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Chemical Review Committee
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Item 5 (b) (xi) of the provisional agenda*

Technical work: review of notifications of final regulatory action: phorate

Phorate: supporting documentation provided by Brazil

Note by the Secretariat

As referred to in document UNEP/FAO/RC/CRC.13/13, the annex to the present note sets out documentation provided by Brazil to support its notification of final regulatory action for phorate in the pesticide category. The present note, including its annex, has not been formally edited.

* UNEP/FAO/RC/CRC.13/1.

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Annex

Phorate: supporting documentation provided by Brazil

List of documents:

1. Final Regulatory Action-Phorate-Final Information.
2. Focused summary.
3. Email clarification from Brazil on exposure data for Phorate.
4. Resolution RDC N° 12 of March 13, 2015 – issued by the National Health Surveillance Agency (ANVISA).
5. Technical notes on the toxicological revaluation on the active ingredient phorate – prepared by National Health Surveillance Agency (ANVISA) with collaboration of Oswaldo Cruz Foundation (FIOCRUZ).
6. Use of Agricultural Pesticides and Prostate Cancer Risk in the Agricultural Health Study Cohort. American Journal of Epidemiology, v. 157, No 9, 2003.
7. Phorate - Review of pesticide poisoning incident data. 29 July 1998. Virginia Dobozy. https://archive.epa.gov/pesticides/chemicalsearch/chemical/foia/web/pdf/057201/057201-031.pdf.
8. In-laws, insecticide—and a mimic of brain death. John Victor Peter, Appaswamy T Prabhakar, Kishore Pichamuthu, Lancet 2008; 371: 622.
9. Pesticide Use Modifies the Association Between Genetic Variants on Chromosome 8q24 and Prostate Cancer. Koutros S. *et al. Cancer Research, v.70. p. 9224-9233, 2010.*
10. Phorate Exposure and Incidence of Cancer in the Agricultural Health Study. Mahajan, R. et al. Environmental Health Perspectives. V. 114, n 8, p. 1205-9, August 2006.
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16. HI-6 in man: efficacy of the oxime in poisoning by organophosphorus insecticides. Kusic, R.; Jovanovic, D.; Randjelovic, S.; Joksovic, D.; Todorovic, V.; Boskovic, B.; Jokanovic, M.; Vojvodic. V. Hum Exp Toxicol, v. 10, n. 2, p. 113-118, 1991.
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ROTTERDAM CONVENTION

SECRETARIAT FOR THE ROTTERDAM CONVENTION
ON THE PRIOR INFORMED CONSENT PROCEDURE
FOR CERTAIN HAZARDOUS CHEMICALS AND PESTICIDES
IN INTERNATIONAL TRADE

FORM FOR NOTIFICATION

OF FINAL REGULATORY ACTION TO BAN OR SEVERELY RESTRICT A CHEMICAL

Country: Brazil

SECTION 1 IDENTITY OF CHEMICAL SUBJECT TO THE FINAL REGULATORY ACTION

1.1	**Common name**	Phorate
1.2	**Chemical name according to an internationally recognized nomenclature (e.g. IUPAC), where such nomenclature exists**	O,O-diethyl S-ethylthiomethyl phosphorodithioate
1.3	**Trade names and names of preparations**	Granutox and Granutox 150 G

1.4 Code numbers

1.4.1	CAS number	298-02-2
1.4.2	Harmonized System customs code	OSHA IMIS Code Number: 2064
1.4.3	Other numbers (specify the numbering system)	

1.5 Indication regarding previous notification on this chemical, if any

1.5.1 ☐ This is a first time notification of final regulatory action on this chemical.

1.5.2 ☒ This notification replaces all previously submitted notifications on this chemical.

Date of issue of the previous notification: 15 august 2016.

SECTION 2 FINAL REGULATORY ACTION

2.1 The chemical is: ☒ **banned** **OR** ☐ **severely restricted**

2.2 Information specific to the final regulatory action

2.2.1 Summary of the final regulatory action

Prohibition of all technical and formulated products based on phorate active ingredient. So, the production, use, trade, import and export of phorate had been banned.

2.2.2 Reference to the regulatory document, e.g. where decision is recorded or published

Resolution RDC No. 12 of 13 March 2015, issued by National Health Surveillance Agency (ANVISA):

BOARD OF DIRECTORS
RESOLUTION RDC No. 12, of 13 MARCH 2015

States about the technical regulation for the active ingredient phorate as the result of toxicological reevaluation

The Board of Directors of the National Health Surveillance Agency (ANVISA), within the use of its attributions given by the
subsections III and IV, article 15 of the Law No. 9.782, of 26 January 1999,
subsection V and paragraphs 1st and 3rd, article 5 of the Internal Statute approved in the terms of the Annex I of the ANVISA Ordinance No. 650, of 29 May 2014, published in the Federal Official Gazette of 2 June 2014, according to what is stated in
the subsection III, article 2, subsections III and IV, article 7 of the Law No. 9.782 from 1999,
and the Program for the Improvement of the Regulation Process of the Agency, instituted by the Ordinance No. 422, of 16 April 2008,
in the meeting held on March 5th, 2015 adopts the following Resolution of the Board of Directors and I, Director-President Substitute, determine its publication.

Article 1st Cancel the toxicological evaluation reports of all technical products and formulated products based on the active ingredient phorate, from the date of publication of this Resolution.

Article 2nd Exclude the monograph of the active ingredient phorate, from the date of publication of this Resolution.

Article 3rd Determine that companies which hold stocks of such products provide its adequate final disposal and that such procedure be previously informed to the

Ministry of Agriculture, Livestock, and Food Supply (MAPA), to ANVISA and to the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA), within the period of thirty days from the date of publication of this Resolution.

Article 4th This Resolution enters into force in the date of its publication.

JAIME CESAR DE MOURA OLIVEIRA

Annex 1 - RDC No. 12 of 13 March 2015. Copy of the Federal Official Gazette of March 16th, 2015.

2.2.3 Date of entry into force of the final regulatory action

March 16th, 2015

2.3 Category or categories where the final regulatory action has been taken

2.3.1 All use or uses of the chemical in your country prior to the final regulatory action

Insecticide authorized exclusively for agricultural use for the following crops: cotton, potato, coffee, beans and corn.

2.3.2 Final regulatory action has been taken for the category ☐ Industrial

Use or uses prohibited by the final regulatory action

Use or uses that remain allowed (only in case of a severe restriction)

2.3.3 Final regulatory action has been taken for the category ☒ Pesticide

Formulation(s) and use or uses prohibited by the final regulatory action

All uses

Formulation(s) and use or uses that remain allowed

(only in case of a severe restriction)

None

2.4 Was the final regulatory action based on a risk or hazard evaluation? ☒ **Yes**

☐ **No** (If no, you may also complete section 2.5.3.3)

2.4.1 If yes, reference to the relevant documentation, which describes the hazard or risk evaluation

Technical Note on the toxicological revaluation on the active ingredient phorate – prepared by National Health Surveillance Agency (ANVISA) with collaboration of Oswaldo Cruz Foundation (FIOCRUZ).

2.4.2 Summary description of the risk or hazard evaluation upon which the ban or severe restriction was based.

2.4.2.1 Is the reason for the final regulatory action relevant to human health? ☒ Yes ☐ No

If yes, give summary of the hazard or risk evaluation related to human health, including the health of consumers and workers

Phorate was an insecticide authorized in Brazil exclusively for agricultural use in cotton, potato, coffee, beans and corn. In 2008 Brazilian Health Surveillance Agency (ANVISA) initiated the toxicological reassessment of phorate due to evidences of high acute toxicity and neurotoxicity of this active ingredient of pesticides. Brazilian law predicts that pesticides may have their registrations cancelled in the country when they fall under the following conditions related to human health: when they have no antidote or effective treatment in Brazil; if found teratogenic, mutagenic or carcinogenic; if they cause hormonal disturbances and damage to the reproductive system or if they are more dangerous to humans than demonstrated in tests with laboratory animals. Phorate and its metabolites are easily absorbed through skin and mucous membranes and irreversibly block the catalytic activity of acetylcholinesterase (AChE), the enzyme responsible for mediating the hydrolysis of acetylcholine in acetic acid and choline acid. Thus, they interrupt the transmission of nerve impulses in the cholinergic synapses of the central nervous system (CNS), autonomic nervous system (ANS) and neuromuscular junction. Inactivation of AChE causes cholinergic hyperstimulation by acetylcholine accumulation in the synaptic cleft. Phorate is considered one of the most toxic organophosphate AChE inhibitors, with mean oral LD50 for mice ranging from 1.4 to 10 mg/kg body weight. Phorate can cause complex neurological clinical manifestations in humans, such as encephalopathy, intermediate syndrome and delayed polyneuropathy, described by various

authors (Young, Jung; Ayer, 1979; Kashyap et al., 1984;. WHO / FAO, 1988; Kusic et al., 1991; Dobozy, 1998; Das and Jena, 2000; Thanal, 2001; Jayakumar, 2002; Mission, 2006; Peter; Prabhakar; Pichamuthu, 2008a; 2008b).

However, in laboratory animals that received phorate there were no cases of intermediate syndrome or late polyneuropathy, what shows this pesticide is more toxic to humans than demonstrated in tests with laboratory animals, a prohibitive criterion for registration of pesticides in Brazil.

Besides its neurotoxic effects, phorate demonstrated potential to cause adverse effects to the endocrine regulation processes of steroid hormones in humans (Usmani, 2003), which may contribute to increased cancer cases (Alavanja, et al., 2002; Mahajan et al., 2006; Koutros et al., 2010).

Regarding human exposure, Usha and Harikrishnan (2004) reported several cases of acute poisoning in communities of Kerala, India. Among these, 5 cases (occurred between 1999 e 2002), were associated to exposure to phorate. According to the authors, in July 1999, about 12 people living in banana crop areas were severely poisoned by phorate. After the product use, it rained on the region, causing the product evaporate quickly and spread to nearby area, reaching the homes. Shortly after application of the product, the symptoms appeared and the affected required hospitalization. In June 2001, a 16-year-old boy died as a result of occupational exposure to phorate for a period of one week. That same year, 40 rural women workers in a tea plantation were intoxicated during harvesting. Symptoms appeared within 30 minutes after exposure, featured by light-headedness, dizziness, blurred vision, vomiting. Thirty-seven women had more severe and remained hospitalized for two days. The authors point out that in July 2002, 31 children from an upper primary school were poisoned by phorate applied in plantation nearby school. The children showed persistent headache, chest pain, breathing difficulty, nausea, giddiness, blurring of vision and stomach pain, and one of them showed uncontrolled muscle twitching and convulsions even after 24 hours of treatment.

On 21 July 2006, 20 residents of Salkiana village, district Jalandhar, India, had to be rushed to a hospital when neurotoxic symptoms of acute exposure to phorate were observed. The product was used in a nearby sugarcane field. The worst affected were the school children of an Elementary School. Teachers and students started complaining of a strange smell and breathlessness. Suddenly one student felt unconscious and then students started to faint. Within ten minutes, 16 students fainted after inhaling something that was toxic. In addition to difficulty breathing, the most frequent symptoms were poorly being, headache, eye irritation, dizziness, nausea, vomiting, lacrimation, salivation excessive, muscle cramps and pain. Six days after exposure to phorate, several patients still had symptoms such as eye irritation, dermal reactions and general uneasiness. (Mission, 2006).

Several studies show that agricultural workers exposed to phorate are victims of poisonings and deaths related to toxicity characteristics of the active ingredient. The exposure

becomes even more dangerous due to the difficulties related to the availability and/or inefficiency of PPE. Moreover, these various social issues (low education, low income) and biological (age and gender) are factors that increase the risk and severity of poisoning caused by this organophosphate.

Therefore, from the revaluation of the health effects of phorate, completed in 2015, ANVISA concluded this active ingredient of pesticides has the potential to cause hormonal disturbances in humans and is more toxic to humans than demonstrated in tests with laboratory animals, which are prohibitive criteria for registration of pesticides in Brazil.

Phorate was banned in Brazil on March 16, 2014, where it was no longer marketed since 2011.

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Expected effect of the final regulatory action

Eliminate the risks posed by phorate

2.4.2.2 Is the reason for the final regulatory action relevant to the environment?

☐ Yes

☒ No

If yes, give summary of the hazard or risk evaluation related to the environment

Expected effect of the final regulatory action

2.5 Other relevant information regarding the final regulatory action

2.5.1 Estimated quantity of the chemical produced, imported, exported and used

	Quantity per year (MT)	Year
produced	Formulated Product (Final Product): 153,9 t	2009
imported	Active Ingredient: 17,15 t	2009
exported	Active Ingredient: 35,96 t	2011
used	2009: Active Ingredient Sells: 26,49 t 2009: Formulated Product (Final Product) Sells: 272,58 t 2010: Formulated Product (Final Product) Sells: 6,72 t	

2011: Formulated Product (Final Product) Sells: 0,01 t 2012, 2013, 2014 and 2015: no production, import, export and sells.	

2.5.2 Indication, to the extent possible, of the likely relevance of the final regulatory action to other states and regions

Similar health and environmental problems are likely to be encountered in other countries where the substance is used.

2.5.3 Other relevant information that may cover:

2.5.3.1 Assessment of socio-economic effects of the final regulatory action

2.5.3.2 Information on alternatives and their relative risks, e.g. IPM, chemical and non-chemical alternatives

The alternatives to phorate applied in cotton crops in Brazil are: acephate, acetamiprid, benfuracarb, methidathion, esfenvarelate, imidacloprid, thiacloprid, permethrin, cypermethrin, azadirachtin, cyfluthrin, pymetrozine, methomyl, beta-cyfluthrin, flonicamid, chlorpyrifos, bifenthrin, deltamethrin, dimethoate, carbosulfan, clothianidin, zeta-cypermethrin, triazophos, fenthion, malathion, diafenthiuron, furathiocarb, thiodicarb, fenvarelate and fenitrothion. The alternatives to phorate applied in potato crops in Brazil are: acephate, acetamiprid, benfuracarb, esfenvarelate, imidacloprid, thiacloprid, alfa-cypermethrin, pymetrozine, methomyl, beta-cyfluthrin, chlorpyrifos, bifenthrin, deltamethrin, carbosulfan, beta-cypermethrin, piridafenthion, diafenthiuron, fipronyl, clorantraniliprole, cadusafos, tebupirinfos, lambda cyalotrine, gama-cyalotrine and chlorphenapir. The alternatives to phorate applied in coffee crops in Brazil are: esfenvarelate, imidacloprid, permethrin, cypermethrin, azadirachtin, cyfluthrin, beta-cyfluthrin, chlorpyrifos, zeta-cypermethrin, alfa-cypermethrin, beta-cypermethrin, novaluron, abamectin, clorantraniliprole, teflubenzuron, lufenuron, cyantraniliprole, pyriproxyfen, fenpropathrin, gamma-cyhalothrin, lambda-cyihalotrin and fluvarinate. The alternatives to phorate applied in bean crops in Brazil are: thiodicarb, imidacloprid, malathion, chlorpyriphes, esfenvarelate, acetate, acetamiprid, bifenthrin, beta-cyfluthrin, thiacloprid, phenopopation, clothanidine, carbosulfan, permethrin and etofenprox. The alternatives to phorate applied in corn crops in Brazil are: chlorpyriphes, fipronyl, bifenthrin and imidacloprid.

2.5.3.3 Basis for the final regulatory action if other than hazard or risk evaluation

2.5.3.4 Additional information related to the chemical or the final regulatory action, if any

SECTION 3 PROPERTIES

3.1 Information on hazard classification where the chemical is subject to classification requirements

International classification systems
e.g. WHO, IARC, etc.

WHO	Ia - Extremely hazardous

Other classification systems
e.g. EU, USEPA

USEPA	I - Highly toxic

3.2 Further information on the properties of the chemical

3.2.1 Description of physico-chemical properties of the chemical

Molecular Formula	**C**7H17O2PS3
Molecular Weight	260,38 *g moL*$^{-}$
Water solubility	50 mg/l @ 25 deg C
Melting point	-42,9ºC
Boiling point	125-127 deg C @ 2.0 mm Hg

Reference

PubChem- https://pubchem.ncbi.nlm.nih.gov/

3.2.2 Description of toxicological properties of the chemical

Summary of critical end-points for phorate

Absorption, distribution, excretion and metabolism in animals

Rate and extent of oral absorption: Rapid, approximately 90% within 24 h

Dermal absorption: Extensive based on acute toxicity

Distribution: Rapid and extensive

Potential for accumulation: None

Rate and extent of excretion: 89% within 24 h; urinary excretion predominated (77%); faecal excretion (12%).

