade endosulfan (purity, 96.5%) at concentrations of 0, 3, 10, or 30 ppm for one year, calculated by the authors to be equivalent to 0, 0.23, 0.77, and 2.3 mg/kg bw per day. In addition, one group was given a diet containing 30-60 ppm endosulfan, increasing in stages from 30 ppm for 54 days, to 45 ppm for 52 days, and 60 ppm for 19-40 days; these dogs were killed *in extremis* before the scheduled completion of the experiment and showed a number of signs of poisoning, including tonic contraction and increased sensitivity to noise and optical stimuli. Some animals given endosulfan at 30 ppm

throughout the 12-month study had violent contractions of the abdominal muscles (without vomiting), and males at this dose had reduced body-weight gains throughout the study and slightly reduced body weights in the latter stages of the study, in comparison with control animals. Cholinesterase activity was measured in serum, erythrocytes, and brain, but difficulty appears to have been experienced in measuring these activities, and there were large variations within groups for the brain enzyme, the group mean of which was increased in dogs at 30 ppm. No other effects related to treatment were observed, and no increase in the incidence of neoplastic or non-

neoplastic lesions was observed in treated animals. The NOAEL was 10 ppm, calculated by the authors to be equivalent to 0.57 mg/kg bw per day, on the basis of clinical signs and reductions in body weight (Brunk 1989, 1990).

Groups of two male and two female mongrel dogs were given technical-grade endosulfan in gelatin capsules at doses of 0, 3, 10, or 30 ppm, equivalent to 0, 0.075, 0.25, or 0.75 mg/kg bw per day, on six days per week for one year. The group receiving 3 ppm was given 100 ppm for the first three days of treatment, but clinical signs of vomiting, tremors, convulsions, rapid respiration, mydriasis, salivation, and tonic-clonic convulsions in one male and both females led to a reduction in the dose for the remainder of the study. No clinical signs or treatment-related effects on body-weight gain were seen. Clinical chemical and haematological end-points were within normal limits, and kidney function was unaffected by treatment. No gross or histopathological changes associated with treatment were noted. The NOAEL was 30 ppm, equivalent to 0.75 mg/kg bw per day, on the basis of clinical signs at the initial high dose (Keller, 1959b).

(d) Genotoxicity

Endosulfan was tested for genotoxicity in a wide range of assays, both *in vitro* (with and without metabolic activation) and *in vivo* (Table 3). There was no evidence of genotoxicty in most of these assays. In an assay for dominant lethal mutation in male Swiss mice given endosulfan of a purity of 97.3%, there was a significant change in the result of mating during the sixth mating week in the group at 16.6 mg/kg bw per day. The total numbers of implants per pregnancy were 9 in controls and 4.5 at the high dose; the numbers of live implants per pregnancy were 9 in controls and 2.2 at the high dose; and the numbers of dead implants per pergnancy were none in controls and 2.25 at the high dose (Pandey et al., 1990). While there is no doubt about the statistical significance of these results, it is unusual to find a true dominant lethal effect appearing so late in an experimental mating schedule; however, it is not unknown, since a reproducible effect of this type was demonstrated with some glycol ethers (McGregor et al., 1983). In a later assay for dominant lethal mutation (Dzwonkowska & Hübner, 1991), much lower doses were used, so the results cannot be used as evidence for a non-reproducible effect. Significant increases in the proportion of morphologically abnormal sperm were also observed in the study of Pandey et al. (1990), as well

Table 3. Results of assays for the genotoxicity of endosulfan

End-point	Test object	Dose	Result	Reference
				(LED or HID)[a]

In vitro					
Differential toxicity	B. subtilis rec strains H17 and M45	2000 µg/disc	Negative[a]	Shirasu et al. (1978)	
Reverse mutation	S. typhimurium TA100, TA1535, TA1537, TA1538, TA98; E. coli WP2 uvrA	5000 µg/plate	Negative[b]	Shirasu et al. (1978)	
Gene conversion	S. cerevisiae, D4	5000 µg/ml	Negative[b]	Mellano & Milone (1984b)	
Forward mutation	S. pombe	500 µg/ml	Negative[b]	Mellano & Milone (1984a)	
Unscheduled DNA synthesis	Male F344 rat primary hepatocytes	51 µg/ml	Negative[a]	Cifone & Myhr (1984b)	
Gene mutation	Mouse lymphoma L5178Y cells, tk locus	75 µg/ml [?]	Negative[b]	Cifone & Myhr (1984a)	
Chromosomal aberration	Human lymphocytes	200 µg/ml	Negative[b]	Asquith & Baillie (1989)	
Chromosomal aberration	Human lymphocytes	200 µg/ml	Negative[b]	Pirovano & Milone (1986)	
In vivo					
Micronucleus formation	NMRI mouse bone-marrow cells	5 mg/kg bw, po × 1	Negative	Jung et al. (1983)	
Micronucleus formation	NMRI mouse bone-marrow cells	10 mg/kg bw, po × 1	Negative	Müller (1988)	

Table 3. (continued)

End-point	Test object	Dose (LED or HID)[a]	Result	Reference
Chromosomal aberration	Albino rat bone-marrow cells	55 mg/kg, po × 5	Negative	Dikshith & Dotta (1978)

Dominant lethal mutation	Male Swiss mice	16.6 mg/kg bw, ip × 5	Equivocal	Pandey et al. (1990)	
Dominant lethal mutation	Male Balb/c mice	0.64 mg/kg bw, ip × 1 and ip × 5	Negative	Dzwonkowska & Hübner (1991)	
Sperm morphology	Mice	16.6 mg/kg bw, ip × 5	Positive	Pandey et al. (1990)	
Sperm morphology	Mice in vivo	3 mg/kg bw, ip × 35	Positive	Khan & Sinha (1996)	

LED, lowest effective dose; HID, highest ineffective dose; po, oral; ip, intraperitoneal

[a] In the absence of exogenous metabolic activation; not tested in the presence of exogenous metabolic activation

[b] In the absence and presence of exogenous metabolic activation

as in a later study (Khan & Sinha, 1996) at lower daily doses of a 35% emulsifiable concentrate.

(e) Reproductive toxicity

(i) *Multigeneration reproductive toxicity*

A preliminary investigation was conducted to determine the doses of endosulfan to be used in a two-generation study of reproductive toxicity. Four groups of 10 male and 10 female seven-week-old Crl: COBS CD Sprague-Dawley rats were given diets containing technical-grade endosulfan (purity, 97%) at concentrations of 0, 50, 75, or 100 ppm for two weeks and subsequently throughout mating and the rearing of offspring to weaning. Food consumption and body weights were decreased in adults at 75 and 100 ppm. At terminal autopsy, the mean weights of the livers were significantly higher than the control value in all treated groups. Mating performance, pregnancy rate, and the duration of gestation were unaffected by treatment. The litter weights of dams were significantly decreased at 75 ppm and to a greater extent at 100 ppm from day 4 *post partum.* No treatment-related abnormalities were found in the young (Edwards et al., 1982).

In a two-generation study of reproductive toxicity with two matings in each generation, four groups of six-week-old Sprague-Dawley rats were fed diets containing technical-grade endosulfan (purity, 97%) at concentrations of 0, 3, 15, or 75 ppm, equal to 0.2-0.23, 1-1.2, and 5-5.7 mg/kg bw per day for males and

0.24-0.26, 1.2-1.3, and 6.2-6.9 mg/kg bw per day for females. The group sizes were 32 of each sex for the F_0 generation and 28 of each sex for the F_{1b} generation. No clinical signs or deaths related to treatment were observed during the study. Single deaths occurred among F_0 females at 0, 3, and 15 ppm and among F_{1b} control females. Mating performance and pregnancy rates were not affected by treatment. Statistically significant decreases in litter weight were occasionally seen, but there was no effect on mean pup weights or on litter size. No treatment-related effect on sex ratios was seen at any dose.

Statistically significantly increased relative kidney weights were seen at 75 ppm in F_0 and F_{1b} males, and statistically significantly increased relative liver weights were observed in F_0 males and females at 75 ppm and in F_{1b} females at 15 and 75 ppm. The effect at 15 ppm in F_{1b} dams was not seen at this dose in any other matings. Yellowish discolouration of cells in the proximal convoluted tubules were observed in male F_{1b} rats at 3, 5, and 75 ppm and in female F_{1b} rats at 75 ppm. The incidence and extent of this effect was dose-related; traces of discolouration were seen at all doses, and minimal discolouration was seen in male rats at 15 and 75 ppm. Granular or clumped pigment was seen in proximal convoluted tubular cells in males at the high dose, but these findings were not associated with histopathological evidence of renal damage. While the increased incidence of cellular discolouration was related to

treatment, the finding is considered not to be toxicologically significant as no adverse effects were seen on cells and the yellow pigment was considered likely to be due to storage of endosulfan and its metabolites in lysosomes before excretion (Annex 1, reference 58). The presence of the pigment is thus an indication of exposure to endosulfan rather than of toxicity. The NOAEL for maternal toxicity was 15 ppm, equal to 1 mg/kg bw per day, on the basis of increased relative liver and kidney weights at higher doses. The NOAEL for reproductive effects was 75 ppm, equal to 6 mg/kg bw per day, the highest dose tested (Edwards et al., 1984; Offer, 1985).

(ii) Developmental toxicity

Rats

Groups of mated female albino rats (strain and age unspecified) were given oral doses of 0 (20 rats), 5 (26 rats), or 10 (32 rats) mg/kg bw per day of endosulfan (purity unspecified) on days 6-14 of gestation. No marked changes in behaviour or appearance were reported, and the body weights of treated animals were similar to those of controls. The numbers of pregnancies were 18, 20, and 21, respectively. The dams were killed on day 21 of gestation. No abortions occurred, but there was a significant increase in the percent of litters with resorptions (5.5% in controls, 20% at 5 mg/kg bw per day, and 23% at 10 mg/kg bw per day) and increased fetal mortality, although this effect was slight and was not dose-related (0, 2, and 1 in the three groups, respectively). Slight increases in

the incidences of cerebral hypoplasia and enlargement of the renal pelvis were observed on visceral examination, but these effects were not considered to be related to treatment as they were also seen in control animals and the increases were small and were not dose-dependent. No other increase in the incidence of visceral abnormalities was reported. Skeletal examination showed statistically significant increases in the incidences of absent fifth sternebrae and of fetuses with incomplete ossification. A slight increase in the incidence of absent fifth metacarpus, although not statistically significant, was also seen in treated animals. These effects were not considered to be related to treatment, as their magnitude was small and they were not dose-dependent. No maternal toxicity was seen at any dose. The level of reporting in this published paper was inadequate for identifying a NOAEL for developmental toxicity (Gupta et al., 1978).