Metabolism in animals - Major pathway: cleavage of phosphorus–sulfur bond, methylation of the liberated thiol group and oxidation of the resulting divalent moiety to the sulfoxide and sulfone.

Toxicologically significant compounds (plants, animals and the environment): Parent, phorate sulfoxide and phorate sulfone

Acute toxicity

Rat, LD50, oral 3.7 mg/kg bw in males, 1.4 mg/kg bw in females

Rat, LD50, dermal 9.3 mg/kg bw in males, 3.9 mg/kg bw in females

Rat, LC50, inhalation 0.06 mg/l of air in males (1 h), 0.011 mg/l of air (1 h) in females

Rabbit, dermal irritation Highly toxic by skin contact—could not be tested

Rabbit, ocular irritation Highly toxic by eye contact—could not be tested

Dermal sensitization Highly toxic by skin contact—could not be tested

Short-term studies of toxicity

Target/critical: effect Brain and erythrocyte acetylcholinesterase activity, and miosis (rats)

Lowest relevant oral NOAEL: 0.07 mg/kg bw per day

Lowest relevant dermal NOAEL: No data

Lowest relevant inhalation NOAEC: No data

Genotoxicity: Negative results in vivo and in vitro

Long-term studies of toxicity and carcinogenicity

Target/critical effect: Inhibition of erythrocyte and brain cholinesterase activity

Lowest relevant NOAEL: 0.07 mg/kg per day (rat)

Carcinogenicity: Not carcinogenic in mice and rats

Reproductive toxicity

Reproduction target/critical effect: Reduced pup growth at maternally toxic dose

Lowest relevant reproductive NOAEL: 2 ppm, equivalent to 0.17 mg/kg bw per day

Developmental target/critical effect: Decreased pup weights and delayed ossification at maternally toxic doses (rats)

Lowest relevant developmental NOAEL: 0.3 mg/kg bw per day (rats)

Neurotoxicity/delayed neurotoxicity

Single dose study of neurotoxicity

Target/critical effect: Signs consistent with acetylcholinesterase inhibition; no neuropathological effects

Relevant NOAEL: 0.25 mg/kg bw

Delayed neuropathy: No delayed neurotoxicity in hens

Medical data: Findings consistent with inhibition of acetylcholinesterase activity; no record of permanent sequelae

Reference

JMPR/FAO Report 2004

3.2.3 Description of ecotoxicological properties of the chemical

Ecological Effects:

Effects on birds: Phorate is very highly toxic to birds. The reported acute oral LD50 values are 12.8 mg/kg in chukar, 7.5 mg/kg in starlings, 0.6 to 2.5 mg/kg in mallards, 7 to 21 mg/kg in northern bobwhite quail, 1 mg/kg in red-winged blackbirds, and 7 mg/kg in ring-neck pheasants. The 5- to 8-day dietary LC50 values are reported as 370 to 580 ppm in Japanese quail, mallard, northern bobwhite quail, and ring-neck pheasant.

Effects on aquatic organisms: Phorate is very highly toxic to fish. Reported 96-hour LC50 values range from 2 to 13 ug/L in cutthroat trout, bluegill sunfish and largemouth bass. Other 96-hour LC50 values are 110 ug/L in northern pike and 280 ug/L in channel catfish. Reported 96-hour LC50 values for the compound in freshwater invertebrates such as stoneflies and scuds are 4 ug/L, also indicating very high toxicity. Other LC50 values are 0.006 ug/L for amphipods and 0.11 to 1.9 ug/L in other freshwater invertebrates. The acute oral LD50 of phorate is 85 mg/kg in bullfrogs.

Effects on other organisms: Phorate is toxic to bees, with a reported topical application LD50 of 10 ug per bee.

Environmental Fate:

Breakdown in soil and groundwater: Phorate is of moderate persistence in the soil environment, with reported field half-lives of 2 to 173 days. A representative value may be approximately 60 days. Actual residence times may be influenced by soil clay and organic matter content, rainfall, and soil pH. Soil treatments often leave more residues in plants than foliar treatments, because the compound persists in the soil and is readily taken up by plant roots. Phorate binds moderately well to most soils and is slightly soluble in water. It should therefore not be highly mobile in most soils, and should mainly be transported with runoff via sediment and water. Phorate has minimal potential to leach through the soil and contaminate groundwater. This is most likely where soils are sandy and aquifers are shallow. Field studies indicate that leaching is very low in soils high in clay and organic matter content, and lower in sandy soils.

Breakdown in water: The half-life of phorate in acidic water solutions is between a few days and a few weeks, depending on temperature; the half-life in alkaline (basic) water may be much shorter. Phorate is degraded by waterborne microorganisms and hydrolysis. As it breaks down in water,

nontoxic, water-soluble products are formed.

Breakdown in vegetation: Phorate itself is not persistent in plants, but plants metabolize phorate to very potent anticholinesterase agents such as the sulfoxide and sulfone derivatives of the compound. This activity will usually peak several days following application before decreasing. Phorate and its soil metabolites are absorbed from the soil by plant roots and are translocated to above-ground portions of the plant. Following treatment with a 10% granular formulation at 1 pound a.i./acre, phorate residues persisted at very low levels for 28 days in the kernels, cobs, or husks. After 83 days, there were no detectable residues of phorate or breakdown products.

Reference

http://extoxnet.orst.edu/pips/phorate.htm

SECTION 4 DESIGNATED NATIONAL AUTHORITY

Institution	Ministry of Environment
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Date, signature of DNA and official seal: _____

PLEASE RETURN THE COMPLETED FORM TO:

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00153 Rome, Italy

OR

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Definitions for the purposes of the Rotterdam Convention according to Article 2:

(a) 'Chemical' means a substance whether by itself or in a mixture or preparation and whether manufactured or obtained from nature, but does not include any living organism. It consists of the following categories: pesticide (including severely hazardous pesticide formulations) and industrial;

(b) 'Banned chemical' means a chemical all uses of which within one or more categories have been prohibited by final regulatory action, in order to protect human health or the environment. It includes a chemical that has been refused approval for first-time use or has been withdrawn by industry either from the domestic market or from further consideration in the domestic approval process and where there is clear evidence that such action has been taken in order to protect human health or the environment;

(c) 'Severely restricted chemical' means a chemical virtually all use of which within one or more categories has been prohibited by final regulatory action in order to protect human health or the environment, but for which certain specific uses remain allowed. It includes a chemical that has, for virtually all use, been refused for approval or been withdrawn by industry either from the domestic market or from further consideration in the domestic approval process, and where there is clear evidence that such action has been taken in order to protect human health or the environment;

(d) 'Final regulatory action' means an action taken by a Party, that does not require subsequent regulatory action by that Party, the purpose of which is to ban or severely restrict a chemical.

Focused Summary of the Notification of Final Regulatory Action for Phorate - Brazil

1. Introduction

1.1. The events that led to the final regulatory action

In February 2008, the National Health Surveillance Agency (ANVISA) published the Resolution RDC No. 10/2008 with a list of active ingredients that should be subjected to a toxicological reevaluation, listing the active ingredient phorate. In the motivation for the need for reevaluation of the active ingredient phorate, ANVISA justified that preliminary studies had demonstrated high acute toxicity and neurotoxicity of that pesticide. Added to that, the reevaluation was necessary due to the suspension of the use of the active ingredient triggered, in 2009, by the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA) which found, during an inspection in a company that holded the pesticide registration, a formulation with a different composition from that one approved/registered. Moreover, the existence of decisions in other countries restricting the use and banning products based on phorate was an important warning that led to the beginning of the reevaluation process.

In January 2012 ANVISA, in combination with experts from Oswaldo Cruz Foundation (FIOCRUZ), prepared a Technical Note on the Toxicological Reevaluation of the Active Ingredient Phorate (Annex 1) and submitted it to public consultation in order to receive comments and suggestions from the general public, along with a proposal to ban the active ingredient as a regulatory referral.

After the public consultation, the Technical Committee on Pesticide Reassessment, composed by representatives from IBAMA, Ministry of Agriculture, Livestock and Food Supply (MAPA) and ANVISA decided to cancel the monograph of the active ingredient phorate.

1.2. The significance of the regulatory action

As a result of the toxicological reevaluation, the Board of Directors of the National Health Surveillance Agency (ANVISA) published the resolution RDC No. 12 of 13 March 2015 (Annex 2), cancelling the registration of phorate. From this day on, no production, import, export, and trade of products based on the active ingredient phorate should be allowed.

1.3. An overview of the regulatory system

The legal reference for the pesticide management in Brazil is Law No. 7.802/89 (Pesticide Law), regulated by Decree No. 4.074/02. According to this law, no pesticide shall be manufactured, imported, exported, traded or used unless it has been registered in Brazil.

To obtain the registration approval, the pesticide shall be subjected to: (1) a toxicological evaluation conducted by the National Health Surveillance Agency; (2) a potential environmental hazards evaluation conducted by the Brazilian Institute of Environment and Renewable Natural Resources; and (3) an agronomic efficiency evaluation performed by the Ministry of Agriculture, Livestock and Food Supply (MAPA). After the evaluations carried out by the three agencies, MAPA gives final approval of label and leaflet and issues the Pesticide Registration Certificate.

The Pesticide Law also establishes the Reevaluation of Registered Pesticides. According to the Brazilian legislation, a pesticide should be banned when there are no methods to deactivate the product; in the absence of antidote or effective treatment; when it causes hormonal disruption or damages to the reproductive system; when they are teratogenic, carcinogenic or mutagenic. In addition, it should be banned when it is more dangerous for human health than for animals.

The reevaluation can be taken by initiative of one or more of the governmental agencies responsible for the pesticides registration (IBAMA, ANVISA or MAPA), in accordance with their respective competences, when there is evidence of reduction of agronomic efficiency and/or change of risks to human health or environment. The occurrence of these evidences can be verified by scientific researches or cases occurred in Brazil or elsewhere, which discourage the use of registered products; or when Brazil had been advised in this regard by international health, food or environmental organizations, of which Brazil is member or signatory of agreements.

A Technical Committee consisting of representatives from the three registrant agencies is formed to carry out the reevaluation, which can invite representatives of the agricultural industry and the scientific community to participate on the group. During the reevaluation process, the holders of the pesticide registration must submit all documents and technical studies that are requested by the registrant agencies.

In the pesticide reevaluation process, the reviewer agencies, in accordance with their respective competences, develop Technical Notes on the toxicology and/or potential environmental hazards of the active ingredient in addition to an economic analysis of pesticide substitutes, based on data collected from studies and surveys conducted by national and international accredited institutions as well as information provided by pesticide holder companies.

In addition, the poisonings reported in the National System of Toxic-Pharmacological Intoxications and Poisonings (SINITOX) can be used as reference for quantifying the incidents involving pesticides in the country, and be reported in the Technical Note on the toxicological reevaluation. The results of the Pesticide Residues in Food Analysis Programme can also be taken into account as exposure data.

The Technical Notes in the reevaluation process assess the potential exposures, the hazard, in accordance to parameters and methodologies adopted internationally, especially the World Health Organization (WHO); Food and Agriculture Organization (FAO); Organization for Economic Co-operation and Development (OECD); the USA Environmental Protection Agency and the European Union.

After the reevaluation, measures to restrict, suspend or prohibit the production and import of pesticides could be taken as well as cancel the registration, if a criterion of prohibition of registration is fulfilled.

1.4. The scope of the regulatory action

The resolution RDC No. 12 of 13 March 2015 from ANVISA prohibited the production, import, export, and trade of products based on the active ingredient phorate in Brazil.

The resolution also obliged that all companies which hold stocks of products based on the active ingredient phorate should provide, within the period of thirty days, its adequate final disposal along with previously informing MAPA, ANVISA and IBAMA about it.

2. Risk Evaluation

2.1. Key findings of the national risk evaluation

Brazilian's risk evaluation took into account toxicology and public health; occupational health and safety, environmental impact and availability of lower-risk alternatives.

The reevaluation identified that phorate has several adverse effects on human health as an association with diabetes mellitus in pregnancy, nephrotoxicity, reproductive toxicity, respiratory system toxicity and neurotoxicity. Phorate is classified as extremely toxic by ANVISA – Class 1 (Toxicologic Classification).

Phorate and its metabolites are easily absorbed through skin and mucous membranes and irreversibly block the catalytic activity of acetylcholinesterase (AChE), the enzyme responsible for mediating the hydrolysis of acetylcholine in acetic acid and choline acid. Thus, they interrupt the transmission of nerve impulses in the cholinergic synapses of the central nervous system (CNS), autonomic nervous system (ANS) and neuromuscular junction. Inactivation of AChE causes cholinergic hyperstimulation by acetylcholine accumulation in the synaptic cleft.

Phorate is considered one of the most toxic organophosphate AChE inhibitors, with mean oral LD50 for mice ranging from 1.4 to 10 mg/kg body weight.

Phorate can cause complex neurological clinical manifestations in humans, such as encephalopathy, intermediate syndrome and delayed polyneuropathy, described by various authors (Young, Jung; Ayer, 1979; Kashyap et al., 1984; WHO / FAO, 1988; Kusic et al., 1991; Dobozy, 1998; Das and Jena, 2000; Thanal, 2001; Jayakumar, 2002; Mission, 2006; Peter; Prabhakar; Pichamuthu, 2008a; 2008b).

Besides its neurotoxic effects, phorate demonstrated potential to cause adverse effects to the endocrine regulation processes of steroid hormones in humans (Usmani, 2003), which may contribute to increased cancer cases (Alavanja, et al., 2002; Mahajan et al., 2006; Koutros et al., 2010).

The reevaluation concluded that the active ingredient phorate has the potential to cause hormonal disruption in humans and is more toxic to humans than demonstrated in tests with laboratory animals, which are prohibitive criteria for registration of pesticides in Brazil. Finally, the Technical Note supported the ban of the phorate active ingredient given the provisions of Brazilian pesticides law.

The complete version of the ANVISA Technical Note on the Toxicological Reevaluation of the Active Ingredient Phorate, in Portuguese, is in Annex 1 and available at: http://portal.anvisa.gov.br/documents/33880/2540793/Conslta%2BP%25C3%25BAblica%2Bn%25C2%25B0%2B9%2BGGTOX%2Bcompleta.pdf/6c929012-e313-41d3-9d7d-4b429846da03

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The complete list of references can be found in the ANVISA Technical Note on the Toxicological Reevaluation of the Active Ingredient Phorate (Annex 1) available at: http://portal.anvisa.gov.br/documents/33880/2540793/Conslta%2BP%25C3%25BAblica%2Bn%25C2%25B0%2B9%2BGGTOX%2Bcompleta.pdf/6c929012-e313-41d3-9d7d-4b429846da03

2.3. Reference to national studies

The toxicological assessment of the active ingredient is described on the Technical Note on the Toxicological Reevaluation of the Active Ingredient Phorate (Annex 1) and was assessed based on the available reports from international agencies or institutes such as the US Environmental Protection Agency (EPA) and the International Program on Chemical Safety (IPCS), as well as the studies submitted to ANVISA in the toxicological dossier to support the registration of technical and formulated products.

The key studies submitted to ANVISA in the toxicological dossier are detailed in Annex 1 and are related to acute toxicity (17 studies); sub chronic toxicity (4 studies); chronic, carcinogenicity and genotoxicity toxicity (9 studies); endocrine system and reproductive toxicity (2 studies); and embryophetal development (5 studies).

2.4. A summary of actual or potential human exposure and/or environmental fate presented in the Technical Note of ANVISA

The Brazilian reevaluation identified that phorate has several adverse effects on human health as an association with diabetes mellitus in pregnancy, nephrotoxicity, reproductive toxicity, respiratory system toxicity and neurotoxicity.

The metabolites phoratoxon, phoratoxon sulfoxide and phoratoxon sulfone are 100 to 1000 times more potent as acetylcholinesterase inhibitors. Phorate demonstrated to be extremely toxic, causing lethality at low doses, for different exposure conditions.

Several studies show that agricultural workers exposed to phorate are victims of poisonings and deaths related to toxicity characteristics of the active ingredient. The exposure becomes even more dangerous due to the difficulties related to the availability and/or inefficiency of personal protective equipment (PPE).

Workers are one of the population groups most affected by pesticides, and much of this is due to productive contexts. A study carried out by Waichman (2008) in municipalities of the state of Amazonas (Manaus, Iranduba, Careiro da Várzea and Manacapuru) concluded that farmers were not prepared for the proper use of pesticides, ignoring the risks of these products to human health and the environment. In their opinion, personal protective equipment is not used because they are expensive, uncomfortable and unsuitable for the hot climate of the region. Lack of training and poor knowledge of the hazards of pesticides contribute to the incorrect handling during the preparation, application and disposal of empty containers. In these conditions the exposure of farmers, their families, consumers and the environment is high.

Moreover, the various social (low education, low income) and biological (age and gender) factors increase the risk and severity of poisoning caused by this organophosphate.

The experimental and epidemiological studies involving the respiratory tract demonstrate that phorate has high toxicity for this system.

Experimental studies, performed at similar doses to occupational human exposure, corroborate the biological plausibility of the findings. Emphysema, bronchopneumonia, inflammatory changes and respiratory distress were the main effects found, and some were shown to be irreversible for the observation period even after the exposure ceased. It is known that these effects may cause increased pulmonary vascular resistance, overwhelm the heart and even cause heart failure. Such effects can not only reduce the work efficiency, but also irreparably harm the quality of life of individuals exposed and lead to death.

The neurotoxicity of phorate has also been demonstrated in epidemiological studies. Neurotoxic manifestations such as vomiting, dizziness, abdominal pain, tachycardia, excessive salivation, miosis and hypotension were observed in cases of intentional intoxication, occupational and accidental exposure to phorate.

More severe symptoms such as convulsions, spasms, tremors, loss of muscle coordination, increased muscle tone of the limbs, respiratory distress, cerebral edema, loss of consciousness and deep coma have also been described. Findings in some patients were consistent with brain death, including absence of corneal, oculocephalic, pupillary and muscular reflexes, absence of reactions

to pain or heat stimuli, and absence of spontaneous respiration, with global suppression of cortical activity. Some intoxication cases have evolved to death.

Phorate can cause complex neurological manifestations such as encephalopathy, intermediate syndrome and delayed polyneuropathy in humans. However, late-onset polyneuropathy has not been described in laboratory animals, characterizing it as being more toxic to humans than animal tests have shown.

Cases of pesticide poisoning occurred in India caused by the exposure to phorate were also presented in the Technical Note.

The conclusions of the Technical Note were that “considering all the toxicological effects associated with the active ingredient phorate and their inclusion among the prohibitive characteristics of registration, especially that of "having characteristics more toxic to humans than animal tests have been able to demonstrate", the active ingredient phorate must have its prohibited use in Brazil, in order to protect the health of exposed workers, consumers and the general population.

3 Risk Reduction and relevance to other States

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

According to Brazilian law, the companies that import, export, produce and formulate pesticides, their components and related shall annually inform data on the quantities of pesticides that were imported, exported, produced, formulated and traded through the Electronic System of National Environmental Agency - IBAMA.

According to the report submitted by the companies, no production, import, export, and trade of products based on phorate had occurred between 2012 and 2015 in Brazil.

	Quantity per year (MT)	Year
Produced	Formulated Product (Final Product): 153,9 t	2009
Imported	Active Ingredient: 17,15 t	2009
Exported	Active Ingredient: 35,96 t	2011
Used	Active Ingredient Sells: 26,49 t	2009
	Formulated Product (Final Product) Sells: 272,58 t	2009
	Formulated Product (Final Product) Sells: 6,72 t	2010
	Formulated Product (Final Product) Sells: 0,01 t	2011
	No production, import, export and sells.	2012, 2013, 2014, 2015

Source: Ibama/2016

3.2. Relevance of the control action to other States

The restriction of use of phorate should be considered by all States because of the high risk associated with all uses and considering all the toxicological effects associated with the active ingredient, especially for "having characteristics more toxic to humans than laboratory animal tests have been able to demonstrate"; the potential for causing endocrine disruption and the absence of antidote or effective treatment in cases of late polyneuropathy.

Considering also the international regulatory trends and the WHO recommendation for banning extremely toxic products to reduce hazards to the exposed population and specially the existence of alternatives less toxic to the use of the active ingredient phorate, the following alternatives are considered to pose lower risks to human health and the environment and should be taking into account by all States:

- Alternatives to phorate applied in cotton crops in Brazil: acephate, acetamiprid, benfuracarb, methidathion, esfenvarelate, imidacloprid, thiacloprid, permethrin, cypermethrin, azadirachtin, cyfluthrin, pymetrozine, methomyl, beta-cyfluthrin, flonicamid, chlorpyrifos, bifenthrin, deltamethrin, dimethoate, carbosulfan, clothianidin, zeta-cypermethrin, triazophos, fenthion, malathion, diafenthiuron, furathiocarb, thiodicarb, fenvarelate and fenitrothion;
- Alternatives to phorate applied in potato crops in Brazil: acephate, acetamiprid, benfuracarb, esfenvarelate, imidacloprid, thiacloprid, alfa-cypermethrin, pymetrozine, methomyl, beta-cyfluthrin, chlorpyrifos, bifenthrin, deltamethrin, carbosulfan, beta-cypermethrin, piridafenthion, diafenthiuron, fipronyl, clorantraniliprole, cadusafos, tebupirinfos, lambda cyalotrine, gama-cyalotrine and chlorphenapir;
- Alternatives to phorate applied in coffee crops in Brazil: esfenvarelate, imidacloprid, permethrin, cypermethrin, azadirachtin, cyfluthrin, beta-cyfluthrin, chlorpyrifos, zeta-cypermethrin, alfa-cypermethrin, beta-cypermethrin, novaluron, abamectin, clorantraniliprole, teflubenzuron, lufenuron, cyantraniliprole, pyriproxyfen, fenpropathrin, gamma-cyhalothrin, lambda-cyihalotrin and fluvarinate;
- Alternatives to phorate applied in bean crops in Brazil: thiodicarb, imidacloprid, malathion, chlorpyriphes, esfenvarelate, acetate, acetamiprid, bifenthrin, beta-cyfluthrin, thiacloprid, phenopopation, clothanidine, carbosulfan, permethrin and etofenprox; and
- Alternatives to phorate applied in corn crops in Brazil: chlorpyriphes, fipronyl, bifenthrin and imidacloprid.

3.3. Comments on the typical use of the chemical the notifying country, with comments on possible misuse if appropriate.

According to the ANVISA's monograph, phorate was an insecticide authorized in Brazil exclusively for agricultural use, having typical and supported uses in soil in cotton, peanut, potato, coffee, beans, corn, tomato and wheat.

After the reevaluation, no production, import, export and sells of phorate was permitted in Brazil, in order to protect the health of exposed workers, consumers and the general population. This was considering all the toxicological effects associated with the active ingredient phorate and its inclusion among the prohibitive characteristics of registration, especially because of "having characteristics more toxic to humans than animal tests could demonstrate".

Epidemiological studies suggest that exposure to organophosphates is associated with psychiatric disorders, particularly depression and suicide. Exposure to these compounds may lead to the development of depression, an important factor in suicide (Steenland et al., 1994; Stephens et al., 1995; AMR et al, 1997; FIEDLER et al, 1997; LONDON et al, 1997; VAN WIJNGAARDEN, 2003; LONDON et al, 2005; JAGA; DHARMANI, 2007; BESELER et al, 2008).

Dear Elisabetta,

In this Toxicological Reevaluation of the active ingredient phorate made by Anvisa, no data on exposure and conditions of use of phorate in Brazil were used, but only general data, as reported in the document.

The 3 studies mentioned (Waichman, 2008; Garcia 2001 and Alves Filho 2002) address the use of pesticides in general in Brazil and not any particularly.

We have available only the Anvisa's Technical Note that was sent to you and, unfortunately, we do not have any additional data.

Finally, it's important to mention that the reevaluation of phorate was finalized when there was no more production, import, export, commercialization or use of products based on the active ingredient phorate in Brazil, so the reevaluation followed a more simplified rite.

We are still available for any questions or clarification you need.

Sincerely,

Mirian de Oliveira
Analista Ambiental

Gerência de Qualidade Ambiental
Departamento de Qualidade Ambiental e Gestão de Resíduos
Secretaria de Recursos Hídricos e Qualidade Ambiental
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BOARD OF DIRECTORS

RESOLUTION RDC No. 12, of 13 MARCH 2015

States about the technical regulation for the active ingredient phorate as the result of toxicological reevaluation

The Board of Directors of the National Health Surveillance Agency (ANVISA), within the use of its attributions given by the

subsections III and IV, article 15 of the Law No. 9.782, of 26 January 1999,

subsection V and paragraphs 1st and 3rd, article 5 of the Internal Statute approved in the terms of the Annex I of the ANVISA Ordinance No. 650, of 29 May 2014, published in the Federal Official Gazette of 2 June 2014, according to what is stated in

the subsection III, article 2, subsections III and IV, article 7 of the Law No. 9.782 from 1999,

and the Program for the Improvement of the Regulation Process of the Agency, instituted by the Ordinance No. 422, of 16 April 2008,

in the meeting held on March 5th, 2015 adopts the following Resolution of the Board of Directors and I, Director-President Substitute, determine its publication.

Article 1st Cancel the toxicological evaluation reports of all technical products and formulated products based on the active ingredient phorate, from the date of publication of this Resolution.

Article 2nd Exclude the monograph of the active ingredient phorate, from the date of publication of this Resolution.

Article 3rd Determine that companies which hold stocks of such products provide its adequate final disposal and that such procedure be previously informed to the Ministry of Agriculture, Livestock, and Food Supply (MAPA), to ANVISA and to the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA), within the period of thirty days from the date of publication of this Resolution.

Article 4th This Resolution enters into force in the date of its publication.

JAIME CESAR DE MOURA OLIVEIRA

Art. 4º Os medicamentos revalidados podem ser consultados, assim como suas apresentações validas no link:

http://www7.anvisa.gov.br/datavisa/Consulta_Produto/consulta_medicamento.asp

Art. 5º Esta Resolução entra em vigor na data de sua publicação.

JAIME CESAR DE MOURA OLIVEIRA

(*) Esta Resolução e o anexo a que se refere serão publicados em suplemento à presente edição.

RESOLUÇÃO - RE Nº 820, DE 13 DE MARÇO DE 2015(*)

O Diretor-Presidente Substituto da Agência Nacional de Vigilância Sanitária no uso das atribuições que lhe confere o Decreto de recondução de 9 maio de 2014, da Presidenta da República, publicado no DOU de 12 de maio de 2014 e a Portaria MS/GM n° 912, de 12 de maio de 2014, tendo em vista o disposto no inciso VII do art. 164 e no inciso I, § 1º do art. 6º do Regimento Interno da ANVISA, aprovado nos termos do anexo I da Portaria n.º 650, de 29 de maio de 2014, publicada no DOU de 02 de junho de 2014, resolve:

Art. 1º Indeferir petições relacionadas à Gerência-Geral de Medicamentos, conforme relação anexa;

Art. 2º Esta Resolução entra em vigor na data de sua publicação.

JAIME CESAR DE MOURA OLIVEIRA

(*) Esta Resolução e o anexo a que se refere serão publicados em suplemento à presente edição.

RESOLUÇÃO - RE Nº 821, DE 13 DE MARÇO DE 2015(*)

O Diretor-Presidente substituto da Agência Nacional de Vigilância Sanitária, no uso das atribuições que lhe conferem o Decreto de recondução de 9 de maio de 2014, publicado no DOU de 12 de maio de 2014, designado para substituir o Diretor-Presidente pela Portaria MS/GM nº 912, de 12 de maio de 2014 e o inciso IX do art. 13 do Regulamento da ANVISA, aprovado pelo Decreto nº 3.029, de 16 de abril de 1999, tendo em vista o disposto no inciso VII do art. 164 e no inciso I, § 1º do art. 6º do Regimento Interno da ANVISA, aprovado nos termos do anexo I da Portaria n.º 650, de 29 de maio de 2014, publicada no DOU de 02 de junho de 2014, resolve:

Art. 1º Indeferir petições relacionadas à Gerência-Geral de Produtos Biológicos, Sangue, Tecidos, Células e Órgãos, conforme relação anexa;

Art. 2º Esta Resolução entra em vigor na data de sua publicação.

JAIME CÉSAR DE MOURA OLIVEIRA

(*) Esta Resolução e o anexo a que se refere serão publicados em suplemento à presente edição.

DIRETORIA COLEGIADA

RESOLUÇÃO - RDC Nº 12, DE 13 DE MARÇO DE 2015

Dispõe sobre regulamento técnico para o ingrediente ativo Forato em decorrência da reavaliação toxicológica

A Diretoria Colegiada da Agência Nacional de Vigilância Sanitária, no uso da atribuição que lhe conferem os incisos III e IV, do art. 15, da Lei nº 9.782, de 26 de janeiro de 1999, inciso V e §§ 1º e 3º do art. 5º do Regimento Interno aprovado nos termos do Anexo I da Portaria nº 650 da ANVISA, de 29 de maio de 2014, publicada no DOU de 02 de junho de 2014, tendo em vista o disposto nos incisos III, do art. 2º, III e IV, do art. 7º da Lei nº 9.782, de 1999, e o Programa de Melhoria do Processo de Regulamentação da Agência, instituído por Portaria nº 422, de 16 de abril de 2008, em reunião realizada em 05 de março de 2015, adota a seguinte Resolução da Diretoria Colegiada e eu, Diretor-Presidente Substituto, determino a sua publicação.

Art. 1º Cancelar os informes de avaliação toxicológica de todos os produtos técnicos e formulados à base do ingrediente ativo forato, a partir da data de publicação desta Resolução.

Art. 2º Excluir a monografia do ingrediente ativo forato, a partir da data de publicação desta Resolução.

Art. 3º Determinar que as empresas que detenham estoques desses produtos providenciem a sua destinação adequada e que esse procedimento seja previamente informado ao MAPA, à ANVISA e ao IBAMA, no prazo de trinta dias a partir da publicação desta Resolução.

Art. 4º Esta Resolução entra em vigor na data da sua publicação.

JAIME CESAR DE MOURA OLIVEIRA

ARESTO Nº 64, DE 13 DE MARÇO DE 2015

Vistos, relatados e discutidos os presentes autos, em sessão realizada em 26 de fevereiro de 2015, ACORDAM os membros da Diretoria Colegiada da ANVISA, com fundamento no inciso VI, do art. 15 da Lei nº 9.782, de 26 de janeiro de 1999, e no art. 64 da Lei nº 9.784, de 29 de janeiro de 1999, aliado ao disposto no inciso VII e no §1º do art. 5º do Regimento Interno aprovado nos termos do Anexo I da Portaria nº 650 da ANVISA, de 29 de maio de 2014, publicada no D. O. U. de 02 de junho de 2014, e em conformidade com a Resolução RDC nº 25 de 04 de abril de 2008, decidir os recursos a seguir especificados, conforme relação anexa, em conformidade com a deliberação aprovada pela Diretoria Colegiada desta Agência.

JAIME CÉSAR DE MOURA OLIVEIRA
Diretor-Presidente
Substituto

ANEXO

Empresa: INTERFÓRMULA FARMÁCIA DE MANIPULAÇÃO LTDA.
CNPJ: 96.161.289/0001-66
Processo: 25000.026213/99-60
Expediente do Processo: 999070/35-7
Expediente do Recurso: 0511296/12-6
Decisão: A DIRETORIA COLEGIADA DECIDIU, POR UNANIMIDADE, DECLARAR A EXTINÇÃO DO RECURSO POR PERDA DE OBJETO NOS TERMOS DO VOTO DO RELATOR.