Groups of 25 mated CD Sprague-Dawley rats were given technical-grade endosulfan (purity, 97.3%) in corn oil by gavage on days 6-19 of gestation at a dose of 0, 0.66, 2, or 6 mg/kg bw per day. The clinical signs in dams at 6 mg/kg bw per day included flaccidity, rough coat, alopecia, and hyperactivity. A dose-related decrease in maternal body-weight gain was seen at 2 and 6 mg/kg bw per day. The number of implantations and litter size were unaffected, but there was a slight reduction in the weight and length of fetuses of dams at the high dose. A non-dose-related reduction in the percent of live fetuses and an increase in the number of resorbed fetuses were seen at 2 mg/kg

bw per day. No statistically significant treatment-related effect on the sex ratio was observed. No external variations or malformations were seen at 0.66 or 2 mg/kg bw per day; at the high dose, 5/405 fetuses exhibited lordosis (anteroposterior curvature of the spine) and six had oedema. All five of the fetuses with lordosis and five of those with oedema were from a single litter. In one fetus from the same litter, the skin of the upper forelimb was webbed to the chest. No significant treatment-related effects were seen on soft-tissue development. Common minor skeletal variations were present in all groups. The incidence of poorly ossified sixth sternebrae was significantly greater in animals at the high dose than in the control group, and two fetuses at this dose had clubbed left hindlimbs. The five fetuses from the same litter that had oedema and lordosis also had wide, thickened vertebral arches, ribs, and clavicles, and the clavicles were shortened, curved, and twisted. Four of these fetuses had shortened pubes, and two had an unossified hyoid bone. The incidence of these effects was generally < 1%, and the effects were largely related to delayed development and confined mainly to a single litter from a single dam that showed numerous signs of poisoning related to administration of endosulfan, including face rubbing, alopecia, flaccidity, and hyperactivity. The developmental effects are therefore probably related to the maternal toxicity of the high dose. The NOAEL for maternal toxicity was 0.66 mg/kg bw per day on the basis of decreased body-weight gain and clinical signs at higher doses. The NOAEL for developmental toxicity was 2 mg/kg bw per day on the basis of delayed development and a low incidence of isolated skeletal

variations (Mackenzie, 1980).

Groups of 20-24 mated female Wistar rats were given technical-grade endosulfan (purity, 97.3%) dissolved in sesame oil by gavage on days 7-16 of gestation at doses of 0, 0.66, 2, or 6 mg/kg bw per day. No clinical signs of toxicity were reported in females at 0.66 or 2 mg/kg bw per day; four dams at 6 mg/kg bw per day died after 6-10 doses of endosulfan, three of these rats having tonic-clonic convulsions for several days before death. Thirteen of the surviving animals had tonic-clonic convulsions for a number of days, generally around day 10 of gestation. Some of these rats also showed hypersalivation on a number of days during treatment. Statistically significant decreases in body weight and body-weight gain were observed at 6 mg/kg bw per day. No statistically significant changes in reproductive or pup parameters were observed at any dose, and the fetal sex ratio was relatively well balanced. No statistically significant increase in the incidence of abnormalities was observed in fetuses. A single oedematous, retarded fetus at 6 mg/kg bw per day presented with superior brachygnathia and a relatively small alveolar cavity in the upper jaw, combined with cleft palate, bending of both hind feet in the tarsal joint, wavy clavicles, and bent and shortened scapulae. These effects were considered to be spontaneous, as no other limb or head defects were observed in any pup in any of the litters at this dose. Skeletal examination revealed a statistically significant increase in the incidence of fragmented thoracic vertebral centra at 6 mg/kg bw per day. This effect was considered to be treatment-related and reflects the frank maternal toxicity of endosulfan at the high

dose. No treatment-related major malformations were observed. The NOAEL for maternal toxicity was 2 mg/kg bw per day on the basis of deaths, clinical signs, and decreased body weights at higher doses. The NOAEL for developmental toxicity was 2 mg/kg bw per day on the basis of the increased incidence of fragmented thoracic vertebral centra (Albrecht & Baeder, 1993).

Rabbits

Groups of 20-26 mated New Zealand white rabbits were given technical-grade endosulfan (purity, 97.3 %) in corn oil by gavage on days 6-28 of gestation at doses of 0, 0.3, 0.7, or 1.8 mg/kg bw per day. There were no changes in mean body weight. None of the does at 0.3 or 0.7 mg/kg bw per day aborted, and there were no signs of toxicity and no deaths. The high dose was associated with signs of maternal toxicity that included noisy, rapid breathing, hyperactivity, and convulsions. The does were killed on day 29 of gestation. The number of implantations, litter size, sex ratio, mean fetal weight and length, and the numbers of live and resorbed fetuses were unaffected by treatment. There were no dead fetuses in any group, and no gross external alterations were reported. The only soft-tissue anomalies occurred in 6/167 fetuses (2/20 litters examined) at the high dose and consisted of the left carotid arising from the innominate; 1/141 control fetuses (1/18 litters examined) also showed this abnormality. Common skeletal variations and minor anomalies occurred at similar

incidences in control and treated fetuses. Endosulfan did not have teratogenic or developmental effects even at the maternally toxic dose of 1.8 mg/kg bw per day. The NOAEL for maternal toxicity was 0.7 mg/kg bw per day on the basis of clinical signs at higher doses (Dickie et al., 1981).

(f) Special studies

(i) *Enzyme induction*

Endosulfan was one of 16 organochlorine pesticides tested for their ability to induce hepatic microsomal enzyme activities. Wistar rats were given diets containing endosulfan at concentrations of 0, 20, 50, or 200 ppm for two weeks. No difference from control enzyme activity was observed at 20 or 50 ppm; at 200 ppm group, the activities in comparison with the control were 123% for aniline hydroxylase (statistically significant, one experiment), 191% for aminopyrene demethylase (statistically significant, one experiment), and 124% for hexobarbital oxidase (not significant, one experiment) (den Tonkelaar & van Esch, 1974).

ICR mice given endosulfan at 5 mg/kg bw per day by oral gavage for three days and killed on the fourth day showed no increase in liver weight or total hepatic cytochrome P450 content. The dearylation of *O*-ethyl *O*- *para*-nitrophenyl phenylphosphorothioate and parathion and NAD(P)H-dependent reductase activity were nonsignificantly increased (Robacker et al., 1981).

(ii) *Promotion*

Enhancement of gamma-glutamyl transpeptidase-positive foci in rat liver was studied as an indicator of promotion. Young male Sprague-Dawley rats were partially hepatectomized and injected intraperitoneally 24 h later with *N*-nitrosodiethylamine at 30 mg/kg bw. One week after the partial hepatectomy, the rats were randomized to groups of 10 or 11 and dosed orally by gavage on five days per week for 10 weeks with endosulfan in corn oil at 0, 1, or 5 mg/kg bw per day. The numbers and volume of enzyme-altered foci were not increased in the treated groups in comparison with controls.

In the same study, inhibition of intercellular communication was studied in Chinese hamster lung V79 cells in a metabolic cooperation assay and in rat liver WB epithelial cells in a scrape loading, dye-transfer assay. At nontoxic concentrations, technical-grade endosulfan, analytical-grade ensosulfan (alpha and ß isomers and a mixture of the two), and endosulfan sulfate inhibited gap-junctional intercellular communication in both systems. In addition, endosulfan ether was effective in the rat liver WB cell system (Flodström et al., 1988).

(iii) Immunotoxicity

In two published studies, endosulfan was administered to male

Wistar rats at dietary doses of up to 50 ppm for six weeks (Banarjee & Hussain, 1987) or up to 20 ppm for 22 weeks (Banarjee & Hussain, 1986) to evaluate humoral and cell-mediated immune responses. In the six-week study, a significant decrease in total serum antibody titre to tetanus toxoid was seen at 30 and 50 ppm, with a slight decrease (not statistically significant) at 10 ppm. A decrease in both immunoglobulin (Ig)M and IgG and in the total gamma-globulin content of rat serum was observed at 50 ppm. Cellular immunity was assayed by measuring inhibition of migration of activated leukocytes and macrophages. Rats exposed to endosulfan and subsequently immunized with tetanus toxoid showed a significant decrease in inhibition of leukocyte and macrophage migration in a dose-dependent pattern, the decrease becoming statistically significant at 30 and 50 ppm. These results indicate that both humoural and cellular immunity was depressed as a result of exposure to endosulfan at 30 and 50 ppm, with no effect at 10 ppm, equivalent to 0.5 mg/kg bw per day. In the 22-week study, the specific response of serum antibody titre to tetanus toxoid showed a marked decrease in rats exposed to 10 or 20 ppm endosulfan throughout the experiment, in a dose- and time-dependent pattern. Treatment at 10 or 20 ppm diminished the inhibition of migration of both leukocytes and macrophages throughout the study.

The effect of endosulfan on the immune system did not appear to be secondary to other toxic effects, since the body weights of the animals were unaffected by treatment and endosulfan is not known to affect the hormonal system. Immune responses were unaffected by treatment at 5 ppm, equivalent to 0.25 mg/kg bw per day. The Committee

noted that the method used to assess cellular immunity in these studies is far from ideal as it is flawed by large inherent errors, lack of objectivity, and, except in very experienced hands, lack of accuracy. Less subjective tests of cellular immunity, such as cytotoxic T cell response to a virus, would have provided more reliable results.

Technical-grade endosulfan (purity, 96%) was administered in sesame oil on 10 occasions by gavage to groups of eight female Wistar rats at doses of 0.5, 1.5, or 4.5 mg/kg bw per day from two days before until seven days after infection by gavage with approximately 500 *Trichonella spiralis* larvae. As a positive control, prednisolone was administered by subcutaneous injection at a dose of 25 mg/kg bw per day two days before and three days after infection. Three rats from each group were killed seven days after infection so that the number of adult worms in the intestine could be counted; the remaining rats were killed 54 days after infection in order to count the number of larvae in the tongue. Thymus and spleen weights and the percentage lymphocytes in the white cell count were measured at both times. Body weights were measured weekly. There were no differences between endosulfan-treated and untreated rats, whereas the prednisolone-treated group had a sevenfold higher tongue larval count and, at seven days, a 25% reduction in thymus weight, a 50% reduction in spleen weight, and lymphocyte counts < 50% of the control value

(Hack & Leist, 1988).

Endosulfan was included in the first part of a study to screen for immunotoxicity, but because no effect was observed it was not examined in greater detail. Groups of six male, weanling Wistar rats were given endosulfan in the diet at concentrations of 20, 100, or 250 ppm for three weeks. Body weights and food intake were recorded weekly. At autopsy, the weights of the liver, kidneys, spleen, thymus, pituitary, adrenals, thyroid, testis, and mesenteric and popliteal lymph nodes were recorded, these organs were also examined histologically. Haematological examination consisted of total and differential leukocyte counts. Serum IgM and IgG were determined by enzyme-linked immunosorbent assay. The only effects induced by endosulfan were considered to be expressions of general toxicity, and there was no evidence for any specifically immunotoxic effects. The most sensitive parameter for the toxicity of endosulfan was a reduction in body-weight gain, which was observed at 100 ppm (Vos et al., 1982).

(iv) Neurobehavioural effects and neurotoxicity

In a number of studies conducted by the same group of investigators, endosulfan (purity, 95%) was given by gavage to rats at a dose of 2 mg/kg bw per day for 90 days (Paul et al., 1993, 1994) or up to 6 mg/kg bw per day for 30 days (Paul et al., 1995), and behavioural and biochemical changes were determined. Signs of frank toxicity (reduced body weights, reduced food consumption, death, increased intensity of tremors, and increased liver enzyme activity) were observed in all studies, and some changes in behaviour were

noted, including increased motor activity and inhibition of conditioned and unconditioned escape and avoidance responses.

Groups of adult domestic hens were given a single oral dose of 96 mg/kg bw endosulfan, the LD_{50}, in corn oil, observed for 21 days, re-dosed, and again observed for 21 days. As was to be expected, a number of deaths occurred that were related to treatment. No signs of ataxia and no treatment-related changes in nervous tissue were seen in vehicle control or endosulfan-treated hens. Seven of 10 hens in a concurrent positive control group given tri- *ortho*-cresyl phosphate developed ataxia and signficant spinal cord and peripheral nerve degeneration (Roberts et al., 1983).

No inhibition of rat brain acetylcholinesterase activity was observed in a preparation incubated with 10 µmol/L alpha-endosulfan for up to 75 min.. A similar concentration of aldicarb produced 15% inhibition within 5 min and 80% inhibition within 75 min (Müllner, 1989).

Technical-grade endosulfan (purity, 98.6%) was administered to groups of 10 rats as a single dose of 25, 50, or 100 mg/kg bw to males and 3, 6, or 12 mg/kg bw to females. Deaths occurred at the highest doses, and there was a dose-related increase in the frequency of

clinical signs, which were reversible and apparent only on the day of dosing. These were assumed to be due to the known affinity of endosulfan for the gamma-aminobutyric acid receptors in the brain. At 50 and 100 mg/kg bw in males and 6 and 12 mg/kg bw in females, various serious neuropharmacological effects were seen, including coarse tremor and tonic-clonic convulsions. At 25 mg/kg bw in males and 3 mg/kg bw in females, the clinical signs seen were typical of general discomfort, such as stilted gait, squatting posture, and irregular respiration. No compound-related effects on motor activity were observed at non-lethal doses. No effects were seen on the rearing frequency, fore- or hindlimb grip strength, or on landing foot-spread. No histopathological effects were found in the central or peripheral nervous system. The study was carried out in accordance with prevailing OECD testing guidelines (April 1996) and the OECD principles of good laboratory practice (12 May 1981 [C(81)30(Final)]) (Bury, 1997).

(v) Effects on sperm

In a study of biochemical changes induced by endosulfan in the testis of Druckrey rats, the authors postulated that endosulfan impairs testicular function by altering the enzyme activities responsible for spermatogenesis, thus affecting the intratesticular spermatid count and resulting in low sperm production and increased sperm deformities. The data presented support the notion that administration of endosulfan at relatively high doses ($\geq$ 2.5 mg/kg bw per day) for several months increases the activity of a number of enzymes in the testes, including lactate dehydrogenase, sorbitol dehydrogenase, gamma-glutamyl transpeptidase, and glucose-6-phosphate dehydrogenase. At doses of 5 mg/kg bw per day and higher, there was a marked reduction in sperm count (up to 47%) in comparison with controls. In the absence of historical control data, it is unclear whether the decrease in sperm count seen at 2.5 mg/kg bw per day (22%) was within the normal biological range for the test animals. The sperm abnormalities and the reductions in spermatid count and sperm production, while statistically significantly different from those in concurrent controls, were only slight. In the absence of consistent dose-response relationships for these effects, they were considered not biologically significant (Sinha et al., 1995).

The effects of endosulfan on gonadal hormones, measured as plasma and testicular testosterone, plasma follicle-stimulating and luteinizing hormones, and plasma and testicular 3ß- and 17ß-hydroxysteroid dehydrogenases, was investigated in Wistar rats given endosulfan in peanut oil at doses up to 10 mg/kg bw per day for up to 30 days. Significant inhibition of 3ß- and 17ß-hydroxysteroid dehydrogenases occurred in the testes of treated animals after 30 days of treatment, and the plasma follicle-stimulating hormone, luteinizing hormone, and testosterone concentrations were significantly ($p < 0.05$) reduced in rats treated for 15 or 30 days at either dose. Plasma and testicular testosterone concentrations were not significantly reduced after 15 days of treatment at 7.5 mg/kg. A

significant decrease in the content or activity of microsomal cytochrome P450 and related mixed-function oxidases was observed in the testes of treated animals, with marked inhibition of the activity of glutathione- *S*-transferase at both doses. The latter changes were reversed when endosulfan was withdrawn, but the testicular testosterone concentrations remained significantly reduced (Singh & Pandey, 1990).

(vi) Endocrine effects

An estrogen is a substance that can induce estrus or a biological response associated with estrus; one such effect is proliferation of cells in the female genital tract. In recent years, naturally occurring and man-made substances in the environment that may be estrogenic have come under increasing scrutiny. Suspicion that endosulfan may have estrogenic properties was stimulated by observation of the reduced sperm counts described above and of testicular atrophy in rats given endosulfan in the diet in long-term studies.

Perhaps the first study of estrogenic effects *in vivo* was conducted by Raizada et al. (1991), who treated groups of eight ovariectomized Wistar rats, weighing an average of 100 g, with endosulfan at 1.5 mg/kg bw per day by gavage, estradiol dipropionate intraperitoneally (dose unspecified), or a combination of these treatments for 30 days. Endosulfan did not change the weights of the uterus, cervix, or vagina, whereas estradiol propionate produced large increases. The increased weights seen with the combined treatment were similar to those with estradiol propionate alone.

Concern that endosulfan might be estrogenic persisted as a result of the findings of an 'E-screen' assay, which was developed to assess the estrogenic effects of environmental chemicals by observing their proliferative effect on a target cell. The numbers of cells present after similar inocula of the human breast cancer cell line, MCF-7, were compared in the absence of estrogens (negative control), in the presence of estradiol-17ß (positive control), and with a range of concentrations of endosulfan. In this assay, endosulfan was estrogenic at concentrations of 10-25 µmol/L, with a proliferative effect about 80% that of 17ß-estradiol at 10 µmol/L; the relative proliferative potency (i.e. the ratio of the doses of endosulfan and estradiol-17ß required to produce the maximum effect) was 0.0001%. In addition, endosulfan competed with estradiol-17ß for binding to the estrogen receptor and increased the concentrations of progesterone receptor and pS2 in MCF-7 cells, as would be expected for a compound that mimics estrogens (Soto et al., 1994, 1995).

As part of a study to optimize investigations of estrogenic activity, endosulfan and nine other chemicals (17ß-estradiol, diethylstilbestrol, tamoxifen, 4-hydroxytamoxifen, methoxychlor, the methoxychlor metabolite 2,2-bis *(para*-hydroxyphenyl)-1,1,1-trichloroethane, nonylphenol, *ortho*, *para*'-DDT, and kepone) with known or suspected estrogenic

activity were tested in three assays: competitive binding to the mouse uterine estrogen receptor, transcriptional activation in HeLa cells transfected with plasmids containing an estrogen receptor and a response element, and the uterotropic assay in mice. The results of the three assays were consistent with respect to the known estrogenic activities of the chemicals tested and their requirements for metabolic activation. There was no evidence from any of these tests that endosulfan is estrogenic (Shelby et al., 1996).

A much publicized report indicated that even estrogens of low potency, such as endosulfan, could have important effects because of interaction with other chemicals. The estrogenic potencies of combinations of chemicals were screened in a system in which the human estrogen receptor sequence is incorporated into the yeast genome. Combinations of two weak environmental estrogens, such as dieldrin, endosulfan, and toxaphene, were 1000 times more potent in human estrogen receptor-mediated transactivation than any chemical alone (Arnold et al., 1996). This result was not reproduced in another laboratory in which the same assay was used or in a uterotropic assay in which sexually immature rats were treated with endosulfan or dieldrin alone or in a combination on three successive days and the uterine mass weighed on the following day. The highest doses used in the human estrogen receptor assay were determined by the solubility of the compounds, and the highest doses in the uterotropic assay were 100 mg/kg bw for endosulfan or dieldrin alone and 75 mg/kg bw of each in combination. Both chemicals were inactive in both assays, and there was no evidence of synergism (Ashby et al., 1997). In a further study with the human estrogen receptor assay, however, 0.1 mmol/L endosulfan increased the activity of ß-galactosidase (Ramamoorthy et al., 1997).

More doubt was cast upon the thesis of synergism by an independent study in which endosulfan and dieldrin showed no additive effect in displacing ^{3}H-17ß-estradiol from rat uterine estrogen receptors or in inducing the proliferation of MCF-7 breast cancer cells. The weak proliferative potential described by Soto et al. (1994, 1995) was, however, confirmed in this assay *in vitro.* Endosulfan or dieldrin alone at 3 mg/kg bw per day or in combination, injected intraperitoneally daily for three days, did not stimulate uterotrophic activity and had no effect on pituitary prolactin or other endocrine-related end-points in immature female rats, indicating that these weakly estrogenic compounds do not interact in a synergistic fashion in binding to estrogen receptors or in activating estrogen receptor-dependent responses in mammalian tissues or cells (Wade et al., 1997). The paper in which synergism was originally proposed was later withdrawn, since the results could not be reproduced, even in the same laboratory (McLachlan, 1997). Overall, these results suggest that concomitant exposure to weakly estrogenic compounds probably does not result in reproductive toxicity related to estrogen action.

3. Observations in humans

In general, the doses of endosulfan involved in cases of

poisoning have been poorly characterized. In a summary of case reports (Lehr, 1996), the lowest reported dose that resulted in death was 35 mg/kg bw; deaths have also been reported after ingestion of 295 and 467 mg/kg bw, within 1 h of ingestion in some cases. Intensive medical treatment within 1 h was reported to be successful after ingestion of doses of 100 and 1000 mg/kg bw. The clinical signs in these patient were consistent with those seen in laboratory animals, dominated by tonic-clonic spasms. In a case in which a dose of 1000 mg/kg bw was ingested, neurological symptoms requiring anti-epileptic therapy were still required one year after exposure.