Empresa: BOIRON MEDICAMENTOS HOMEOPÁTICOS LTDA.
CNPJ: 07.498.711/0001-87
Processo: 25351.029869/2007-61
Expediente do Processo: 037897/07-6
Expedientes dos Recursos: 0606724/12-7 e 0606729/12-8
Decisão: A DIRETORIA COLEGIADA DECIDIU, POR UNANIMIDADE, DECLARAR A EXTINÇÃO DOS RECURSOS POR PERDA DE OBJETO NOS TERMOS DO VOTO DO RELATOR.

Empresa: ABÍLIO A. TOZANI-ME
CNPJ: 04.412.895/0001-22
Processo: 25351.381663/2012-59
Expediente do Processo: 0545519/12-7
Expediente do Recurso: 1035741/12-6
Parecer: 100/2014-COARE/SUINP
Decisão: POR UNANIMIDADE, CONHECER E DAR PROVIMENTO AO RECURSO, ACOMPANHANDO A POSIÇÃO DA RELATORIA QUE ACATA O PARECER DA ÁREA TÉCNICA.

Empresa: FARMA DIET DA PENHA FARMÁCIA DE MANIPULAÇÃO E COMÉRCIO LTDA.-ME
CNPJ: 03.985.687/0003-11
Processo: 25351.263102/2007-60
Expediente do Processo: 337303/07-7
Expediente do Recurso: 987826/11-2
Parecer: 051/2013-COARE/GGIMP
Decisão: POR UNANIMIDADE, CONHECER E NEGAR PROVIMENTO AO RECURSO, ACOMPANHANDO A POSIÇÃO DA RELATORIA QUE ACATA O PARECER DA ÁREA TÉCNICA.

Empresa: BALM-LABOR INDÚSTRIA FARMACÊUTICA LTDA.-ME
CNPJ: 04.712.572/0001-54
Processo: 25351.280196/2005-70
Expediente do Processo: 332262/05-9
Expediente do Recurso: 0006738/14-5
Parecer: 057/2014-COARE/SUINP
Decisão: POR UNANIMIDADE, CONHECER E NEGAR PROVIMENTO AO RECURSO, ACOMPANHANDO A POSIÇÃO DA RELATORIA QUE ACATA O PARECER DA ÁREA TÉCNICA.

Empresa: DIAGNOSTEK INDÚSTRIA E COMÉRCIO DE PRODUTOS CIENTÍFICOS LTDA.
CNPJ: 06.223.055/0001-47
Processo: 25351.080369/2007-13
Expediente do Processo: 102577/07-5
Expediente do Recurso: 1007320/12-5
Parecer: 086/2014-COARE/GGIMP
Decisão: POR UNANIMIDADE, CONHECER E DAR PROVIMENTO AO RECURSO, ACOMPANHANDO A POSIÇÃO DA RELATORIA QUE ACATA O PARECER DA ÁREA TÉCNICA.

Empresa: COLLECT IMPORTAÇÃO E COMÉRCIO LTDA.
CNPJ: 53.452.157/0001-14
Processo: 25351.675847/2013-66
Expediente do Processo: 0968022/13-5
Expediente do Recurso: 0525157/14-5
Decisão: A DIRETORIA COLEGIADA DECIDIU, POR UNANIMIDADE, PELA EXTINÇÃO DO RECURSO SEM JULGAMENTO DO MÉRITO EM RAZÃO DE DESISTÊNCIA SOLICITADA PELA REQUERENTE.

ARESTO Nº 65, DE 13 DE MARÇO DE 2015

Vistos, relatados e discutidos os presentes autos, ACORDAM os membros da Diretoria Colegiada da ANVISA, com fundamento no inciso VI, do art. 15 da Lei n.º 9.782, de 26 de janeiro de 1999, e no art. 64 da Lei n.º 9.784, de 29 de janeiro de 1999, aliado ao disposto no inciso VII e no § 1º do art. 5º do Regimento Interno aprovado nos termos do Anexo I da Portaria nº 650, de 29 de maio de 2014, publicada no DOU de 02 de junho de 2014, e em conformidade com a Resolução RDC n.º 25, de 4 de abril de 2008, decidir os recursos, a seguir especificados, conforme relação anexa, em conformidade com as deliberações aprovadas pela Diretoria Colegiada desta Agência em Reunião Ordinária Pública - ROP 004/2015 realizada em 26 de Fevereiro de 2015.

JAIME CESAR DE MOURA OLIVEIRA
Diretor-Presidente
Substituto

ANEXO

Empresa: Biomet 3I do Brasil Ltda.
CNPJ:02.913.684/0001-48
Processo nº.: 25351.061181/2012-63
Expediente da Reconsideração de Indeferimento n.º: 0604509/13-0
Decisão: por unanimidade, CONHECER e NEGAR PROVIMENTO ao recurso, acompanhando a posição da relatoria que acata o Parecer 78/2014- Corca/SUALI.

Empresa: Famara Brasil Indústria e Comércio Ltda.
CNPJ: 61.152.856/0001-77
Processo nº.: 25351.373488/2012-13
Expediente da Reconsideração de Indeferimento n.º: 0536782/13-4
Decisão: por unanimidade, CONHECER e NEGAR PROVIMENTO ao recurso, acompanhando a posição da relatoria que acata o Parecer 131/2014 - Corca/Suali.

Empresa: Signo Vinces Equipamentos Odontológicos Ltda.
CNPJ: 03.717.757/0001-99
Processo nº.: 25351.024587/2012-04
Expediente da Reconsideração de Indeferimento n.º: 0601873/13-4
Decisão: por unanimidade, CONHECER e NEGAR PROVIMENTO ao recurso, acompanhando a posição da relatoria que acata o Parecer 77/2014 - Corca/Suali.

Empresa: Medtronic Comercial Ltda.
CNPJ: 01.772.798/0001-52
Processo nº.: 25351.107445/2013-21
Expediente da Reconsideração de Indeferimento n.º: 0536545/13-7
Decisão: por unanimidade, CONHECER e NEGAR PROVIMENTO ao recurso, acompanhando a posição da relatoria que acata o Parecer 135/2014 - Corca/Suali.

ARESTO Nº 66, DE 13 DE MARÇO DE 2015

Vistos, relatados e discutidos os presentes autos, ACORDAM os membros da Diretoria Colegiada da ANVISA, com fundamento no inciso VI, do art. 15 da Lei n.º 9.782, de 26 de janeiro de 1999, e no art. 64 da Lei n.º 9.784, de 29 de janeiro de 1999, aliado ao disposto no inciso VII e no § 1º do art. 5º do Regimento Interno aprovado nos termos do Anexo I da Portaria nº 650, de 29 de maio de 2014, publicada no DOU de 2 de junho de 2014, e em conformidade com a Resolução RDC n.º 25, de 4 de abril de 2008, decidir os recursos, a seguir especificados, conforme relação anexa, em conformidade com as deliberações aprovadas pela Diretoria Colegiada desta Agência no Circuito Deliberativo - CD 091/2015, de 09/03/2015.

JAIME CESAR DE MOURA OLIVEIRA
Diretor-Presidente
Substituto

ANEXO

1) Empresa: Companhia Bandeirantes de Armazéns Gerais.
CNPJ: 58.128.174/0002-03
Número do Processo: 25759.142145/2007-20
Expediente do Processo: 180513074
Expediente do Recurso: 0156847147
Decisão: A Diretoria Colegiada decidiu, por unanimidade, ACATAR os pedidos de desistência da recorrente, acompanhando a posição da Corep/Supaf.

2) Empresa: Companhia Bandeirantes de Armazéns Gerais.
CNPJ: 58.128.174/0001-14
Número do Processo: 25759.142268/2007-61
Expediente do Processo: 180657072
Expediente do Recurso: 0179184142
Decisão: A Diretoria Colegiada decidiu, por unanimidade, ACATAR os pedidos de desistência da recorrente, acompanhando a posição da Corep/Supaf.

SUPERINTENDÊNCIA DE CORRELATOS E ALIMENTOS

RESOLUÇÃO - RE Nº 727, DE 10 DE MARÇO DE 2015(*)

O Superintendente de Correlatos e Alimentos, no uso de suas atribuições legais conferidas pela Portaria nº 1.666, de 10 de outubro de 2014, tendo em vista o disposto no art.59 e no inciso I, § 1º do art. 6º do Regimento Interno aprovado nos termos do Anexo I da Portaria nº 650, de 29 de maio de 2014, publicada no DOU de 2 de junho de 2014.

Considerando o art. 12 e o art. 33 e seguintes da Lei n.º 6.360, de 23 de setembro de 1976, bem como o inciso IX, do art. 7º da Lei n.º 9.782, de 26 de janeiro de 1999, resolve:

Art. 1º Deferir as petições dos produtos saneantes, conforme relação anexa.

Art. 2º Esta Resolução entra em vigor na data de sua publicação.

JOÃO TAVARES NETO

(*) Esta Resolução e o anexo a que se refere serão publicados em suplemento à presente edição.

RESOLUÇÃO - RE Nº 728, DE 10 DE MARÇO DE 2015(*)

O Superintendente de Correlatos e Alimentos, no uso de suas atribuições legais conferidas pela Portaria nº 1.666, de 10 de outubro de 2014, tendo em vista o disposto no art.59 e no inciso I, § 1º do art.

Agência Nacional de Vigilância Sanitária

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Consulta Pública nº 9, de 19 de janeiro de 2012
D.O.U de 23/01/2012

A Diretoria Colegiada da Agência Nacional de Vigilância Sanitária, no uso da atribuição que lhe confere o inciso IV do art. 11 do Regulamento da ANVISA, aprovado pelo Decreto no. 3.029, de 16 de abril de 1999, c/c o inciso II, e §§ 1º e 3º do art. 54 do Regimento Interno aprovado pela Portaria no. 354 da ANVISA, de 11 de agosto de 2006, republicada em 21 de agosto de 2006, em reunião realizada em 17 de janeiro de 2012,

adota a seguinte Consulta Pública e eu, Diretor-Presidente, determino a sua publicação:

Art. 1º Fica aberto, a contar da data de publicação desta Consulta Pública, o prazo de 60 (sessenta) dias para que sejam apresentadas críticas e sugestões relativas à proposta de Regulamento Técnico, para o ingrediente ativo Forato, contido na Relação de Monografias dos Ingredientes Ativos de Agrotóxicos, Domissanitários e Preservantes de Madeira.

Art. 2º Informar que a proposta Regulamento Técnico, bem como a Nota Técnica do Ingrediente Ativo Forato estará disponível, na íntegra, durante o período de consulta no endereço eletrônico www.anvisa.gov.br e que as sugestões deverão ser encaminhadas por escrito para o seguinte endereço: Agência Nacional de Vigilância Sanitária, SIA, Trecho 5, Area Especial 57, Lote 200, Brasília, DF, CEP 71.205.050 ou Fax: (061)3462-5726 ou E-mail: toxicologia@anvisa.gov.br.

Art. 3º Findo o prazo estipulado no art. 1º a Agência Nacional de Vigilância Sanitária articular-se-á com os Órgãos e Entidades envolvidos na reavaliação toxicológica de acordo com a RDC 48, de 07 de julho de 2008, visando à consolidação do texto final.

DIRCEU BRÁS APARECIDO BARBANO

ANEXO DE PROPOSTA DE REGULAMENTO TÉCNICO

RESOLUÇÃO RDC N.º , DE DE DE 2012

Proposta de regulamento técnico para o ingrediente ativo Forato em decorrência da reavaliação toxicológica

A Diretoria Colegiada da Agência Nacional de Vigilância Sanitária, no uso da atribuição que lhe confere o inciso IV do art. 11 do Regulamento aprovado pelo Decreto nº 3.029, de 16 de abril de 1999, e tendo em vista o disposto no inciso II e nos §§ 1º e 3º do art. 54 do Regimento Interno aprovado nos termos do Anexo I da Portaria nº 354 da ANVISA, de 11 de agosto de 2006, republicada no DOU de 21 de agosto de 2006, em reunião realizada em.....de ..... de 20.., e

considerando o disposto na Constituição Federal, de 05 de outubro de 1988, em seu artigo 5º, XXXIII e LX, relativos ao direito à informação e publicidade dos atos da administração pública;

considerando o disposto na Constituição Federal, de 05 de outubro de 1988, em seu artigo 200, incisos I, II e VII;

considerando o disposto na Lei nº. 8.080, de 19 de setembro de 1990, em seu art. 6º, incisos I e alíneas, VII, IX e § 1º e incisos;

considerando o disposto na Lei nº. 9.782, de 26 de janeiro de 1999, em seu artigo 8º e parágrafos, que determina a regulamentação, o controle e a fiscalização dos produtos que envolvam risco à saúde pública;

considerando o disposto na Lei n° 9.784, de 29 de janeiro de 1999; que regula o processo administrativo no âmbito da Administração Pública Federal;

considerando a Lei nº 10.603, de 17 de dezembro de 2002, que dispõe sobre a informação não divulgada submetida para aprovação da comercialização de produtos;

considerando o disposto na Lei nº. 7.802, de 11 de julho de 1989, art. 3º, § 6º, alíneas, combinado com disposto no Decreto nº 4.074, de 04 de janeiro de 2002, artigos 2º, inciso VI; art. 6º, inciso I; art. 19, parágrafo e incisos e art. 31, incisos e

considerando o disposto na Instrução Normativa Conjunta nº. 02, de 27 de setembro de 2006, que estabelece procedimentos para fins de reavaliação agronômica ou toxicológica ou ambiental dos agrotóxicos, seus componentes e afins;

considerando a RDC nº 10, de 22 de fevereiro de 2008, estabelecendo a reavaliação toxicológica de produtos técnicos e formulados à base do ingrediente ativo Forato;

considerando a RDC nº 48, de 07 de julho de 2008, estabelecendo os procedimentos administrativos para a reavaliação toxicológica;

considerando o impacto dos agrotóxicos de forma difusa e coletiva e a importância da ampla participação da sociedade através do instrumento de consulta pública;

considerando que o ingrediente ativo forato é extremamente tóxico, é neurotóxico e é mais tóxico para humanos do que para animais de laboratório;

considerando que o ingrediente ativo forato se enquadra dentre os agrotóxicos com características proibitivas de registro;

considerando a recomendação da Organização Mundial de Saúde - OMS para proibição de produtos extremamente tóxicos, com vista a redução de perigos à população exposta à este produto;

considerando que o forato no cenário internacional, tem sido alvo de severas restrições em diversos países devido aos riscos para a saúde humana;

adota a seguinte Resolução e eu, Diretor-Presidente, determino a sua publicação:

Art. 1º Cancelar os informes de avaliação toxicológica de todos os produtos técnicos e formulados à base do ingrediente ativo forato a partir da data de publicação desta Resolução.

Art. 2º Manter a monografia do ingrediente ativo forato até a data de 30 de junho de 2012 para fins de programas de monitoramento de resíduos de agrotóxicos nos alimentos.

Art. 3º Indeferir os pleitos de avaliação toxicológica, em tramitação nesta Agência, de produtos técnicos e formulados à base de forato, com vistas à obtenção de registro de produtos, devido ao enquadramento do ingrediente ativo dentre as proibições de registro do art. 3º, § 6º, alínea “e", da Lei 7.802, de 11 de julho de 1989.

Art.4º Solicitar ao Ministério da Agricultura, Pecuária e do Abastecimento que suspenda as importações de produtos técnicos e formulados à base de forato a partir da publicação desta Resolução.

Art. 5º Esta Resolução entra em vigor na data da sua publicação.

DIRCEU BRÁS APARECIDO BARBANO
Diretor-Presidente

ANVISA	**Agência Nacional de Vigilância Sanitária** **Gerência Geral de Toxicologia**	Ministério da Saúde **FIOCRUZ** **Fundação Oswaldo Cruz**

NOTA TÉCNICA
REAVALIAÇÃO TOXICOLÓGICA DO INGREDIENTE ATIVO FORATO

1. Apresentação e motivações para reavaliação

A Nota Técnica visa cumprir uma das etapas da reavaliação toxicológica, prevista na RDC nº 10/2008 e detalhada na RDC n° 48/2008. Esta nota foi elaborada pelos especialistas da Fundação Oswaldo Cruz – FIOCRUZ.