Comments

More than 90% of an oral dose of endusulfan was absorbed in rats, with maximum plasma concentrations occurring after 3-8 h in males and about 18 h in females. Elimination occurs mainly in the faeces and to a lesser extent in the urine, more than 85% being excreted within 120 h. The highest tissue concentrations were in the kidneys. The metabolites of endosulfan include endosulfan sulfate, diol, hydroxy-ether, ether, and lactone but most of its metabolites are polar substances which have not yet been identified. Endosulfan would not be expected to accumulate significantly in human tissues. No data on plant metabolites were available to the Meeting.

A battery of tests for acute toxicity in several species with technical-grade endosulfan showed that it is highly toxic after oral or dermal administration, with respective LD_{50} values of 10-160 mg/kg bw and 45-135 mg/kg bw. The LC_{50} value for rats in a single study was 13 mg/m^3 in females and 35 mg/m^3 in males. Endosulfan, administered by any route, is more toxic to female than to male rats. Clinical

signs of acute intoxication include piloerection, salivation, hyperactivity, respiratory distress, diarrhoea, tremors, hunching, and convulsions.

WHO has classified endosulfan as moderately hazardous (WHO, 1996).

The kidney is the target organ for toxicity. The renal effects include increased renal weights and granular pigment formation after short-term administration and progressive, chronic glomerulo-nephrosis or toxic nephropathy after long-term exposure, although the observation of progressive glomerulonephrosis is complicated by the fact that this is a common lesion in ageing laboratory rats and occurs at high incidence in control rats.

In a 90-day feeding study in rats, the cytoplasm of isolated cells in the renal proximal convoluted tubules had a yellowish colour, particularly in males, at all dietary concentrations from 10 ppm. The presence of this yellow pigmentation was largely reversible during a four-week recovery period, and it did not appear to indicate nephrotoxicity. A darker, more particulate, granular and/or clumped pigment was also observed, predominantly in cells of the straight

portions and occasionally in the proximal convoluted tubules, at dietary concentrations of 30 ppm and above. This darker pigment was more persistent than the yellow one, and urinalysis revealed darker urine and marginally more ketones at doses from 60 ppm, and marginally more protein, particularly in males, indicating renal damage at doses of 360 ppm and above. Similar findings emerged from a multigeneration study but not from a two-year study of carcinogenicity in rats. The changes in pigmentation were considered to be due to the presence of endosulfan and/or its metabolites in the enlarged lysosomes. To test this hypothesis, a four-week feeding study was conducted in which male rats were given dietary concentrations of 360 or 720 ppm endosulfan. Light and electron microscopy of the kidneys of these animals clearly showed increases in the number of lysosomes and the size of cells in the convoluted tubule, probably as a result of accumulation of the test material and/or its metabolites. Lysosomal changes were not observed in either brain or liver, and the renal changes receded appreciably during a 30-day recovery period. Chemical analysis of the kidneys indicated the presence of alpha-endosulfan and, to a lesser extent ß-endosulfan sulfate, and endosulfan lactone. The concentrations of the dominant alpha-endosulfan in the kidneys were about 50 times those in the liver. The concentrations in blood were usually below the level of detection. After the 30-day recovery period, renal alpha-endosulfan was detected only in traces and ß-endosulfan not at all. Similar analysis of tissues from rats in the two-year study of toxicity and carcinogenicity did not reveal the presence of these substances in the kidney, although measurable alpha-endosulfan was found in the liver at 75 ppm. The yellow colour therefore indicates the presence of endosulfan and/or its metabolites, rather than either a stage in the pathogenesis of nephropathy or an independent expression of toxicity. It was postulated that in longer

studies its removal from lysosomes is accelerated by enzyme induction, which has not been investigated.

In a 78-week study, exposure of rats to endosulfan at a high dose of 20 mg/kg bw per day resulted in testicular atrophy, characterized by degeneration and necrosis of the germinal cells lining the seminiferous tubules. In addition, decreased sperm counts accompanied by an increased incidence of sperm abnormalities have been reported in mice, again at high doses of endosulfan. Reductions in the activities of some testicular xenobiotic-metabolizing enzymes and some hormones that are necessary for normal testicular function were also seen in a 30-day study in rats at 10, but not at 7.5 mg/kg bw per day. The functional significance of these findings was not clear, as studies of reproductive and developmental toxicity in rats and rabbits showed neither impaired fertility nor any increase in the incidence of defects or abnormalities in offspring. Given the high doses at which these testicular effects were observed, it would appear that they are of little human significance.

No genotoxic activity was observed in an adequate battery of tests for mutagenicity and clastogenicity *in vitro* and *in vivo.* The Meeting concluded that endosulfan is not genotoxic.

No carcinogenic effect was observed in mice at 18 ppm for 24 months, in female rats at 445 ppm for 78 weeks in one study or in male or female rats at 75 ppm or 100 ppm for two years in two other studies. The Meeting noted the differences in the dietary concentrations used in these studies, but non-neoplastic responses were seen even at the lower doses.

Endosulfan at dietary concentrations of 0, 3, 15, or 75 ppm did not affect reproductive performance or the growth or development of the offspring of rats over the course of a two-generation study. The NOAEL was 75 ppm, the highest dose tested, equal to 5 mg/kg bw per day for males and 6.2 mg/kg bw per day for females. The NOAEL for parental toxicity was 15 ppm, equal to 1 mg/kg bw per day for males and 1.2 mg/kg bw per day, on the basis of increased liver and kidney weights at 75 ppm.

In two studies of developmental toxicity in rats given oral doses of 0, 0.66, 2, or 6 mg/kg bw per day, the NOAEL for maternal toxicity was 0.66 mg/kg bw per day in one study and 2 mg/kg bw per day in the other. In the first case, the basis was decreased body-weight gain at 2 mg/kg bw per day and decreased body-weight gain and clinical signs of toxicity at 6 mg/kg bw per day; in the second case, the basis was mortality, clinical signs of toxicity, and decreased body-weight gain at 6 mg/kg bw per day. In both studies, the NOAEL for developmental toxicity was 2 mg/kg bw per day, in the first case on the basis of delayed development and a low incidence of skeletal variations seen at 6 mg/kg per day and in the second on the basis of an increased incidence of fragmented thoracic vertebral centra seen at 6 mg/kg bw per day. In neither study was there any treatment-related major malformation.

In a study of developmental toxicity in rabbits given oral doses of 0, 0.3, 0.7, or 1.8 mg/kg bw per day, the NOAEL for maternal tocicity was 0.7 mg/kg bw per day on the basis of clinical signs of toxicity at 1.8 mg/kg bw per day. The NOAEL for developmental toxicity was 1.8 mg/kg bw per day, the highest dose tested.

Several recent studies have shown that endosulfan, alone and in combination with other pesticides, may bind to estrogen receptors and may perturb the endocrine system. The available studies show only very weak binding to hormone receptors *in vitro,* and the evidence for their relevance to adverse physiological effects *in vivo* is extremely limited. Long-term assays of toxicity and studies of reproductive and developmental toxicity in experimental mammals did not indicate that endosulfan induces functional aberrations that might result from loss of endocrine homeostasis.

The absence of immunotoxic effects in a large number of bioassays with endosulfan suggested that it does not have an adverse effect on the immune function of laboratory animals. However, in two studies, rats given endosulfan in the diet at 30 or 50 ppm for 6 weeks or 20 ppm for 22 weeks had reduced serum titres of tetanus toxoid antibody

and reduced immunoglobulins G and M, and inhibition of migration of both leukocytes and macrophages. These findings have not been confirmed.

In a summary of case reports of human poisoning incidents, the lowest reported dose that caused death was 35 mg/kg bw. Higher doses caused death within 1 h. The clinical signs in these patients were dominated by tonic-clonic convulsions, consistent with the observations in experimental animals.

An ADI of 0-0.006 mg/kg bw was established on the basis of the NOAEL of 0.6 mg/kg bw per day in the two-year dietary study of toxicity in rats and a safety factor of 100. The ADI is supported by similar NOAEL values in the 78-week dietary study of toxicity in mice, the one-year dietary study of toxicity in dogs, and the study of developmental toxicity in rats.

An acute RfD of 0-0.02 mg/kg was established on the basis of the NOAEL of 2 mg/kg bw per day in the study of neurotoxicity in rats and a safety factor of 100.

Toxicological evaluation

Levels that cause no toxic effect

Mouse: 3.9 ppm, equal to 0.58 mg/kg bw per day (females in a 78-week study of toxicity)

Rat: 15 ppm, equal to 0.6 mg/kg bw per day (two-year dietary study of toxicity)
75 ppm, equal to 6 mg/kg bw per day (reproductive toxicity)
0.66 mg/kg bw per day (maternal toxicity in a study of developmental toxicity)
2 mg/kg bw per day (fetotoxicity in a study of developmental toxicity)

Rabbit: 0.7 mg/kg bw per day (maternal toxicity in a study of developmental toxicity)

Dog: 10 ppm, equivalent to 0.57 mg/kg bw per day (one-year study of toxicity)

Estimate of acceptable daily intake for humans

0-0.006 mg/kg bw

Estimate of acute reference dose for humans

0.02 mg/kg bw

Studies that would provide information useful for continued evaluation of the compound

1. Studies of immunotoxicity with standard test protocols

2. Studies of the significant sex difference in acute toxicity, particularly in rats

3. Further observations in humans

List of end-points relevant for setting guidance values for dietary and non-dietary exposure

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Absorption, distribution, excretion, and metabolism in mammals

Rate and extent of absorption	Rat: oral, > 90% absorption; max. concentration at 3-8 h (males) 18 h (females)
Distribution	Mainly in kidney and liver
Potential for accumulation	Low
Rate and extent of excretion	Biphasic; urinary half-life was 6 h for 1st phase, 33-68 h for 2nd phase; faecal half-life was 10 h and 30 h; > 85% excretion within 120 h
Metabolism in animals	Oxidation and hydrolysis; unidentified polar metabolites
Toxicologically significant compounds (animals, plants and environment)	Parent; no data on plant metobolites

Acute toxicity

Rat: LD_{50} oral	10 mg/kg bw (female)
Rat: LD_{50} dermal	500 mg/kg bw (female)
Rat: LC_{50} inhalation	13 mg/m^3 4 h (female)
Skin irritation	Not irritating
Eye irritation	Not irritating
Skin sensitization	Not sensitizing

Short-term toxicity

Target/critical effect	Reduced survival, convulsions, salivation
Lowest relevant oral NOAEL	Rat: 0.64 mg/kg bw per day, dietary
Lowest relevant dermal NOAEL	Rat: 3 mg/kg bw per day
Lowest relevant inhalation NOAEL	Rat: 2 mg/m^3, no effect (highest concentration)

Genotoxicity	Not genotoxic

Long-term toxicity and carcinogenicity

Target/critical effect	Kidney

Lowest relevant NOAEL	Rat: 0.6 mg/kg bw per day, 2-year study
Carcinogenicity	Not carcinogenic
Reproductive toxicity	
Reproduction target: critical effect	None identified
Lowest relevant reproductive NOAEL	Rat: 6 mg/kg bw per day
Developmental target /critical effect	Fetoxicity at maternally toxic doses
Lowest relevant developmental NOAEL	Rat: 2 mg/kg bw per day
Neurotoxicity/Delayed neurotoxicity	Rat: 1.5 mg/kg bw (female); 12.5 mg/kg bw (male)
	no effect
Other toxicological studies	Immunotoxicity in certain special assays, not
	confirmed in sensitization test or histologically
	Some conflicting evidence of interaction with
	estrogen receptors in vitro; none in vivo
Medical data	Lowest lethal dose: 35 mg/kg bw, oral

Summary	Value	Study	Safety factor
ADI	0-0.006 mg/kg bw	Several different species and end-points	100
Acute reference dose	0.02 mg/kg bw	Study of neurotoxicity in rats	100

References

Albrecht, M. & Baeder, C. (1993) Hoe 002671-substance technical (code: Hoe 002671 00 ZD98 0005). Testing for embryotoxicity in the Wistar rat after oral administration. Unpublished report No. 93.0716, document A51695. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Arai, M., Hiromori ,T., Ho, S., Okuno, Y. & Miyamoto, J. (1981) Life span chronic toxicity study of endosulfan in mice -- 12 month report. Unpublished report No. 0285, at-10 from Sumitomo Chemical Co Ltd, Japan, 28 April 1981. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Arcelin, G. (1996) Contact hypersensitivity to endosulfan (Code: Hoe 002671 00ZD99 0008) in albino guinea pigs maximumization test. Unpublished report No. 630753 from RCC, Itingen, Switzerland. Hoechst document A58132.A51695. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Arnold, S.F., Klotz, D.M., Collins, B.M., Vonier, P.M., Guilette, L.J. & McLachlan, J.A. (1996) Synergistic activation of oestrogen receptor with combinations of environmental chemicals. *Science,* 272, 1489-1492.

Ashby, J., LeFevre, P.A., Odum, J., Harris, C.A., Routledge, E.J. & Sumpter, J.P. (1997) Synergy between synthetic oestrogens? *Nature,* 385, 494.

Asquith, J.C. & Baillie, J.H. (1989) Endosulfan substance technical (code Hoe 002671 0I ZD95 0005). Metaphase analysis of human lymphocytes. Unpublished report No. m/hl/1307 from Toxicol Laboratories, United Kingdom, 16 March 1989. Hoechst document A40411. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Banarjee, B.D. & Hussain, Q.Z. (1986) Effect of subchronic endosulfan exposure on humoral and cell-mediated immune responses in albino rats. *Arch. Toxicol.,* 59, 279-284.

Banarjee, B.D. & Hussain, Q.Z. (1987) Effects of endosulfan on humoral and cell-mediated immune responses in rats. *Bull. Environ. Contam. Toxicol.,* 38, 438-441.

Barnard, A.V., Atkinson, J.S., Heywood, R., Street, A.E, Gibson, W.A., Rao, R., Offer, J.M. & Gopinath, A.R.H. (1984) 13 week toxicity study in mice. Unpublished report No. Hst 229/831052, 25 September 1984 from Huntingdon Research Centre, Huntingdon, Cambridgeshire, United Kingdom. Hoechst document A29663. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Barnard, A.V., Jones, D.R., Powell, L.A.J., Heywood, R., Street, A.E., Gibson, W.A., Gopinath, C., Majeed, S.K. & Almond, R.H. (1985) 13-week toxicity study in rats followed by a 4-week withdrawal period. Unpublished report No. Hst 230/84176, 25 March 1985 from Huntingdon Research Centre, Huntingdon, Cambridgeshire, United Kingdom. Hoechst document A30700 Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt- am-Main, Germany.

Bhide, M.B. & Naik, P.Y. (1984a) The acute oral toxicity (LD_{50}) of Excel Industries Ltds endosulfan technical No. 2 to the albino rats. Unpublished report from Indian Institute of Toxicology (report No. and date not given). Submitted to WHO by the National Registration Authority for Agricultural and Veterinary Chemicals, Australia.

Bhide, M.B. & Naik, P.Y. (1984b) The acute oral toxicity (LD_{50}) of Excel Industries Ltds endosulfan technical No. 1 to the albino mice. Unpublished report from Indian Institute of Toxicology (report No. and date not given). Submitted to WHO by the National Registration Authority for Agricultural and Veterinary Chemicals, Australia.

Bhide, M.B. & Naik, P.Y. (1984c) The acute dermal toxicity (LD_{50}) of Excel Industries Ltds endosulfan technical No. 2 to the albino

rabbits. Unpublished report from Indian Institute of Toxicology (report No. and date not given). Submitted to WHO by the National Registration Authority for Agricultural and Veterinary Chemicals, Australia.

Bowman, J.S. (1959) Preliminary report: Subacute feeding -- dairy cows. Unpublished report from Hazleton Laboratories, USA. Hoechst document No. A14205. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Bracha, P. (1977) Thionex tech: Acute oral toxicity study in rats, skin irritation study in rabbits, acute dermal toxicity study in rabbits, eye irritation study in rabbits and acute inhalation study in rats. Unpublished report No. 6111820 from Warf Institute Inc. Madison, Wisconsin, USA. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Bremmer, J.N. (1997a) Endosulfan: Substance technical (code: Hoe 002671 00 ZD99 0008) Testing for primary dermal irritation in the rabbit. Unpublished Hoechst Marion Roussel preclinical development study No. 96.0837. Hoechst document A58442. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Bremmer, J.N. (1997b) Endosulfan: Substance technical (code: Hoe 002671 00 ZD99 0008) Testing for primary eye irritation in the rabbit. Unpublished Hoechst Marion Roussel preclinical development study No. 96.0838. Hoechst document A58443. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Bremmer, J.N. & Leist, K.-H. (1998) Endosulfan (AE F002671, substance technical). Evaluation of the acute oral and dermal toxicity. Unpublished report No. TOX98/003 from Hoechst Schering AgrEvo GmbH. Hoechst document A59823. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Brunk, R. (1989) Endosulfan-substance technical (code: Hoe 002671 00 ZD96 0002). Testing for toxicity by repeated oral administration (1-year feeding study) to beagle dogs. Unpublished report No. 87.0643 from Pharma Research Toxicology and Pathology. Hoechst report No. 89.0188; document No. A40441 Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Brunk, R. (1990) Addendum to Hoechst report 89.0188; document No. A44605. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Bury, D. (1997) Endosulfan; substance, technical (code: Hoe 002671 00 ZD99 0008). Neurotoxicological screening in the male and female Wistar rat. Acute toxicity. Unpublished report No. 96.0373 from Hoechst Marion Roussel Preclinical Development, Germany. Hoechst report No. A59088. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Christ, O. & Kellner, H.M. (1968) Investigations with endosulfan-^{14}C in mice. Unpublished report No. ch/he-8412 from Hoechst Radiochemical, Frankfurt, 31 December 1968. Hoechst document A53842, translation of document A14217. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Cifone, A.M. & Myhr, B.C. (1984a) Evaluation of Hoe 002671-substance technical in the rat primary hepatocyte unscheduled DNA synthesis assay. Unpublished LBI project No. 20991 from Litton Bionetics Inc., USA, November 1984. Hoechst document No. A29800. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Cifone, A.M. & Myhr, B.C. (1984b) Mutagenicity evaluation of Hoe 002671-substance technical in the mouse lymphoma forward mutation assay. Unpublished LBI project No. 20980 from Litton Bionetics Inc., USA, November 1984. Hoechst document No. A29801. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Craine, E.M. (1986) A dermal absorption study in rats with ^{14}C-endosulfan. Unpublished project No. Wil-39028, 11 December 1986 from Wil Research Lab, USA. Wil-39028. 11 Decemnber, 1986. Hoechst document No. A35730. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Craine, E.M. (1988) A dermal absorption study in rats with ^{14}C-endosulfan with extended test duration. Unpublished project No. Wil-39029 from Wil Research Lab, USA. Hoechst document No. A39677. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Deema, P. Thompson, E. & Ware, G. (1966) Metabolism, storage and excretion of ^{14}C-endosulfan in the mouse. *J. Econom. Entomol.,* 59, 546-550.

Dickie, S.M., Mackenzie K.M. & Rao, G.N. (1981) Teratology study with FMC 5462 in rabbits. Unpublished study No. 80070, 27 July 1981 from Raltech Scientific Services. Hoechst document No. A23192. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Diehl, K.H. & Leist, K.H. (1988a) Hoe 002671: Active ingredient technical (code: Hoe 002671 0I ZD96 0002). Testing for acute oral toxicity in the male and female Wistar rat. Unpublished study No. 88.0551, 27 July 1988, from Pharma Research Toxicology and Pathology, Frankfurt, Germany. Hoechst document No. A39680. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Diehl, K.H. & Leist, K.H. (1988b) Endosulfan-active ingredient technical (code: Hoe 002671 0I ZD96 0002). Testing for acute dermal toxicity in the male and female Wistar rat. Unpublished study No. 88.0552, 22 August 1988, from Pharma Research Toxicology and Pathology, Germany. Hoechst document A39397, 1 September 1988. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Dikshith, T.S. & Datta, K.K. (1978) Endosulfan: Lack of cytogenetic effects in male rats. Unpublished document No. A17140 from Industrial Toxicology Research Centre. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Dikshith, T.S.S., Raizada, R.B., Kumar, S.N., Srivastava, M.K., Kaushal, R.A., Singh, R.P. & Gupta, K.P. (1988) Effect of repeated dermal application of endosulfan to rats. *Vet. Hum. Toxicol.,* 30, 219-224.