A partir da data de sua publicação, a mesma ficará em Consulta Pública por 60 dias conforme disposto na RDC nº 48/2008, e as contribuições, após consolidadas, serão analisadas conjuntamente pela comissão de reavaliação integrada pela ANVISA (Agência Nacional de Vigilância Sanitária), IBAMA (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis) e MAPA (Ministério da Agricultura, Pecuária e Abastecimento).

O controle da produção, da comercialização e do emprego de técnicas, métodos e substâncias que comportem risco para a vida, para a qualidade de vida e para o meio ambiente, são incumbências do Poder Público, atribuídas pelo artigo 225 da Constituição Federal, e regulamentado no caso específico dos agrotóxicos, pela Lei n° 7.802, de 11 de julho de 1989.

De acordo com a Lei n° 7.802/89, os agrotóxicos, como o nome aduz, são substâncias que comportam risco à vida e à saúde, tanto para os trabalhadores expostos a essas substâncias, quanto para os consumidores de culturas tratadas e para a população em geral. Estes produtos necessitam de uma detalhada avaliação para obtenção de registro, a qual é procedida pelos Ministérios da Agricultura, da Saúde e do Meio Ambiente, cada um em suas respectivas áreas de atuação.

O legislador reconheceu a possibilidade de efeitos danosos dos agrotóxicos ao estabelecer, no § 6º do art. 3° da Lei nº 7.802/89, as proibições de registro. Dessa forma os agrotóxicos, para a obtenção do registro, são avaliados quanto aos impactos à saúde humana e ao meio ambiente e com relação à eficácia agronômica.

A ANVISA é o órgão responsável no âmbito do Ministério da Saúde pela avaliação da toxicidade dos agrotóxicos e seus impactos à saúde humana; emite o parecer toxicológico favorável ou desfavorável à concessão do registro pelo Ministério da Agricultura. Os estudos exigidos para efetuar a avaliação toxicológica dos agrotóxicos seguem parâmetros e metodologias adotadas internacionalmente, em particular pela ONU/OMS – Organização Mundial de Saúde, OECD – Organization for Economic Co-operation and Development, USA/EPA – Environmental Protection Agency e ONU/FAO – Food and Agriculture Organization. A avaliação toxicológica

leva também em conta as condições brasileiras de uso e consumo de culturas tratadas com agrotóxicos e o impacto desses produtos na saúde humana de trabalhadores e consumidores.

No Brasil, uma vez concedido o registro de determinado agrotóxico, este possui validade *ad eternum,* sem previsão de qualquer prazo para renovação ou revalidação do mesmo, até que os órgãos reguladores decidam reavalia-lo. O conhecimento técnico científico sobre os ingredientes ativos e especialmente sobre o surgimento de perigos e riscos associados ao uso é dinâmico e pode apresentar novas evidências impondo a reavaliação toxicológica e de efeitos sobre a saúde e ao ambiente. A Lei n° 7.802/89 e o Decreto n° 4.074/02 amparam este procedimento.

Em relação aos aspectos toxicológicos, a reavaliação de agrotóxicos ocorre quando há alguma indicação de perigo ou risco à saúde humana, em comparação a avaliação feita para a concessão de registro. As novas evidências podem ser apresentadas mediante novos estudos e pelo avanço dos conhecimentos científicos. Alertas em função de observações epidemiológicas, clínicas ou por eventuais acidentes podem servir como evidências, mesmo quando os estudos experimentais conduzidos em animais de laboratório não são suficientes para concluir sobre a nocividade do produto técnico em humanos.

A ANVISA, diante de alertas de efeitos adversos do forato, que se configuram dentre os proibitivos de registro, publicou a reavaliação deste agrotóxico cuja análise é o objeto da presente nota.

2. Introdução

O forato, assim como diversos outros compostos inseticidas (por ex.: parationa etílica e metílica, fosmete, metamidofós, triclorfom, malationa, clorpirifós, acefato), pertence ao grupo químico dos organofosforados (OP), que são inibidores irreversíveis da acetilcolinesterase (AChE) e provocam efeitos tóxicos sobre os diferentes sistemas dos seres vivos expostos (EDWARDS; TCHOUNWOU, 2005).

Os primeiros compostos organofosforados foram preparados por alquimistas na Idade Média, mas seu estudo sistemático teve início no século XIX, por Lassaigne em 1820, com a esterificação do ácido fosfórico. Vinte e cinco anos mais tarde, uma série de derivados de fosfinas foi preparada por Thinard e colaboradores e a partir destes

trabalhos o progresso da investigação dos compostos de fósforo foi acelerado (SANTOS, 2007).

A partir da segunda metade do século XIX, seu desenvolvimento foi dominado por pesquisadores britânicos e alemães (TOY, 1976; STODDART, 1979). A descoberta das propriedades tóxicas e inseticidas de alguns compostos de fósforo por Schrader e colaboradores, em 1930, criou novos compostos organofosforados nas indústrias (STODDART, 1979).

Observou-se durante a I Guerra Mundial que indivíduos asfixiados com o gás mostarda, bis (2- cloroetil) sulfeto tinham como conseqüências danos na medula óssea e no tecido linfocitário. Estudos em animais durante a II Guerra Mundial demonstraram que a exposição à mostarda nitrogenada, análoga ao composto bis (2-cloroetil) amino, a mecloretamina, destrói os tecidos linfócitos (TEICHER; SOTOMAYOR, 1994).

A qualidade inseticida dos organofosforados foi primeiramente observada na Alemanha durante a II Guerra Mundial em um estudo de gases (*Sarin*, *Soman* e *Tabun*), extremamente tóxicos para o sistema nervoso (ROSATI et al, 1995).

Os compostos organofosforados – OP - foram introduzidos como biocidas na década de 1970, inicialmente apresentados como substitutivos dos organoclorados por serem menos persistentes no ambiente, porém com alta toxicidade (WOODWELL et al, 1967; PEAKALL et al, 1975; MURPHY, 1988). Foi também a partir dessa época que aumentou de forma drástica o número de casos de intoxicação por OP, mesmo em baixas doses (ARAUJO et al, 2007).

Os OP são ésteres fosfóricos compostos por um átomo de fósforo pentavalente, derivado do ácido fosfórico, do ácido tiofosfórico ou do ácido ditiofosfórico (BRASIL, 1997). Sua estrutura química está representada na figura 1.

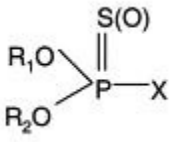


Figura 1: Estrutura química geral dos organofosforados (OP)

Cocker et al (2002) estudaram a importância das características estruturais dos compostos organofosforados e mostraram que estão relacionadas com suas diferentes atividades tóxicas, tais como o tipo de heteroátomo ou grupo funcional ligado ao átomo de fósforo e seu estado de oxidação. Assim, na estrutura geral dos OP a parte ‘X’ da

molécula (ver figura 1) possibilita a sua diferenciação em produtos específicos. Os inseticidas OP são usados frequentemente na forma "tio" (P=S) que por dessulfuração metabólica oxidativa produz a forma P=O.

Foi comprovado que a toxicidade elevada para a espécie humana de diversos organofosforados está relacionada às ligações P=O presentes em sua estrutura molecular ou em seus metabólitos. Esta ligação possibilita maior transferência de elétrons do fósforo para o oxigênio, resultando em cargas mais intensas nos dois elementos e, como conseqüência, interações mais fortes entre o organofosforado com o centro esterásico da enzima acetilcolinesterase (COCKER et al, 2002).

2.1 Identidade química do forato

O forato apresenta as seguintes características:

Nome comum: Forato

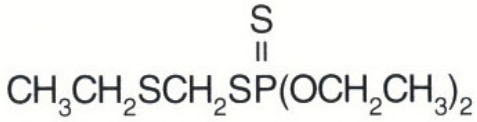
Sinonímia: EI 3911; CL 35,024; AC 35024

Nome químico: O,O-diethyl S-ethylthiomethyl phosphorodithioate

Número de registro no *CAS* (*Chemical Abstracts Service*): 298-02-2

Fórmula empírica: $C_7H_{17}O_2PS_3$

Fórmula estrutural:



Grupo Químico: Organofosforado

Classe Agronômica: Inseticida, acaricida e nematicida

Classificação toxicológica: I – Extremamente tóxico

Fonte: ANVISA, 2010

2.2 Produção e uso

A utilização dos agrotóxicos no Brasil tem trazido sérias conseqüências, tanto para o meio ambiente como para a saúde do trabalhador rural. Essas conseqüências são, na maioria das vezes, condicionadas por fatores como alta toxicidade dos produtos, uso inadequado e falta de utilização de equipamentos de proteção coletiva e individual. Esta situação é agravada pelas precárias condições socioeconômicas e culturais da grande

maioria dos trabalhadores rurais, o que amplia sua vulnerabilidade frente à toxicidade dos agrotóxicos (SILVA et al, 1999; SOBREIRA; ADISSI, 2003).

O Brasil está entre os países com maior consumo de agrotóxicos no mundo, com um mercado no ano de 2010 de US$7,3 bilhões de comercialiazação de agrotóxicos e 790 mil toneladas de produtos. É o maior consumidor da América Latina, com consumo estimado em 84% da quantidade comercializada nesta região.

De acordo com a EPA mais de um milhão de toneladas de forato foram utilizadas nos EUA no ano de 1999 (BANO; Musarrat, 2003).

O forato, segundo a monografia da ANVISA (ANVISA, 2009) pode ser aplicado no solo nas culturas de algodão, amendoim, batata, café, feijão, milho, tomate, trigo (Tabela 1). A Ingestão Diária Aceitável (IDA) do forato está estabelecida atualmente em 0,0005 mg/kg de peso corpóreo e os Limites Máximos de Resíduos (LMR) expressos como forato conforme constam a seguir.

Tabela 1: Limites Máximos de Resíduos - LMR e respectivos intervalos de segurança para o forato, por cultura agrícola

Cultura	**LMR (mg/kg)***	**Intervalo de Segurança (dias)**
Algodão	0,05	(1)
Amendoim	0,05	(1)
Batata	0,05	(1)
Café	0,05	90
Feijão	0,1	(1)
Milho	0,05	(1)
Tomate	0,1	(1)
Trigo	0,05	(1)

(1): Intervalo de segurança não determinado devido à modalidade de emprego.

*: Os LMRs referem-se à soma de forato, seu análogo oxigenado, e seus sulfóxidos e sulfonas, expressos como forato.

Fonte: BRASIL, 2009

2.3 Relevância para a saúde pública

A partir do uso disseminado dos organofosforados, vários efeitos adversos foram descritos em populações humanas e em outras espécies animais (GALLOWAY;

HANDY, 2003). Dentre os efeitos tóxicos associados aos organofosforados encontram-se a neurotoxicidade, a imunotoxicidade, a carcinogenicidade, a desregulação endócrina e alterações no desenvolvimento do indivíduo.

Algumas condições como idade, gênero, via e dose de exposição contribuem para uma maior suscetibilidade individual, de maneira que crianças, idosos e mulheres em idade fértil constituem grupos populacionais de especial risco aos agrotóxicos (OLIVEIRA, 2004).

Regiões onde não existe infra-estrutura suficiente para regular e controlar eficazmente o uso de agrotóxicos, como a América Latina, África e Ásia, problemas decorrentes do uso de agrotóxicos na agricultura são ainda mais graves (NUNES; RIBEIRO, 1999).

Garcia (2001) encontrou uma relação direta entre as curvas de crescimento de registro de intoxicações e as vendas de agrotóxicos. Alves Filho (2002) corrobora estes dados de relação entre a quantidade de agrotóxicos utilizada com os valores das vendas dos produtos e os índices de intoxicação.

Em relação ao contexto de vulnerabilidades quanto à exposição, há grande subnotificação de intoxicações por agrotóxicos no Brasil. Estima-se que para cada caso registrado de intoxicação por agrotóxico ocorrem outros 50 sem notificação, ou com notificação errônea (OPAS, 1996; SOBREIRA; ADISSI, 2003). Segundo estimativas da Organização Mundial da Saúde, 70% das intoxicações por agrotóxicos ocorridas no mundo são devidas a exposições ocupacionais (OLIVEIRA-SILVA, 2001). Segundo dados do IBGE (2004), das 84.596.294 pessoas com mais de 10 anos ocupadas no Brasil, 17.733.835 (cerca de 20%) tinham o trabalho agrícola como principal ramo de atividade, revelando o grande potencial de exposição a substâncias tóxicas na população brasileira do campo.

Com relação aos óbitos registrados no SINITOX - Sistema Nacional de Informações Tóxico-Farmacológicas, do Ministério da Saúde e da ANVISA, (disponibilizado pela FIOCRUZ desde 1996 e uma das fontes de informação sobre notificação de casos de intoxicações por agentes químicos) os três principais agentes químicos responsáveis por intoxicações são agrotóxicos de uso agrícola, raticidas e medicamentos. O percentual de letalidade por agrotóxicos, no período de 1997 a 2001 foi em torno de 3% (SINITOX, 2003).

Com relação aos casos de intoxicação ocupacional por agrotóxicos, o percentual de intoxicações foi bem maior, em média 28% do total de casos nos anos apresentados, revelando a enorme vulnerabilidade dos trabalhadores (Tabela 2) (SINITOX, 2007).

Tabela 2: Distribuição do número de casos de intoxicações por agrotóxicos de uso agrícola no período de 1997-2007, no Brasil, segundo dados do SINITOX (Série 1997-2009)

Ano	Casos de intoxicação humana por agrotóxicos	Casos em circunstâncias ocupacionais
2009	5,204	1.158
2007	6.260	1.514
2006	6.757	1.926
2005	6.870	1.745
2004	6.034	1.763
2003	5.945	1.748
2002	5.591	1.788
2001	5.384	1.378
2000	5.127	1.378
1999	4.674	1.499
1998	5.268	1.663
1997	5.474	1.457

Fonte: Série SINITOX, 1997 -2009 (http://www.fiocruz.br/sinitox).

Os trabalhadores são um dos grupos populacionais mais afetados pelos agrotóxicos, e muito disso se deve aos contextos produtivos. Um estudo realizado por Waichman (2008) em municípios do Estado do Amazonas (Manaus, Iranduba, Careiro da Várzea e Manacapuru) verificou que os agricultores vêm usando intensivamente os agrotóxicos na produção de hortaliças. O estudo concluiu que os agricultores não estavam preparados para o uso adequado desta tecnologia, ignorando os riscos dos agrotóxicos para saúde humana e para o ambiente. Não são utilizados equipamentos de proteção individual porque estes são caros, desconfortáveis e inadequados para o clima quente da região. A falta de treinamento e o escasso conhecimento sobre os perigos dos agrotóxicos contribuem para a manipulação incorreta durante a preparação, aplicação e disposição das embalagens vazias. Nestas condições é alta a exposição dos agricultores, suas famílias, consumidores e o ambiente. A tabela 3 relaciona alguns dos principais problemas existentes no Brasil em relação à exposição a agrotóxicos.

Um estudo realizado em seis propriedades produtoras de tomate em Camocim de São Félix – PE revelou que 13,2 % (n=159) dos trabalhadores entrevistados informavam ter sofrido algum tipo de intoxicação. Desses, 45 referiram mal-estar durante a aplicação de produtos, 70% das mulheres citaram problemas na gestação acarretando perda do

feto e ainda 39,4% fizeram referência à perda de um filho no primeiro ano e vida (ARAUJO; NOGUEIRA; AUGUSTO, 2000).

Em Minas Gerais, entre 1991 e 2001, um estudo realizado por Soares et al (2003) apontou o alto grau de risco de agravos à saúde a que estão sujeitos trabalhadores rurais em contato com agrotóxicos, encontrando 50% dos entrevistados (n=1064) moderadamente intoxicados.

Oliveira-Silva (2001), em estudo realizado em Nova Friburgo – RJ, identificou que 10% dos trabalhadores investigados apresentavam sinais e sintomas de intoxicação. Esse mesmo autor estimou que o número esperado de intoxicações agudas por agrotóxicos entre trabalhadores agrícolas brasileiros seria de 360.000 casos a cada ano somente no meio rural.