Donaubauer, H.H. (1988) Endosulfan-substance technical (code: Hoe 002671 OI ZD97 0003). Carcinogenicity study in mice: 24 months feeding study. Unpublished study No. 745 from Pharma Research Toxicology and Pathology, Germany. Hoechst document No. A38008. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Donaubauer, H.H. (1989) Amendment to the report No. 88.0278 endosulfan-substance technical (code: Hoe 002671 OI ZD97 0003). Carcinogenicity study in mice: 24 months feeding study. Unpublished study No. 745 from Pharma Research Toxicology and Pathology, Germany. Hoechst document No. A41617. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Donaubauer, H.H., Leist, K. & Kramer, M. (1985) Endosulfan-substance technical (code: Hoe 002671 01 ZD97 0003). 42-Day feeding study in mice. Unpublished study No. 744 from Pharma Research Toxicology, Germany. Hoechst document No. A38104. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Dorn, E. & Werner, H.-J. (1989) Endosulfan (Hoe 002671 OI ZD97 0003): Determination of residues in the liver and kidneys of rats after chronic (2-years) feeding (week 104). Unpublished Produktentwicklung, GB-C Ökologie II Hoechst AG Study No. (Analytical part) CR065/88. Hoechst document No. A41265. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Dorough, M.W., Huhtanen, K., Marshall, T.C. & Bryant, H.E. (1978) Fate of endosulfan in rats and toxicological considerations of apolar metabolites. *Pest. Biochem. Physiol.,* 8, 241-252.

Dzwonkowska, A. & Hübner, H. (1991) Studies on commercial insecticides with the dominant lethal mutations test. *Polish J. Occup. Med. Environ. Health,* 4, 43-53.

Ebert, E. & Weigand, W. (1984) Testing of the therapeutic effect of diazapam (Valium R) and phenobarbital (Luminal R) in the event of acute poisoning with endosulfan-active ingredient technical (code: Hoe 002671 oi ZD97 0003) in Wistar rats. Unpublished report No. 84.0062 from Pharma forschung Toxikologie. Hoechst document A29211. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Ebert, E., Weigand, W. & Kramer, P. (1985a) Endosulfan-active

ingredient technical (code: Hoe 002671 01 ZD97 0003). Testing for subchronic dermal toxicity (21 applications over 30 days) in SPF Wistar rats. Unpublished study No. 83.0118 from Pharma forschung Toxicologie. Hoechst document No. A30754, translation of document A30751. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Ebert, E., Leist, K.H. & Kramer, P. (1985b) Endosulfan-active ingredient technical (code: Hoe 002671 01 ZD97 0003). Testing for subchronic dermal toxicity (21 applications over 30 days) in Wistar rats. Toxicological review of studies 721 and 729 (reports 84.0321 and 84.0223). Unpublished Hoechst document No. A30755, translation of A30752. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Ebert, E., Leist ,K.H. & Kramer, P. (1985c) Endosulfan-active ingredient technical (code: Hoe 002671 01 ZD97 0003). Testing for subchronic dermal toxicity (21 applications over 30 days) in Wistar rats. Unpublished study No. 83.0508 from Pharma forschung Toxicologie. Hoechst document No. A30753, translation of document A30750. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Edwards, J.A., Hughes, E.W. & Almond, R.H. (1982) Preliminary investigation of the effect of endosulfan (code, Hoe 02671 OI AT 209) on reproduction of the rat. Unpublished report No. Hst 203/82252 from Huntingdon Research Centre, Huntingdon, Cambridgeshire, United Kingdom. Hoechst document No. A29563. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Edwards, J.A., Reid, J.Y., Offer, M.J., Almond, H.R. & Gibson, A.W. (1984) Effect of endosulfan-technical (code, Hoe 02671OI AT209) on reproductive function of multiple generations in the rat. Unpublished report No. Hst 204/83768 from Huntingdon Research Centre, Huntingdon, Cambridgeshire, United Kingdom. Hoechst document No. A29428. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Ehling, G. & Leist, K.-H. (1991a) Hoe 051327: substance technical (code HOE 051327 00 ZB99 0002): Testing for acute oral toxicity in the male and female Wistar rat. Pharma Research Toxicology, Germany. Hoechst document No. A46286. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Ehling, G. & Leist, K.-H. (1991b) Hoe 051327: substance technical (code HOE 051327 00 ZB99 0002): Testing for acute dermal toxicity in the male and female Wistar rat. Pharma Research Toxicology, Germany. Unpublished Hoechst document No. A45783. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Ehling, G. & Leist, K.-H. (1991c) Hoe 051329: substance technical (code HOE 051329 00 ZD98 0001): Testing for acute oral toxicity in the male and female Wistar rat. Pharma Research Toxicology, Germany. Unpublished Hoechst document No. A45783. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Ehling, G. & Leist, K.-H. (1991d) Hoe 051329: substance technical (code HOE 051329 00 ZD98 0001): Testing for acute dermal toxicity in the male and female Wistar rat. Pharma Research Toxicology, Germany. Unpublished Hoechst document No. A45829. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Elsea, J.R. (1958) Thiodan technical: Acute oral administration: Rats. Hazleton Laboratories, USA, 28 February, 1958. Unpublished Hoechst document No. A13686. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Flodström, S., Wärngård, L., Hemming, H., Fransson, R. & Ahlborg, U.G. (1988) Tumour promotion related effects by the cyclodiene insecticide endosulfan studies *in vitro* and *in vivo. Pharmacol Toxicol.,* 62, 230-235.

Goebel, H., Gorbach, S., Knauf, W., Rimpau, R.H. & Uttenbach, H. (1982) Properties, effects, residues and analytics of the insecticide endosulfan. *Res. Rev.,* 83, 41. Submitted to WHO by the National Registration Authority for Agricultural and Veterinary Chemicals, Australia.

Gopinath, C. & Cannon, M.W.J. (1990) Photomicrographic addendum to histopathology report No. Hst/289 Endosulfan, active ingredient technical (code: Hoe 002671 OI ZD97 0003) combined chronic toxicity/carcinogenicity study (104-week feeding in rats). Huntingdon Research Centre Ltd, Huntingdon, Cambridgeshire, United Kingdom. Unpublished Hoechst document No. A44604. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Gorbach, S., Christ, O., Kellner, H.M., Kloss, G. & Börner, E. (1968) The metabolism of endosulfan in milk sheep. *J. Agric. Food Chem.,* 16, 950-953.

Gupta, P.K., Chandra, S.V. & Saxena, D.K. (1978) Teratogenic and embryotoxic effects of endosulfan in rats. *Acta Pharmacol. Toxicol.,* 42, 150-152.

Hack, R. & Leist, K.-H. (1988) Endosulfan-substance technical (code: Hoe 002671 0I ZD96 0002) Testing of host resistance in the female Wistar rat (immunological screening with *Trichonella spiralis).* Unpublished study No. 87.1125 from Pharma Research Toxicology and Pathology, Germany. Hoechst report A43829. Submitted to WHO by Agrevo GmbH, Frankfurt-am-Main, Germany.

Hack, R., Ebert, E. & Leist, K.H. (1995) Chronic toxicity and carcinogenicity studies with the insecticide endosulfan in rats and mice. *Food Chem. Toxicol.,* 33, 941-950.

Hollander, H. & Kramer, P. (1975a) Endosulfan sulfate = NIA 7985. Acute oral toxicity in female SPF-Wistar rats (vehicle: starch suspension). Unpublished Hoechst document No. A06966. Submitted to WHO

by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Hollander, H. & Kramer, P. (1975b) Endosulfan sulfate = NIA 7985. Acute oral toxicity in male beagle dogs. Unpublished Hoechst document No. A06965. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Hollander, H. & Kramer, P. (1975c) Comparative test on the acute toxicity of endosulfan ether and endosulfan alcohol in female SPF-Wistar rats (vehicle: starch suspension). Unpublished Hoechst document No. A07170; translation of A05271. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Hollander, H. & Kramer, P. (1975d) 1-Hydroxy endosulfan ether. Acute oral toxicity in female SPF-Wistar rats (vehicle: starch suspension). Unpublished Hoechst document No. A06967; translation of A05276 Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Hollander, H. & Kramer P. (1975e) Endosulfan lactone: Acute oral toxicity in female SPF-Wistar rats (vehicle: starch suspension). Unpublished Hoechst document No. A07171; translation of A05273. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Hollander, H. & Kramer, P. (1975f) Endosulfan lactone. Acute oral toxicity in male SPF-Wistar rats. Unpublished Hoechst document No. A06964.25. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Hollander, H. & Weigand, W. (1983) Acute aerosol toxicity in male and female SPF Wistar rats 4 hours-LC50. Unpublishd Hoechst report No. 83.0397; document No. A32087. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Hollander, H., Weigand, W. & Kramer, P. (1984) Endosulfan-active ingredient technical (code: Hoe 002671 0I ZD97 0003) Testing for subchronic inhalation toxicity--21 exposures in 29 days -- in SPF Wistar rats. Unpublished study No. 762; document No. A29823, translation of A29766. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Indraningsih, McSweeney, C.S. & Ladds, P.W. (1993) Residues of endosulfan in the tissues of lactating goats. *Aust. Vet. J.*, 70, 59-62.

Jung, Weigand, W. & Kramer, P. (1983) Mouse micronucleus test following oral administration. Unpublished Hoechst AG report No. 83.0458; document No. A31628. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Keller, J.G. (1959a) Subacute feeding--dairy cows. Hazleton Laboratories, USA. Unpublished Hoechst document No. A14206. Submitted

to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Keller, J.G. (1959b) One-year oral study in dog. Hazleton Laboratories, 12 May 1959. Unpublished Hoechst document No. A13924. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Keller, J.G. (1959c) 2 year dietary study in rats. Hazleton Laboratories, USA, 22 May 1959. Unpublished Hoechst document No. A14037. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Kellner, H.M. & Eckert (1983) Hoe 02671-14C. Pharmacokinetics and residue determinations after oral and intravenous administration to rats. Unpublished study No. tep 74/1; bereich c/analytisches project oe 87/45; Hoechst report 01-l42-0382-83, 15 February 1983; document No. A49475, translation of document A27971. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Khan, P.K. & Sinha, S.P (1996) Ameliorating effect of vitamin C on murine sperm toxicity induced by three pesticides (endosulfan, phosphamidon and mancozeb). *Mutagenesis,* 11, 33-36

Kramer, P. & Weigand, W. (1971) Endosulfan lactone (vehicle: sesame oil). Acute oral toxicity in male and female SPF-Wistar K-rat. Unpublished Hoechst document No. A18276, 21 May 1971; translation of A14326. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Lachmann, G. & Siegemund, B. (1987) Hoe 002671-(5a, 9a-^{14}C). Dermal absorption of ^{14}C- endosulfan in rhesus monkeys. Battelle-Institut, Frankfurt. Unpublished Hoechst document No. A36685. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Lehr, W (1996) Summary of intoxications with endosulfan. Clinical cases and poisoning incidents. Unpublished Hoechst report No. psr 96/006, 12 March 1996; document A56361. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Leist, K.H. (1989a) Endosulfan-substance technical (code: Hoe 002671 oi ZD97 0003). Carcinogenicity study in mice, 24 months feeding study -- Residue determination. Pharma Research Toxicology and Pharmacology. Unpublished Hoechst document No. A41284. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Leist, K.-H. (1989b) Amendment to report No. HST 289/881067. Endosulfan, active ingredient technical (code: Hoe 002671 OI ZD97 0003). Combined chronic toxicity/carcinogenicity study (104-week feeding in rats). Residue determination. Unpublished Hoechst report No. 89.1105; document No. A41265. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Leist, K.-H. & Bremmer, J. (1998) Endosulfan (AE F002671, substance

technical). Re-evaluation of the NOAEL in the 90-day subchronic toxicity study in rats. Unpublished AgrEvo document No. 59825. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Leist, K.-H. & Mayer, D. (1987) Endosulfan-active ingredient technical (code: Hoe 002671 01 ZD97 0003). 30-Day feeding study in adult male Wistar rats. Unpublished study No. 84 0585 from Pharma Research Toxicology and Pharmacology. Hoechst report No. 87.0129, 27 March 1987; document No., A37112. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Lightowler, J.E. & Gardner, J.R. (1978) Acute oral toxicity study in rats. Unpublished report No. 78/mak1/428. 20 November 1978 from Life Science Research. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Mackenzie, K.M. (1980) Teratology study with FMC 5462 in rats. Raltech Scientific Services, study No. 79041, 2 October 1980. Hoechst document No. A21393. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Maier-Bode, H. (1966) Investigations on the persistence of the insecticide endosulfan in the vegetable and animal organism. Pharmakologisches Institut der Rheinischen Friedrich Wilhelms Universität. Unpublished Hoechst document No. A4047. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

McGregor, D.B., Willins, M.J., McDonald, P., Holström, M., McDonald, D. & Niemeier, R.W. (1983) Genetic effects of 2-methoxyethanol and bis(2-methoxyethyl)ether. *Toxicol. Appl. Pharmacol.,* 70, 303-316.

McLachlan, J.A. (1997) Synergistic effect of environmental estrogens: Report withdrawn. *Science,* 277, 462-463.

Mellano, D. & Milone, M.F. (1984a) Mutagenic activity of the compound endosulfan-technical with *Saccharomyces cerevisiae* gene conversion-DNA repair test. Instituto di Recherche Biomediche. Unpublished Hoechst document No. A29313. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Mellano, D. & Milone, M.F. (1984b) Mutagenic activity in vitro of the compound endosulfan-technical with *Schizosaccharomyces pombe.* Unpublished experiment No. M 708, 18 June 1984, from Instituto di Recherche Biomediche. Hoechst document No. A29312. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Müller, W. (1988) Endosulfan-substance technical (code: Hoe 002671 0I ZD95 0005). Micronucleus test in male and female NMRI mice after oral administration. Pharma Research Toxicology and Pathology. Unpublished Hoechst document No. A38059. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Müllner, H. (1989) Effects of endosulfan and aldicarb on rat brain acetylcholinesterase. Unpublished Hoechst report 16 June 1989; document No. A43395. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Nath, G., Datta, K.K., Dikshith, T.S.S., Tandon, S.K. & Pandya, K.P. (1978) 30 day oral administration in rats. Interaction of endosulfan and metepa in rats. Industrial Toxicology Research Centre. Unpublished Hoechst document No. A17906. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Noctor, J.C. & John, S.A. (1995) (^{14}C)-Endosulfan: Rates of penetration through human and rat skin determined using an in vitro system. Hazleton Europe, United Kingdom. Unpublished Hoechst document No. A54103. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Nogami, K. (translator, no other name available) (1970) Testing report on the toxicity of endosulfan (Malix) to dogs through acute oral administration (LD_{50}). Unpublished Hoechst document No. A13834. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Offer, J.M. (1985) Addendum to HST 204. Effect of endosulfan-technical (code: Hoe 02671 OI AT209) on the reproductive function of multiple generations in the rat. Histopathological review of the kidneys in adult rats of the F_{1b} generation and in weanling rats of the F_{2b} generation. Huntingdon Research Centre, Huntingdon, Cambridgeshire, United Kingdom. Unpublished Hoechst document No. A30757. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Pandey, N., Gundevia, F., Prem, A.S. & Ray, P.K. (1990) Studies on the genotoxicity of endosulfan, and organochlorine insecticide, in mammalian germ cells. *Mutat. Res.,* 242, 1-7.

Paul, V., Sheela, S., Balasubramaniam, E. & Kazi ,M. (1993) Behavioural and biochemical changes produced by repeated oral administration of the insecticide endosulfan in immature rats. *Indian J. Physiol. Pharmacol.,* 37, 204-208.

Paul, V., Easwaramoorthy, B. & Kazi, M. (1994) The neurobehavioural toxicity of endosulfan: A serotonergic involvement in learning impairment. *Eur.J. Pharmacol. Environ. Toxicol. Pharmacol.,* 270, 1-7.

Paul, V., Easwaramoorthy, B., Arumugam, R.J. & Kazi, M. (1995) A sex-related difference in the neurobehavioural and hepatic effects following chronic endosulfan treatment in rats. *Eur. J. Pharmacol. Environ. Toxicol. Pharmacol.,* 293, 355-360.

Pirovano, R. & Milone, M.F. (1986) Study of the capacity of the test article endosulfan, substance technical to induce chromosome aberrations in human lymphocytes cultured in vitro. Unpublished RBM

experiment No. m 822, 20 March 1986. Hoechst document A33127. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Raizada, R.B., Srivastava, M.K. & Dikshith, T.S.S. (1991) Lack of estrogenic effects of endosulfan: An organochlorine insecticide in rat. *Natl Acad. Sci. Lett.,* 14, 103-107.

Ramamoorthy, K., Wang, F., Chen, I.-C., Norris, J.D., McDonnel, D.P., Leonard, L.S., Gaido, K.W., Bocchinfuso, W.P., Korach, K.S. & Safe, S. (1997) Estrogenic activity of a dieldrin/toxaphene mixture in the mouse uterus, MCF-7 human breast cancer cells, and yeast-based estrogen receptor assays: No apparent synergism. *Endocrinology,* 138, 1520-1527.

Robacker, K.M., Kulkarni, A.P. & Hodgson, E. (1981) Pesticide induced changes in the mouse hepatic microsomal cytochrome P-450-dependent monooxygenase system and other enzymes. *J. Environ. Sci. Health,* B16, 529-545.

Reno, F.E. (1975) Acute oral toxicity study in rats. Endosulfan technical. Unpublished final report, 18 December 1975, from Hazleton Laboratories America. Hoechst document No. A33732. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Roberts, N.L., Phillips, N.K. & Gopinath, C. (1983) Acute delayed neurotoxicity study with endosulfan-technical (code: Hoe 002671 OI ZD97 0003) in the domestic hen. Huntingdon Research Centre, Huntingdon, Cambridgeshire, United Kingdom. Unpublished Hoechst document No. A32153. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Ruckman, S.A., Waterson, L.A., Crook, D., Gopinath, C., Majeed, S.K., Anderson, A. & Chanter, D.O. (1989) Endosulfan, active ingrediant technical (code: Hoe 002671 OI ZD97 0003). Combined chronic toxicity/carcinogenicity study (104-week feeding study in rats). Huntingdon Research Centre, Huntingdon, Cambridgeshire, United Kingdom. Unpublished Hoechst document No.A40440. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Shelby, M.D., Newbold, R.R., Tully, D.B., Chae, K. & Davis, V.L. (1996) Assessing environmental chemicals for estrogenicity using a combination of in vitro and in vivo assays. *Environ. Health Perspectives,* 104, 1296-1300.

Shirasu, Y., Moriya, M. & Ohta, T. (1978) Microbial mutagenicity testing on endosulfan. Institute of Environmental Toxicology, Japan. Unpublished Hoechst document No. A21215, 1978. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

Singh, S.K. & Pandey, R.S. (1990) Effect of sub-chronic endosulfan exposures on plasma gonadotrophins, testosterone, testicular testosterone and enzymes of androgen biosynthesis in rat. *Indian J.*

Exp. Biol., 28, 953-956.

Sinha, N., Narayan, R., Shanker, R. & Saxena, D.X. (1995) Endosulfan-induced biochemical changes in the testis of rats. *Vet. Hum. Toxicol.,* 37, 547-549.

Soto, A.M., Chung, K.L. & Sonnenschein, C. (1994) The pesticides endosulfan, toxaphene and dieldrin have oestrogenic effects on human oestrogen-sensitive cells. *Environ. Health Perspectives,* 102, 380-383.

Soto, A.M., Sonnenschein, C., Chung, K.L., Fernandez, M.F., Olea, N. & Serrano, F.O. (1995) The E-screen assay as a tool to identify estrogens: An update on estrogenic environmental pollutants. *Environ. Health Perspectives,* 103 (Suppl. 7), 113-122.

Stumf, K. & Lehr, W. (1993) Amendment to document A49584. Unpublished Hoechst document A49584, 2 February 1993. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany.

den Tonkelaar, E.M. & van Esch, G.J. (1974) No-effect levels of organochlorine pesticides based on induction of microsomal liver enzymes in short-term toxicity experiments. *Toxicology,* 2, 371-380.

US National Cancer Institute (1978) 78-week dietary study in Osborne-Mendel rats and B6C3F1 mice. NCI study No. NCI-CG-TR62, Technical Report Series No. 62, Bethesda, Maryland, USA.

Vos, J.G., Krajnc, E.I., Beekhof, P.K. & van Logten, M.J. (1982) Methods for testing immune effects of toxic chemicals: Evaluation of the immunotoxicity of various pesticides in the rat. In: Matsunaka, S., Hutson, D.H. & Murphy, S.D., eds, *Pesticide Chemistry: Human Welfare and the Environment,* Oxford, Pergamon Press, pp. 497-504.

Wade, M.G., Desaulniers, D., Leingartner, K. & Foster, W.G. (1997) Interactions between endosulfan and dieldrin on estrogen-mediated processes *in vitro* and *in vivo. Reprod. Toxicol.,* 11, 791-798.

Weigand, W. (1982a) Acute oral toxicity of Hoe 51329 in albino rats. Pharma Research Toxicology, Germany. Unpublished Hoechst document No. A23296. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany (in German).

Weigand, W. (1982b) Acute oral toxicity of Hoe 51330 in albino rats. Pharma Research Toxicology, Germany. Unpublished Hoechst document No. A23297. Submitted to WHO by Hoechst Schering AgrEvo GmbH, Frankfurt-am-Main, Germany (in German).