A exposição aos organofosforados ocorre tanto em áreas rurais quanto em zonas urbanas, o que coloca a população geral expostas aos danos causados por essas substâncias. Exemplo de exposição urbana é dado por um estudo de coorte retrospectivo que apontou o uso de organofosforados em orquidário na área urbana de Petrópolis (RJ) como responsável pela intoxicação de pelo menos 16 moradores de locais próximos ao orquidário. Esse mesmo estudo aponta que pessoas que ficaram mais tempo expostas às substâncias, por passarem mais tempo em casa, tiveram mais chance de se intoxicar (OLIVEIRA; GOMES, 1990).

No meio urbano do Estado do Rio de Janeiro foram registrados 12,6% de casos fatais de intoxicações pelo Instituto Médico Legal – IML entre os anos de 2000-2001, com evidências científicas de associação com agrotóxicos (OLIVEIRA-SILVA, 2003).

No Rio Grande do Sul, um estudo de base populacional, descreveu o perfil sócio-demográfico e a prevalência de algumas morbidades. Entre os resultados obtidos destaca-se que 75% dos trabalhadores utilizavam agrotóxicos, a maioria organofosforados (FARIA et al, 2000). A utilização caracterizou-se como intensa durante sete meses do ano (em 85% dos estabelecimentos); o tipo de agrotóxico utilizado variou conforme a cultura e 12% dos trabalhadores que utilizavam estes produtos referiram intoxicação pelo menos uma vez na vida e a prevalência de transtornos psiquiátricos foi de 36%. Nas propriedades maiores (25 a 100 ha) e onde se utilizavam mais agrotóxicos, observou-se um aumento do risco para intoxicações. Nesse mesmo Estado, um estudo transversal sobre saúde mental de agricultores da Serra Gaúcha mostrou uma forte associação entre intoxicações por agrotóxicos e o desenvolvimento de transtornos psiquiátricos menores (FARIA et al, 1999).

Pires, Caldas e Recena (2005) estudaram no Mato Grossso do Sul, no período de 1992 a 2002, as intoxicações provocadas por agrotóxicos na microrregião de Dourados. Foi observada correlação entre a prevalência de intoxicações e de tentativas de suicídio pela exposição a agrotóxicos, principalmente nas culturas de algodão e feijão. Os municípios de Dourados, Fátima do Sul e Vicentina se apresentaram como mais críticos na microrregião de Dourados. Os inseticidas foram a principal classe de agrotóxicos envolvidos nas ocorrências, principalmente organofosforados e carbamatos, corroborando outros estudos (SENANAYAKE; PEIRES, 1995; SAADEH et al, 1996; SOTH; HOSOKAWA, 2000; SOARES; ALMEIDA; MORO, 2003).

A utilização de um agrotóxico deve obedecer às indicações e recomendações estabelecidas pela monografia da ANVISA, conforme sua classe toxicológica.

O forato é considerado um dos mais tóxicos organofosforados inibidores da acetilcolinesterase, com média de DL_{50} variando entre 2 e 4 mg/kg de peso corpóreo (Hazardous Substances Data Bank, 1988). Outro agravante para a saúde humana é que o forato e seus metabólitos ou derivados são facilmente absorvidos através do contato com a pele e mucosas.

Devido à sua comparativa alta solubilidade em água (50mg/L), o forato possui grande potencial de contaminação de águas superficiais destinadas ao consumo humano (RANI et al, 2009), sendo a concentração máxima aceitável de forato em água de consumo humano 0,002 mg/L (CANADA, 1990).

O forato teve seu uso restrito pela EPA em razão de sua alta toxicidade dérmica, oral e inalatória e pelos efeitos sobre os ecossistemas, especialmente em relação aos pássaros e animais aquáticos. Entre diversas restrições, foi proibida a aplicação aérea, bem como o seu uso no inverno e também em áreas com a presença de mananciais destinados ao abastecimento humano. As formulações podem conter no máximo 5% de forato. (ENVIRONMENTAL PROTECTION AGENCY, 2006). Em relação à exposição ocupacional o forato encontra-se como o mais importante determinante de acidentes de trabalho com agrotóxicos nos Estados Unidos (ENVIRONMENTAL PROTECTION AGENCY, 2006).

3. Toxicocinética

3.1. Vias de exposição e absorção

O forato é um organofosforado lipossolúvel (PETER, PRABHAKAR; PICHAMUTHU, 2008b) rapidamente absorvido por mamíferos através das vias oral, inalatória e dérmica, apresentando alta toxicidade através dessas vias de exposição (BOSHOFF; PRETORIUS, 1979; WORLD HEALTH ORGANIZATION/FOOD AND AGRICULTURE ORGANIZATION, 1988; CANADÁ, 1990; EXTENSION TOXICOLOGY NETWORK, 1996; BHARGAVA; KULDEEP; SARASWAT, 1998; HEALTH COUNCIL OF THE NETHERLANDS, 2003; JOINT/FAO/WHO MEETING ON PESTICIDE RESIDUES, 2006; UNITED NATIONS ENVIRONMENT PROGRAMME/ FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS, 2009).

Estudos demonstram que o forato é rapidamente absorvido por via oral (gavagem) em ratos (JOINT/FAO/WHO MEETING ON PESTICIDE RESIDUES, 2006), bem como pelo trato gastrointestinal (CANADÁ, 1990; HEALTH COUNCIL OF THE NETHERLANDS, 2003).

Diversos fatores podem interferir na absorção dos organofosforados, modificando a toxicocinética e toxicidade desses compostos. A temperatura ambiental elevada e alta umidade relativa aumentam a absorção cutânea, possivelmente em consequência do aumento da taxa de respiração, da frequência e do fluxo sanguíneo para os tecidos que ocorrem nestas condições. Fatores genéticos ou comportamentais como ingestão de bebidas alcoólicas também modificam a absorção e distribuição desses compostos (CALIFORNIA DEPARTMENT OF PESTICIDE REGULATION, 1999; ATHANASOPOULOS; KYRIAKIDIS; STAVROPOULOS, 2004).

3.2. Distribuição

Os compostos organofosforados atravessam facilmente a barreira hematoencefálica, provocando manifestações neurológicas (FERRER, 2003). Também têm a capacidade de transpor facilmente a placenta (VILLENEUVE et al, 1972; ABU-QARE et al, 2000).

Após a absorção, o forato é rapidamente distribuído em mamíferos. Seis horas após a administração oral (gavagem) de uma dose única (0,44 mg/kg) de ^{14}C-forato (pureza > 98%) em ratas, resíduos do composto foram detectados no sangue (0,168 ppm), rins (0,163 ppm), fígado (0,142 ppm), pele (0,109 ppm), músculo (0,100 ppm) e tecidos adiposos (0,031 ppm). Após 192 horas (8 dias), resíduos no fígado e rins ainda

puderam ser detectados (MILLER; WU, 1990 apud JOINT/FAO/WHO MEETING ON PESTICIDE RESIDUES, 2006).

Em ratos machos tratados com uma dose única (0,8 mg/kg) de ^{14}C-forato, resíduos não fosforilados foram detectados no fígado, rins e músculos (HUSSAIN, 1987 apud INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY, 1996).

3.3. Biotransformação

O forato pode ser oxidado a oxon forato, sulfóxido de forato e forato sulfona (ETO, 1994 apud HAJJAR; HODGSON, 1982; CHAPMAN ET AL, 1982; SZETO ET AL, 1990 apud HONG; PEHKONEN; BROOKS, 2000; HODGSON, 1983). O forato também sofre hidrólise, através do ataque nucleofílico no átomo de fósforo central ou no carbono da cadeia lateral (HONG; PEHKONEN; BROOKS, 2000).

A formação de sulfóxido de forato é catalisada, principalmente, pela ação da enzima monooxigenase dependente de flavina-adeninda-dinucleotídeo (FAD) (HAJJAR; HODGSON, 1982; HODGSON, 1983; HODGSON; LEVI, 1992). Entretanto a sulfoxidação do forato também pode ocorrer por enzimas da família CYP, que também catalizam reações de oxidação desse organosfosforado (LEVI; HODGSON, 1988). As enzimas CYP também estão envolvidas na produção dos metabólitos forato sulfona, oxon sulfóxido e oxon sulfona forato, através de reações de oxidação ou de dessulfuração (LEVI; HODGSON, 1988).

3.4. Excreção

Mamíferos expostos ao forato eliminam tanto o composto original quanto os seus metabólitos principalmente através da urina, e, em menor proporção, pelas fezes (BROKOPP; WYATT; GABICA, 1981; WORLD HEALTH ORGANIZATION/FOOD AND AGRICULTURE ORGANIZATION, 1988). Os percentuais de eliminação variam de acordo com a espécie (INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY, 1996; HEALTH COUNCIL OF THE NETHERLANDS, 2003; JOINT/FAO/WHO MEETING ON PESTICIDE RESIDUES, 2006).

A administração oral de uma dose única de 2 mg/kg de forato radiomarcado em ratos machos resultou em eliminação do composto através da urina (35%) e nas fezes (3,5%) dentro de um período de 06 dias (144 horas). Ratos machos tratados com 6 doses diárias de 1 mg/kg, excretaram 12% do fósforo radiomarcado na urina e 6% nas fezes

dentro de 7 dias (BOWMAN; CASIDA, 1958 apud INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY, 1996).

Após a administração oral (gavagem) de uma dose única (0,44 mg/kg) de ^{14}C-forato (pureza > 98%) em ratas, 78% da dose administrada foi eliminada através da urina em 24 horas. A eliminação através das fezes correspondeu a apenas 8% da dose administrada. Ao final do estudo, mais de 94% da dose administrada foi convertida a metabólitos não fosforilados (MILLER; WU, 1990 apud JOINT/FAO/WHO MEETING ON PESTICIDE RESIDUES, 2006).

Os metabólitos O,O-dimetilfosfato, O,O-dietilfosfato, O,O-dimetiltiofosforotioato e O,O-dietiltiofosforotioato foram encontrados em amostras de urina de agricultores expostos a produtos formulados com o ingrediente ativo forato (SHAFIK et al, 1973).

4. Avaliação Toxicológica

4.1 Aspectos gerais das manifestações clínicas em seres humanos

Em geral os efeitos agudos dos OP surgem poucas horas após a exposição. O quadro clínico dessas intoxicações pode variar quanto à gravidade, rapidez de instalação e/ou duração dos sintomas, dependendo da via de absorção e da magnitude da exposição (ECOBICHON, 2001; KAMANYIRE; KARALLIEDDE, 2004).

Os distúrios neurocomportamentais são os mais frequentemente observados em indivíduos cronicamente intoxicados. Os sintomais do tipo neuro-comportamentais em geral são insônia, sonambulismo, sono excessivo, ansiedade, retardo de reações, dificuldade de concentração e uma variedade de sequelas neuropsiquiátricas, labilidade emocional, distúrbios de linguagem, apatia, irritabilidade, alucinações, delírios, tremores, reações esquizofrênicas, alterações no EEG, neuropatia periférica, parestesias, hiporreflexia, deficiência na coordenação neuro-motora e depressão (KLAASSEN, 1991; ALMEIDA; SOARES, 1992; BRASIL, 1997; ECOBICHON, 2001). A maioria desses sintomas muitas vezes deixa de ser relacionada com a exposição aos agrotóxicos, sendo confundidos com agravos à saúde por outras causas.

Os mecanismos de ação dos organofosforados e sua toxicidade aguda são bem conhecidos e caracterizam-se pelos efeitos muscarínicos (ou colinérgicos), nicotínicos e neurológicos. O principal efeito da exposição aguda se relaciona à inibição da enzima

acetilcolinesterase e seu consequente acúmulo nas fendas sinápticas (ECOBICHON, 2001).

Conforme Kamanyire e Karalliedde (2004) embora a inibição da acetilcolinesterase seja o principal mecanismo na toxicologia dos organosfosforados, a suscetibilidade individual, a inibição de outros sistemas enzimáticos e os efeitos diretos dos organofosforados nos tecidos também são importantes. As consequências da inibição de outros sistemas enzimáticos por compostos organofosforados ainda são incertos, entretanto já se tem conhecimento do comprometimento de carboxiesterases tissulares no soro, fígado, intestino e outros tecidos. As carboxiesterases parecem contribuir para a degradação metabólica dos organofosforados e a inibição dessas enzimas contribui para potencializar sua toxicidade. Esses autores citam alguns efeitos já evidenciados em animais e que também podem acometer humanos:

- Inativação por fosforilação de outra beta esterase,
- Alteração da recomposição de neurotransmissores, como, por exemplo, o GABA e glutamato;
- Aumento do número de receptores GABA e dopaminérgicos,
- Atuação como agonista dos receptores muscarínicos M2/M4,
- Inibição de enzimas mitocondriais e da geração de ATP,
- Indução a degranulação celular, provavelmente causando a liberação de histamina e compostos histamínicos,
- Inibição de óxido nítrico,
- Interferência com o surfactante nos pulmões,
- Inibição da fosfolipase A2,
- Interferência na imunidade celular e humoral, por exemplo, na função dos linfócitos T.

Os sinais e sintomas das intoxicações agudas por organofosforados variam em relação ao tipo de ação e ao órgão alvo. No Sistema Nervoso Autônomo, os efeitos muscarínicos ocorrem no aparelho digestivo, com perda de apetite, náuseas, vômitos, dores abdominais, diarréia e defecação involuntária; no aparelho respiratório: rinorréia, hiperemia de vias aéreas superiores, broncoespasmo e aumento da secreção brônquica, edema pulmonar; no sistema circulatório: bradicardia, bloqueio aurículo-ventricular; no sistema ocular: lacrimejamento, dor ocular, congestão da conjuntiva, distúrbio de visão, espasmo ciliar, dor no supercílio e miose; no aparelho urinário: diurese frequente e

involuntária; nas glândulas exócrinas: transpiração excessiva, salivação extrema. Outras alterações observadas são: micção involuntária, sudorese, ereção peniana, bradicardia e hipotensão (ECOBICHON, 2001).

Na síndrome nicotínica, o quadro clínico se constitui geralmente pela presença de fadiga e fraqueza generalizada, cãibras, contrações involuntárias, fasciculações disseminadas e paralisia muscular, incluindo dos músculos respiratórios, e hipertensão arterial transitória (ECOBICHON, 2001).

A ação no Sistema Nervoso Central (SNC) pela neurotoxidade leva aos sintomas de distúrbios do sono, dificuldade de concentração, comprometimento da memória, ansiedade, agitação, tremores, disartria, confusão, ataxia, fala indistinta, perda dos reflexos, convulsões generalizadas, torpor, depressão respiratória, paralisia respiratória central com respiração de Cheyne-Stokes e coma. Observa-se também ação vasomotora em outros centros cardiovasculares e no bulbo que provocam hipotensão, podendo evoluir para coma e morte (KLAASSEN, 1991; BRASIL, 1997; ECOBICHON, 2001).

Diversos casos de intoxicação aguda envolvendo comunidades de Kerala, Índia, foram registrados por Usha e Harikrishnan (2004). Dentre esses casos, cinco registros de casos envolvendo a exposição ao forato no período entre 1999 e 2002 são dignos de nota. Conforme os autores, em julho de 1999 aproximadamente 12 pessoas residentes de região de plantação de banana foram severamente intoxicadas por forato. Em seguida à utilização do produto, houve chuva na região, fazendo com que o produto evaporasse rapidamente e se espalhasse pela área vizinha, atingindo as residências. Os sintomas apareceram logo após a aplicação do produto e os intoxicados necessitaram de hospitalização. Em junho de 2001, um rapaz de 16 anos foi a óbito em decorrência da exposição ocupacional ao forato por um período de uma semana. Nesse mesmo ano, 40 mulheres trabalhadoras rurais em uma plantação de chá foram intoxicadas durante a colheita das folhas. Os sintomas apareceram nos primeiros 30 minutos após a exposição, caracterizados por tontura, vertigem, visão turva, vômitos. Trinta e sete mulheres apresentaram quadros mais graves e permaneceram hospitalizadas por dois dias. Os autores destacam que em julho de 2002, 31 crianças de uma escola do ensino fundamental foram contaminadas por forato aplicado em plantação nas proximidades da escola. As crianças apresentaram quadro de cefaléia, dor torácica, dificuldade respiratória, náusea, vertigem, visão turva e dor abdominal, sendo que um deles apresentou convulsões mesmo após 24h de tratamento. Em junho de 2003 houve o

registro de óbito de uma criança, de um ano e meio, filha de agricultores, em decorrência da intoxicação por forato após ingestão acidental do produto.