WHO (1996) *The WHO Recommended Classification of Pesticides by Hazard and Guidelines to Classification 1996-1997* (WHO/PCS/96.3), International Programme on Chemical Safety, Geneva.

See Also:

Toxicological AbbreviationsEndosulfan (EHC 40, 1984)Endosulfan (HSG 17, 1988) Endosulfan (PDS)Endosulfan (PIM 576)Endosulfan (FAO Meeting Report PL/1965/10/1) Endosulfan (FAO/PL:1967/M/11/1)Endosulfan (FAO/PL:1968/M/9/1)Endosulfan (WHO Pesticide Residues Series 1)Endosulfan (WHO Pesticide Residues Series 4)Endosulfan (WHO Pesticide Residues Series 5)Endosulfan (Pesticide residues in food: 1982 evaluations) Endosulfan (Pesticide residues in food: 1989 evaluations Part II Toxicology)

must be introduced when the pest is at a very low density. Several introductions are made until one month after the first black scales are seen. **Phytotoxicity** Non-phytotoxic and non-phytopathogenic. **Formulation type** Parasitised pupae of whitefly on cards. **Compatibility** Use is entirely compatible with the use of other biological agents, but they are susceptible to a wide range of insecticides and some fungicides. **Principal tradename** 'Bunting *Encarsia formosa*' (Bunting), 'En-Strip' (Koppert).

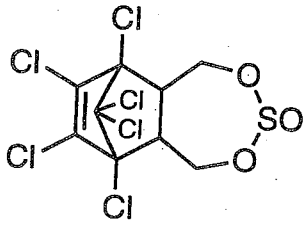
MAMMALIAN TOXICOLOGY

There is no evidence of acute or chronic toxicity, eye or skin irritation or hypersensitivity to mammals. No allergic response or health problems have been observed in production staff or horticultural workers.

262 endosulfan

Insecticide Acaricide

organochlorine



NOMENCLATURE

Common name endosulfan (BSO, E-ISO, (*m*) F-ISO, ANSI, ESA), thiodan (Iran, USSR), benzoepin (JMAF), no name (Italy).
IUPAC name (1,4,5,6,7,7-hexachloro-8,9,10-trinorborn-5-en-2,3-ylenebismethylene) sulfite; 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-= benzodioxathiepine 3-oxide.
C.A. name 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-= benzodioxathiepine 3-oxide. **CAS RN** *[115-29-7]* endosulfan; *[959-98-8]* formerly *[33213-66-0]*, alpha-endosulfan; *[33213-65-9]* formerly *[891-86-1]* and *[19670-15-6]*, beta-endosulfan **Development code** Hoe 02 671; FMC 5462 **Official code** OMS 204 (α); OMS 205 (β); OMS 570; ENT 23979.

PHYSICO-CHEMICAL PROPERTIES

Composition Endosulfan is a mixture of two stereoisomers: alpha-endosulfan, endosulfan (I), stereochemistry 3α,5aβ,6α,9α,9aβ-, comprises 64-67% of the tech. grade; beta-endosulfan, endosulfan (II), stereochemistry 3α,5aα,6β,9β,9aα-, 29-32%. Earlier reports on the stereochemistry of these isomers gave conflicting reports (W. Riemschneider, *World Rev. Pest Control*, 1963, 2(4), 29).
Mol. wt. 406.9 **Mol. formula** $C_9H_6Cl_6O_3S$
Form Colourless crystals (tech., cream to brown, mostly beige). **M.p.** ≥80 °C (tech.); α- 109.2 °C; β- 213.3 °C **V.p.** 0.83 mPa (20 °C) for 2:1 mixture of α- and β- isomers **SG/density** *c.* 1.8 (20 °C) (tech.) K_{ow} logP for α- = 4.74; β- = 4.79 (both at pH 5)

Solubility In water alpha-endosulfan 0.32, beta-endosulfan 0.33 (both in mg/l, 22 °C). In ethyl acetate, dichloromethane, toluene 200, ethanol *c.* 65, hexane *c.* 24 (all in g/l, 20 °C). **Stability** Stable to sunlight. Slowly hydrolysed in aqueous acids and alkalis, with the formation of the diol and sulfur dioxide.

COMMERCIALISATION

History Insecticide reported by W. Finkenbrink (*Nachrichtenbl. Dtsch. Pflanzenschutzdienstes* (*Braunschweig*), 1956, **8**, 183). Introduced by Hoechst AG (now AgrEvo GmbH) and, in the USA, by FMC Corp. **Patents** DE 1015797; US 2799685; GB 810602 all to Hoechst **Manufacturer** AgrEvo; Excel; Hindustan Insecticides; Makhteshim-Agan.

APPLICATIONS

Mode of action Non-systemic insecticide and acaricide with contact and stomach action. **Uses** Control of sucking, chewing, and boring insects and mites on a very wide range of crops, including fruit (including citrus), vines, olives, vegetables, ornamentals, potatoes, cucurbits, cotton, tea, coffee, rice, cereals, maize, sorghum, oilseed crops, hops, hazels, sugar cane, tobacco, alfalfa, mushrooms, forestry, glasshouse crops, etc. Also controls tsetse flies. **Phytotoxicity** Glasshouse geraniums and chrysanthemums, alfalfa, and lima beans may be injured. **Formulation type** EC; WP; DP; GR; UL; FT; SC; EO; Powder concentrate. **Compatibility** Compatible with most pesticides, but incompatible with strongly alkaline materials. **Principal tradename** 'Fan' (FMC), 'Thiodan' (AgrEvo), 'Endocel' (Excel), 'Thionex' (Makhteshim-Agan). **Mixtures** [*endosulfan* +] dimethoate; malathion; methomyl; monocrotophos; pirimicarb; triazophos; fenoprop; parathion; parathion-methyl; amitraz; deltamethrin; heptenophos; lindane + oxine-copper; petroleum oils; thiometon; anthraquinone + lindane + oxine-copper.

ANALYSIS

Product analysis by i.r. spectrometry (*CIPAC Handbook,* 1970, **1**, 360) or by glc (*ibid.,* 1985, **1C**, 2110; *AOAC Methods,* 1990, 983.08). **Residues** determined by glc with MCD (*ibid.,* 976.23; *Pestic. Anal. Man.,* 1979, 201-A, 201-G, 201-I; 405; A. Ambrus *et al., J. Assoc. Off. Anal. Chem.,* 1981, **64**, 773; *Man. Pestic. Residue Anal.,* 1987, **I**, 5, 6, S19; *Anal. Methods Residues Pestic.,* 1988, Part I, M1, M12). Further methods available on request from AgrEvo.

MAMMALIAN TOXICOLOGY

Reviews *Pesticide residues in food - 1989.* FAO Plant Production and Protection Paper 99, 1989. *Pesticide residues in food - 1989 evaluations. Part II - Toxicology.* FAO Plant Production and Protection Paper 100/2, 1990. *Environmental Health Criteria* 40 (WHO, 1984). **Acute oral** LD_{50} for rats 70 mg (in aqueous suspension)/kg, 110 mg tech. (in oil)/kg, 76 mg alpha-isomer/kg, 240 g beta-isomer/kg; for dogs 77 mg tech./kg. **Skin and eye** Acute percutaneous LD_{50} for rabbits 359 mg (in oil)/kg; for male rats >4000 mg/kg, female rats 500 mg/kg. **Inhalation** LC_{50} (1 h) for rats >21 mg/l air; (4 h) for male rats 0.0345, female rats 0.0126 mg/l. **NOEL** In 2 y feeding trials rats receiving 30 mg/kg diet showed no ill-effect; in 1 y trials NOEL for dogs 3 mg/kg diet. **ADI** (JMPR) 0.006 mg/kg b.w. [1989]. **Toxicity class** WHO II; EPA I (tech.).

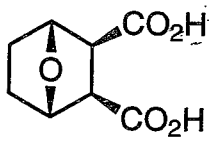
ECOTOXICOLOGY

Birds Acute oral LD_{50} for mallard ducks 205-245, ring-necked pheasants 620-1000 mg/kg. **Fish** Highly toxic (LC_{50} (96 h) for golden orfe 0.002 mg/l water) but, in practical use, should be harmless to wildlife. **Bees** Not toxic to bees under field conditions at an application rate of 1.6 l/ha (560 g endosulfan/ha). **Daphnia** EC_{50} (48 h) 75-750 µg/l.

ENVIRONMENTAL FATE

Animals The principal route of elimination is faeces. Most of the radioactivity is excreted within the first 48 hours. The amounts excreted are independent of dose level, number of dosages and isomerism. There are indications of species-specificity. Residues of endosulfan accumulate in the kidneys rather than in fat. Elimination from the kidneys takes place with half-lives of 7 days, but there is no sign of accumulation in the kidneys even after long-term feeding. Endosulfan is metabolised rapidly in mammalian organisms to less toxic metabolites and to polar conjugates. **Plants** The plant metabolites (mainly endosulfan sulfate) were also found in animals and have thus been investigated from a toxicological point of view. 50% of residues are lost in 3-7 days (depending on plant species). **Soil and water** Endosulfan (alpha- and beta-) is degraded in soil with DT_{50} 30 to 70 days. The main metabolite usually found was endosulfan sulfate, which is degraded more slowly and is for this reason the most important metabolite. DT_{50} for total endosulfan (alpha- and beta- endosulfan and endosulfan sulfate) in the field is 5-8 months. No leaching tendency was observed. Log K_{oc} 3.48-4.30. K_D < 3%.

263 endothal *Herbicide Algicide Plant growth regulator*



NOMENCLATURE

Common name endothal (BSI, France, New Zealand, since 1990 E-ISO (*m*) F-ISO), endothall (ANSI, Canada, WSSA).
IUPAC name 7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid.
C.A. name 7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid. **CAS RN** *[145-73-3]* endothal, unstated stereochemistry; *[28874-46-6]* endothal *rel*-(1*R*,2*S*,3*R*,4*S*)-isomer; *[17439-94-0]* endothal-diammonium, unstated stereochemistry
Other names 1,2-dicarboxy-3,6-*endo*-cyclohexane.

PHYSICO-CHEMICAL PROPERTIES

Composition Of the 4 theoretical stereoisomers of endothal, the *rel*-(1*R*,2*S*,3*R*,4*S*)-isomer is the most effective herbicide (US 2550494).
Mol. wt. 186.2 **Mol. formula** $C_8H_{10}O_5$
Form Colourless crystals (monohydrate). **M.p.** 144 °C (monohydrate) **V.p.** Negligible