Embora não seja muito frequente, casos de intoxicação subcrônica podem ser observados em sujeitos expostos ao forato. Das e Jena (2000) relataram caso clínico em que houve associação entre encefalopatia, síndrome intermediária e polineuropatia retardada induzida por organofosforados.

Conforme Peter et al. (2008a) descreveram, alterações da consciência ou coma são fenômenos que podem ocorrer em casos de intoxicação aguda severa e apresentam prognóstico ruim. Esses autores desenvolveram um estudo de coorte prospectivo, onde acompanharam durante um ano 35 pacientes admitidos em unidade hospitalar com intoxicação aguda grave por OP, dentre os quais o forato. O quadro clínico agudo foi constituído por sinais e sintomas colinérgicos (miose, salivação, lacrimejamento, sudorese e bradicardia). Os pacientes evoluíram com comprometimento encefálico grave, caracterizado por coma profundo por volta do quinto dia após a intoxicação aguda. Dentre os pacientes, quatro foram intoxicados por forato, dos quais um evoluiu para coma profundo.

4.2. Toxicidade Aguda

A toxicidade aguda do ingrediente ativo (IA) forato foi avaliada com base nos dados disponíveis em relatórios de agências ou institutos internacionais, tais como a EPA (Environmental Protection Agency) e o IPCS (International Programme on Chemical Safety), bem como nos estudos encaminhados à ANVISA com o intuito de suportar o registro dos produtos técnicos e formulados à base desse IA. Os estudos foram conduzidos em animais experimentais (ratos, coelhos e camundongos) através de exposição pelas vias oral, inalatória, dérmica, ocular, intravenosa e intraperitoneal. Os dados de doses letais (oral e dérmica) e concentrações letais estão sumarizados na tabela 3.

Tabela 3 – Estudos agudos sobre o ingrediente ativo forato

Espécie	Linhagem	Via	Pureza (%)	DL50 (mg/kg) ou CL50 (mg/l)	Referência
Rato	Wistar	Oral	90	<50 (♂)	Dossiê de registro submetido à ANVISA
Rato	-	Oral	-	♂ - 3,7 ♀ - 1,4	EPA (2006)

Rato	-	Oral	-	1,9 - 10	Blinn, R.C. (1992) *Apud* IPCS (1994)
Rato	-	Oral	-	♂ - 2,3 ♀ - 1,1	Gaines, T.B. (1969) *Apud* IPCS (1994)
Rato	-	Oral	-	♂ - 2,8 ♀ - 1,6	Anon (1976) *Apud* IPCS (1994)
Rato	-	Oral	-	♂ - 3,7 ♀ - 1,4	Newell, G.W. & Dilley, J.V. (1978) *Apud* IPCS (1994)
Camundongo	-	Oral	-	11	Blinn, R.C. (1992) *Apud* IPCS (1994)
Camundongo	-	Intraperitoneal	-	3	Blinn, R.C. (1992) *Apud* IPCS (1994)
Rato	Wistar	Dérmica	90	<200 (♂)	Dossiê de registro submetido à ANVISA
Rato	-	Dérmica	-	♂ - 9,3 ♀ - 3,9	EPA (2006)
Rato	-	Dérmica	-	3	Blinn, R.C. (1992) *Apud* IPCS (1994)
Rato	-	Dérmica	-	♂ - 6,2 ♀ - 2,5	Gaines, T.B. (1969) *Apud* IPCS (1994)
Rato	-	Dérmica	-	♂ - 5,7	American Cyanamid Co., 1976 *Apud* IPCS (1994)
Rato	-	Dérmica	-	♂ - 9,3 ♀ - 3,9	Newell, G.W. & Dilley, J.V. (1978) *Apud* IPCS (1994)
Coelho	-	Dérmica	-	♂ - 5,2	Anon (1976) *Apud* IPCS (1994)
Rato	-	Inalatória	-	♂ - 0,06 0,011	EPA (2006)
Rato	-	Inalatória (1h)	-	♂ - 60 mg/m³ ♀ - 11 mg/m³	Newell, G.W. & Dilley, J.V. (1978) *Apud* IPCS (1994)

Os estudos agudos, conduzidos em animais experimentais, mostraram que o forato apresenta elevada toxicidade pelas vias oral, dérmica e inalatória. As principais alterações observadas nos testes de DL_{50} oral foram: tremores, convulsões, salivação, inibição da atividade, lacrimejamento, exoftalmia, hemorragia no trato gastrointestinal, fígado escuro, entre outras.

O forato foi considerado levemente irritante para os olhos de coelhos. Com relação aos estudos de DL_{50} démica, os tremores foram os principais sinais clínicos observados nos animais expostos ao forato. Os testes de sensibilização dérmica feitos com a substância em questão foram considerados negativos.

Dessa forma, o forato é classificado como Classe I – Extremamente Tóxico.

4.3. Toxicidade Subcrônica

Estudo 1

Ano:1990

Espécie: Camundongo (Crl:CD-1)

Número de animais: 20/grupo/sexo

Doses: 1, 3 e 6 ppm – Equivalente a 0,18; 0,55 e 1,10 mg/kg/dia para machos e 0,23; 0,67 e 1,38 mg/kg/dia para fêmeas
Via: Oral (dieta)
Tempo de exposição: 13 semanas
Concentração do ingrediente ativo (pureza): 92,1%
Referência: Trutter, J.A. (1990) 13-Week dietary toxicity study in albino mice with AC 35.024. Final report, Project ID No. 362-201. Unpublished report dated 16 May 1990 from Hazleton Laboratories America Inc. *Apud* IPCS 1994.

Os animais expostos às maiores doses exibiram significativa inibição da atividade da colinesterase plasmática, o mesmo achado também foi observado em fêmeas tratadas com 1 ppm de forato. Houve significativo decréscimo na atividade da colinesterase eritrocitária nos machos e fêmeas que receberam a maior dose, a inibição em relação ao grupo controle foi de 50% nos machos e 61% nas fêmeas. Houve ligeira redução (-17%) na atividade da colinesterase eritrocitária nas fêmeas expostas a 3 ppm da substância teste. Foi observada inibição em cerca de 50% na atividade da colinesterase cerebral nos machos e fêmeas tratados com 6 ppm, quando expostos a 3 ppm os animais exibiram mais de 10% de inibição na atividade dessa enzima em relação ao grupo não tratado. O NOAEL não pôde ser estabelecido.

Estudo 2
Ano:1956
Espécie: Rato
Número de animais: 50/grupo/sexo e 25 /grupo/sexo
Doses: 0,22; 0,66; 2 e 6 ppm (equivalente a 0,01; 0,03; 0,1 e 0,3 mg/kg/dia) e 12 e 18 (equivalente a 0,6 e 0,9 mg/kg/dia)
Via: Oral (dieta)
Tempo de exposição: 13 semanas
Concentração do ingrediente ativo (pureza): 92%
Referência: Tusing, T.W., Kindzin, W., Hanzal, R. & Howard, J. (1956) Repeated oral administration (dogs). Experimental insecticide 3911, 92%. Unpublished report from Hazleton Labs, Inc. Submitted to WHO by Submitted to WHO by American Cynamid Co., Wayne, NJ, USA. *Apud* IPCS 1994.

Ocasionalmente, foram observados tremores intermitentes e excitabilidade nas fêmeas expostas a 6 ppm de forato. Os machos e as fêmeas que receberam 12 e 18 ppm apresentaram severa excitabilidade, tremores intermitentes e ataxia, culminando na morte de 50% dos animais tratados com 12 ppm e de todos os expostos a 18 ppm. Os animais que receberam 6 ppm exibiram significante inibição da atividade da colinesterase plasmática, eritrocitária e cerebral. As fêmeas expostas a 2 ppm exibiram redução na atividade da colinesterase eritrocitária. O NOAEL estabelecido para o estudo foi 0,66 ppm ou 0,03 mg/kg/dia baseado na inibição da colinesterase eritrocitária nas fêmeas.

Estudo 3

Ano: 1987

Espécie: Cães (Beagle)

Número de animais: 2/grupo/sexo

Doses: 0, 01; 0,05; 0,10, 0,25 e 0,50 mg/kg/dia

Via: Oral (cápsulas)

Tempo de exposição: 14 dias

Concentração do ingrediente ativo (pureza): 92,1%

Referência: Piccirillo, V.J., Shellenberger, T.E. & Dauvin, E. M. (1987) 14-Day range-finding oral toxicity study in the dog with AC 35,024. Revised final report No. 85013. Unpublished report dated 11 February 1987 from Tegeris Laboratories Inc., Princeton, NJ, USA. Submitted to WHO by American Cyanamid Co., Princeton, NJ, USA. *Apud* IPCS 1994.

Uma fêmea exposta a 0,50 mg/kg/dia exibiu excessiva salivação e tremores. Os animais (machos e fêmeas) tratados com a maior dose apresentaram decréscimo no ganho de peso corpóreo em relação ao grupo controle.

A atividade da colinesterase plasmática foi significativamente reduzida nos machos e fêmeas tratados com doses iguais ou acima de 0,10 mg/kg/dia, os machos que receberam 0,05 mg/kg/dia também apresentaram inibição na atividade dessa enzima em relação aos valores observados antes do tratamento e nos animais não tratados (grupo controle). Houve significante diminuição (>20%) na atividade da colinesterase eritrocitária nos machos e fêmeas tratados com a maior dose. Quanto à atividade da colinesterase cerebral, foi observada inibição entre 31 e 69% nos animais que receberam 0,10 mg/kg/dia ou mais da substância teste. O NOAEL estabelecido foi 0,01 mg/kg/dia

baseado na inibição da atividade da colinesterase plasmática nos machos tratados com 0,05 mg/kg/dia.

Estudo 4

Ano: 1987

Espécie: Cães (Beagle)

Número de animais: 6/grupo/sexo

Doses: 0, 005; 0,01; 0,05 e 0,25 mg/kg/dia

Via: Oral (cápsulas)

Tempo de exposição: 1 ano

Concentração do ingrediente ativo (pureza): 92,1%

Referência: Shellenberger, T.E. & Tegeris, A.S. (1987) One-year oral toxicity study in purebred beagle dogs with AC 35,024. Final report Nº 85015. Unpublished report dated 20 February 1987 from Tegeris Laboratories Inc., Laurel, MD, USA. Submitted to WHO by American Cyanamid Co., Princeton, NJ, USA. *Apud* IPCS 1994.

Tremores moderados foram observados em um macho e duas fêmeas durante a 23ª e 52ª semana de tratamento com a maior dose. A média do peso corpóreo e o ganho de peso foi 26% menor, em relação ao grupo controle, nos machos tratados com 0,25 mg/kg/dia.

Machos tratados com a maior dose apresentaram significante (p<0,05) redução nos níveis de proteínas totais, essas observações foram realizadas durante a 6ª semana, 3º e 6º meses e ao término do tratamento.

Os animais tratados com 0,25 mg/kg/dia apresentaram inibição de mais de 20% na atividade da colinesterase eritrocitária e entre 43 e 54% da colinesterase cerebral. As doses iguais ou superiores a 0,05 mg/kg/dia reduziram significativamente a atividade da colinesterase plasmática nos machos e fêmeas expostos. O NOAEL estabelecido foi 0,01 mg/kg/dia baseado na inibição da atividade da colinesterase plasmática nos animais tratados com 0,05 mg/kg/dia.

4.4. Toxicidade crônica, carcinogenicidade e genotoxicidade

Na fisiopatologia dos efeitos crônicos decorrente da exposição aos agrotóxicos estão envolvidos, além dos aspectos toxicológicos próprios de cada produto, as características da exposição tais como a intensidade, a duração e a interação com outros

produtos químicos, com os quais pode haver potencialização da ação tóxica, e outros condicionantes biossociais do exposto.

A exposição crônica aos organofosforados também está relacionada, entre outros, ao câncer, efeitos teratogênicos, neuropatias periféricas tardias e toxicidade reprodutiva (CALDAS; SOUZA, 2000).

A mutação no DNA é a alteração genuína do processo de carcinogenicidade (RIBEIRO; SALVADORI; MARQUES, 2003). Essa mutação pode ser causada por agentes químicos, como os agrotóxicos, que podem induzir o câncer por mecanismos variados como genotoxicidade e promoção de tumores (RODVALL; DICH; WIKLUND, 2003).

A maioria dos carcinógenos apresenta uma propriedade em comum: são eletrofílicos altamente reativos que interagem com locais nucleofílicos na célula; sendo o DNA alvo de preferência (SANTOS et al., 2008). Nessa ligação, os adutos de DNA são formados por ligações covalentes. Essa formação pode mutar proto-oncogenes ou genes supressores de tumor e iniciar o processo de carcinogênese (KINZLER; VOGELSTEIN, 1996, apud LOUREIRO; MASCIO; MEDEIROS, 2002). Após essa mutação ocorrem alterações no processo de divisão celular que resultam na perda de características funcionais e na formação de tumores (CUNNINGHAM; MATTHEWS, 1995).

A correlação entre câncer e agrotóxico está mais bem caracterizada nos cânceres de pulmão, de mama, dos testículos, da tireóide, da próstata, do ovário, e do sistema hematopoiético (linfomas não-Hodgkin, leucemias e mieloma múltiplo) (PIMENTEL, 1996).

Um estudo prospectivo realizado com 52.395 aplicadores de agrotóxicos na Carolina do Norte e em Iowa nos EUA, de 1993 a 1997 (ALAVANJA, et al., 2002 apud EPA, 2002) mostrou associação de risco para câncer e o uso de agrotóxicos, entre eles o forato. Em 2006, Mahajan et al, verificaram um maior risco de desenvolvimento de câncer de próstata quando o histórico familiar era positivo para essa neoplasia em agricultores expostos ao forato do mesmo grupo citado acima.

A seguir estão descritos os estudos sobre o potencial carcinogênico do ingrediente ativo forato, aportados no dossiê toxicológico da ANVISA.

Estudo 1

Ano:1981

Espécie: Rato - Crl:COBS CD (SD) BR

Nº de animais: 50/grupo/sexo

Doses: 1, 3 e 6 ppm – equivalente a 0,05; 0,16 e 0,32 mg/kg/dia para machos e 0,07; 0,19 e 0,43 mg/kg/dia para fêmeas

Via: Oral (dieta)

Tempo de exposição: 2 anos (24 meses)

Concentração do ingrediente ativo (pureza): 84,5%

Referência: Manus, A. G. et al. (1981). 24-Month Chronic Toxicity and Potential Carcinogenicity Study in Rats. Litton Bionetics. Dossiê de registro submetido à ANVISA.

O objetivo do estudo foi avaliar o potencial carcinogênico do forato. Ratos receberam a substância teste incorporada à dieta nas doses de 1, 3 ou 6 ppm durante 24 meses. Foi observado um aumento no número de animais em condições moribundas ou que foram encontrados mortos, em todos os grupos, inclusive no controle, contudo esse aumento foi maior no grupo de fêmeas tratadas com a maior dose, sendo que apenas 36% dos animais desse grupo sobreviveram até o final do estudo. Durante a 9ª semana de tratamento os animais apresentaram tremores, esse sinal clínico, segundo o diretor do estudo, foi atribuído a uma superdosagem (327% a mais da dose que deveria ser administrada). As fêmeas que receberam na dieta a dose de 6 ppm de forato apresentaram redução no ganho de peso, em comparação ao grupo controle, durante as primeiras 26 semanas e entre a 74ª e 102ª semanas. Com relação aos parâmetros hematológicos avaliados, as fêmeas tratadas com a maior dose apresentaram, aos 12 meses de estudo, diminuição na quantidade de eritrócitos, hemoglobina e hematócritos. Já machos tratados com 6 ppm de forato exibiram reduções na quantidade de hemoglobina e de leucócitos. Quanto aos parâmetros bioquímicos avaliados, os machos tratados com 1 ppm da substância teste apresentaram, aos 6 meses de tratamento, redução nos níveis da enzima aspartato aminotransferase (AST).

Foi observada inibição da atividade da colinesterase plasmática, maior que 20% e dose-relacionada, nos machos tratados com 6 ppm na avaliação realizada aos 12 meses, em todos os machos tratados ao final do estudo e nas fêmeas que receberam 3 e 6 ppm em todos os períodos amostrados (3, 6, 12 e 24 meses).

Machos que receberam a maior dose e fêmeas tratadas com 3 ou 6 ppm apresentaram inibição, maior que 20%, na atividade da colinesterase cerebral. A colinesterase eritrocitária não foi significativamente inibida, apresentando menos de 20% de inibição nos períodos observados.

Dados de necropsia revelaram aumento na razão entre o peso corpóreo e as adrenais, cérebro, coração, fígado e baço nas fêmeas tratadas com 6 ppm da substância teste. Nos exames patológicos e histopatológicos foi observado aumento na incidência de inflamação e hiperplasia epitelial da porção dianteira do estômago dos animais de ambos os sexos, mas especialmente dos machos, tratados com a maior dose. Esse efeito foi aparentemente relacionado ao tratamento com o forato.

O NOEL estabelecido para o estudo foi 1 ppm, equivalente a 0,05 mg/kg de peso corpóreo por dia.

Estudo 2

Ano:1981

Espécie: Camundongos - CD-1

Nº de animais: 50/grupo/sexo

Doses: 1, 3 e 6 ppm - equivalente a 0,15; 0,45 e 0,90 mg/kg/dia

Via: Oral (dieta)

Tempo de exposição: 18 meses

Concentração do ingrediente ativo (pureza): 91,7%

Referência: Manus, A. G. et al. (1981). 18-Month chronic toxicity and potential carcinogenicity study in mice. Litton Bionetics. Dossiê de registro submetido à ANVISA.

O objetivo do estudo foi avaliar o potencial carcinogênico do ingrediente ativo forato quando administrado na dieta de camundongos nas doses de 1, 3 ou 6 ppm pelo período de 18 meses. Os animais tinham 41 dias de idade quando iniciaram o tratamento.

Durante o curso do estudo 91 camundongos morreram ou foram sacrificados em condições moribundas. Foi observado mortalidade em todos os grupos tratados, inclusive no controle [Machos: controle – 5/50 (10%); 1 ppm – 7/50 (14%); 3 ppm – 11/50 (22%) e 6 ppm – 9/5 (18%). Fêmeas: controle – 13/50 (26%); 1 ppm – 13/50 (26%); 3 ppm – 16/50 (32,7%) e 6 ppm – 17/50 (34,7%)].

Os animais que receberam a substância teste (em todas as doses), bem como os do grupo controle apresentaram perda de pêlo em regiões restritas do corpo ou em múltiplas áreas. Os machos tratados com a maior dose apresentaram redução do peso corpóreo nas primeiras semanas do estudo (1ª, 3ª e 5ª semana), já as fêmeas que receberam 6 ppm da substância teste exibiram diminuição de peso em praticamente todas as 25 primeiras semanas do estudo. Com relação ao consumo de alimentos, todos os grupos tratados (machos e fêmeas) apresentaram reduções no consumo da dieta.

Houve alta incidência de alguns sinais clínicos como tremores, hiperatividade e salivação excessiva, os mesmos foram mais frequentes nos animais tratados com 6 ppm em relação ao grupo controle.

Foram observados linfomas malignos nos animais tratados, sendo que estes foram relativamente comuns, particularmente em fêmeas e geralmente envolveram o trato gastrointestinal, timo, fígado, rins e nódulos linfáticos, a incidência desses achados não foi relacionada à quantidade da substância teste administrada. Os animais tratados apresentaram adenomas, contudo esses achados não foram considerados relacionados ao tratamento, uma vez que o grupo controle também exibiu essas alterações. A incidência de adenoma alveolar e bronquiolar foi maior nos machos tratados com 6 ppm (8/50, comparado a 3/50 do controle), contudo esse efeito não foi atribuído ao tratamento, uma vez que, segundo o diretor do estudo, o aumento da incidência não foi estatisticamente significante e esse tumor é frequentemente observado em camundongos da linhagem CD-1.

Outras alterações que foram observadas nos animais tratados e não tratados (controle) foram: amiloidose; acúmulo multifocal de neutrofilos, linfócitos e macrófagos, frequentemente associado à hepatócitos degenerados ou necróticos; estômago exibindo uma mistura de células inflamatórias infiltradas, edema e hiperplasia com formação de criptas císticas; mielofibrose; infiltrados linfocíticos foram geralmente vistos nos rins e na bexiga urinária em todos os grupos; hiperplasia endometrial cística; lesões nos olhos (a maioria consistiu de catarata). O diretor do estudo concluiu que as alterações patológicas não foram atribuídas à administração prolongada do forato na dieta. Segundo o mesmo, todas as alterações foram consideradas achados incidentais ou parte de complexas doenças espontâneas em camundongos.

Baseado no decréscimo do peso corpóreo e nos sinais clínicos de toxicidade na maior dose. O NOEL estabelecido para esse estudo foi 3 ppm, equivalente a 0,45 mg/kg de peso corpóreo por dia.

Dados na literatura indicam que vários organofosforados são mutagênicos (MOHAMMED, 1999; MATSUHITA; MATSUI; MATSUI, 2006; MATSUHITA et al, 2005). Entre os dados apresentados destaca-se a mutagenicidade dos metabólitos dos organofosforados (CORTEZ-ESLAVA et al, 2001; MATSUHITA; MATSUI; MATSUI, 2005; MATSUHITA et al, 2005;), que muitas vezes são mais mutagênicos do que o ingrediente ativo.

Também é destacado o sinergismo desses compostos e seus metabólitos com aminas aromáticas, moléculas pré-mutagênicas e promotoras de câncer. As aminas aromáticas são utilizadas em vários processos da indústria têxtil e na produção de fármacos, agrotóxicos e plásticos. Esse sinergismo provoca um aumento da mutagenicidade dessas aminas.

Ensaios biológicos *in vitro* e *in vivo*, mediante análise genotóxica e carcinogênica de organofosforados apontam efeitos decorrentes de mutações gênicas, cromossômicas e de lesões na estrutura bioquímica do DNA humano, mostrando assim o potencial mutagênico e/ou carcinogênico desse grupo de agrotóxicos.

Os órgãos internacionais como EPA (1999) e IPCS (1994) classificam o forato como não carcinogênico e não mutagênico baseados em resultados de estudos *in vitro* de mutação reversa e recombinação mitótica e *in vivo* de aberrações cromossômicas e mutação dominante letal. Alguns desses estudos podem ser observados na Tabela 4.

Tabela 4: Estudos de genotoxicidade do forato

Estudo	Microorganismo / Animal / Células	Dose / Concentração	Pureza (%)	Resultado	Referência
Mutação reversa	*S.typhimurium* (TA100, 1535, 1537, 1538); *E. Coli* WP2	Acima de 1.000 mg/placa	-	Negativo[a]	Simmon et. al. (1977) *Apud* IPCS (1994)
Mutação reversa	*E. coli* (p3478 e w3110); *B. subtilis*	1 mg/placa	-	Negativo[b]	Simmon et. al. (1977) *Apud* IPCS (1994)
Mutação reversa	Células do ovário de hamster (locus hprt)	30, 40, 50, 80 e 100 nl/ml 5, 10, 12, 14, 16, 18 e 20 nl/ml	92,1	Negativo[b] Negativo[c]	Thilager & kumarop (1985) *Apud* IPCS (1994)
Recombinação mitótica	*S. cerevisiae* D3	5% peso/volume incubado por 4h antes do "plaquamento"	-	Negativo[a]	Simmon et. al. (1977) *Apud* IPCS (1994)
Síntese de DNA	Fibroblastos humanos (WI – 38)	Acima de $1x10^{-3}$	-	Negativo[a]	Simmon et. al. (1977) *Apud* IPCS (1994)
Aberrações cromossômicas	Ratos (♂ e ♀) – Sprague-Dawley[d]	♂ - 0,25; 1,25 e 2,5 mg/kg/dia ♀ - 0,13; 0,63 e 1,25 mg/kg/dia	92,1	Negativo	Ivett & Myhr, 1986 *Apud* IPCS (1994)
Dominante letal	Camundongo (♂)	5, 10 e 20 mg/kg/dia[e]	-	Negativo	Simmon et. al.

					(1977) *Apud* IPCS (1994)

a – na presença e ausência de ativação metabólica
b – na ausência de ativação metabólica
c – na presença de ativação metabólica
d – os animais foram sacrificados após 6, 18 e 30h.
e – doses administradas na dieta por 7 semanas

Porém, segundo Malhi, Grover (1987) o forato provoca aberrações cromossômicas *in vivo* em células da medula óssea de ratos, como troca entre cromátides, quebra e deleção. Ainda segundo esse autor o trabalho de Sobti et al (1982) mostrou que o forato provoca aumento de recombinação (SCE) em células de linfócitos humanas. Grover, Malhi (1985) também relatam positividade para teste de micronúcleo nessas mesmas células.

Estudo 1

Ano:1981

Espécie: Rato - Crl:COBS CD (SD) BR

Nº de animais: 50/grupo/sexo

Doses: 1, 3 e 6 ppm – equivalente a 0,05; 0,16 e 0,32 mg/kg/dia para machos e 0,07; 0,19 e 0,43 mg/kg/dia para fêmeas

Via: Oral (dieta)

Tempo de exposição: 2 anos (24 meses)

Concentração do ingrediente ativo (pureza): 84,5%

Referência: Manus, A. G. et al. (1981). 24-Month Chronic Toxicity and Potential Carcinogenicity Study in Rats. Litton Bionetics. Dossiê de registro submetido à ANVISA.

O objetivo do estudo foi avaliar o potencial carcinogênico do forato. Ratos receberam a substância teste incorporada à dieta nas doses de 1, 3 ou 6 ppm durante 24 meses. Foi observado um aumento no número de animais em condições moribundas ou que foram encontrados mortos, em todos os grupos, inclusive no controle, contudo esse aumento foi maior no grupo de fêmeas tratadas com a maior dose, sendo que apenas 36% dos animais desse grupo sobreviveram até o final do estudo. Durante a 9ª semana de tratamento os animais apresentaram tremores, esse sinal clínico, segundo o diretor do estudo, foi atribuído a uma superdosagem (327% a mais da dose que deveria ser administrada). As fêmeas que receberam na dieta a dose de 6 ppm de forato apresentaram redução no ganho de peso, em comparação ao grupo controle, durante as

primeiras 26 semanas e entre a 74ª e 102ª semanas. Com relação aos parâmetros hematológicos avaliados, as fêmeas tratadas com a maior dose apresentaram, aos 12 meses de estudo, diminuição na quantidade de eritrócitos, hemoglobina e hematócritos. Já machos tratados com 6 ppm de forato exibiram reduções na quantidade de hemoglobina e de leucócitos. Quanto aos parâmetros bioquímicos avaliados, os machos tratados com 1 ppm da substância teste apresentaram, aos 6 meses de tratamento, redução nos níveis da enzima aspartato aminotransferase (AST).

Foi observada inibição da atividade da colinesterase plasmática, maior que 20% e dose-relacionada, nos machos tratados com 6 ppm na avaliação realizada aos 12 meses, em todos os machos tratados ao final do estudo e nas fêmeas que receberam 3 e 6 ppm em todos os períodos amostrados (3, 6, 12 e 24 meses).

Machos que receberam a maior dose e fêmeas tratadas com 3 ou 6 ppm apresentaram inibição, maior que 20%, na atividade da colinesterase cerebral. A colinesterase eritrocitária não foi significativamente inibida, apresentando menos de 20% de inibição nos períodos observados.

Dados de necropsia revelaram aumento na razão entre o peso corpóreo e as adrenais, cérebro, coração, fígado e baço nas fêmeas tratadas com 6 ppm da substância teste. Nos exames patológicos e histopatológicos foi observado aumento na incidência de inflamação e hiperplasia epitelial da porção dianteira do estômago dos animais de ambos os sexos, mas especialmente dos machos, tratados com a maior dose. Esse efeito foi aparentemente relacionado ao tratamento com o forato.

O NOEL estabelecido para o estudo foi 1 ppm, equivalente a 0,05 mg/kg de peso corpóreo por dia.

Estudo 2

Ano:1981

Espécie: Camundongos - CD-1

Nº de animais: 50/grupo/sexo

Doses: 1, 3 e 6 ppm - equivalente a 0,15; 0,45 e 0,90 mg/kg/dia

Via: Oral (dieta)

Tempo de exposição: 18 meses

Concentração do ingrediente ativo (pureza): 91,7%

Referência: Manus, A. G. et al. (1981). 18-Month chronic toxicity and potential carcinogenicity study in mice. Litton Bionetics. Dossiê de registro submetido à ANVISA.

O objetivo do estudo foi avaliar o potencial carcinogênico do ingrediente ativo forato quando administrado na dieta de camundongos nas doses de 1, 3 ou 6 ppm pelo período de 18 meses. Os animais tinham 41 dias de idade quando iniciaram o tratamento.

Durante o curso do estudo 91 camundongos morreram ou foram sacrificados em condições moribundas. Foi observado mortalidade em todos os grupos tratados, inclusive no controle [Machos: controle – 5/50 (10%); 1 ppm – 7/50 (14%); 3 ppm – 11/50 (22%) e 6 ppm – 9/5 (18%). Fêmeas: controle – 13/50 (26%); 1 ppm – 13/50 (26%); 3 ppm – 16/50 (32,7%) e 6 ppm – 17/50 (34,7%)].

Os animais que receberam a substância teste (em todas as doses), bem como os do grupo controle apresentaram perda de pêlo em regiões restritas do corpo ou em múltiplas áreas. Os machos tratados com a maior dose apresentaram redução do peso corpóreo nas primeiras semanas do estudo (1ª, 3ª e 5ª semana), já as fêmeas que receberam 6 ppm da substância teste exibiram diminuição de peso em praticamente todas as 25 primeiras semanas do estudo. Com relação ao consumo de alimentos, todos os grupos tratados (machos e fêmeas) apresentaram reduções no consumo da dieta.

Houve alta incidência de alguns sinais clínicos como tremores, hiperatividade e salivação excessiva, os mesmos foram mais frequentes nos animais tratados com 6 ppm em relação ao grupo controle.

Foram observados linfomas malignos nos animais tratados, sendo que estes foram relativamente comuns, particularmente em fêmeas e geralmente envolveram o trato gastrointestinal, timo, fígado, rins e nódulos linfáticos, a incidência desses achados não foi relacionada à quantidade da substância teste administrada. Os animais tratados apresentaram adenomas, contudo esses achados não foram considerados relacionados ao tratamento, uma vez que o grupo controle também exibiu essas alterações. A incidência de adenoma alveolar e bronquiolar foi maior nos machos tratados com 6 ppm (8/50, comparado a 3/50 do controle), contudo esse efeito não foi atribuído ao tratamento, uma vez que, segundo o diretor do estudo, o aumento da incidência não foi estatisticamente significante e esse tumor é frequentemente obervado em camundongos da linhagem CD-1.

Outras alterações que foram observadas nos animais tratados e não tratados (controle) foram: amiloidose; acúmulo multifocal de neutrofilos, linfócitos e macrófagos, frequentemente associado à hepatócitos degenerados ou necróticos; estômago exibindo uma mistura de células inflamatórias infiltradas, edema e hiperplasia com formação de criptas císticas; mielofibrose; infiltrados linfocíticos foram geralmente vistos nos rins e na bexiga urinária em todos os grupos; hiperplasia endometrial cística; lesões nos olhos (a maioria consistiu de catarata). O diretor do estudo concluiu que as alterações patológicas não foram atribuídas à administração prolongada do forato na dieta. Segundo o mesmo, todas as alterações foram consideradas achados incidentais ou parte de complexas doenças espontâneas em camundongos.

Baseado no decréscimo do peso corpóreo e nos sinais clínicos de toxicidade na maior dose. O NOEL estabelecido para esse estudo foi 3 ppm, equivalente a 0,45 mg/kg de peso corpóreo por dia.

4.5. Toxicidade sobre o sistema endócrino, reprodutivo e desenvolvimento

4.5.1. Toxicidade sobre o sistema endócrino

O sistema endócrino desempenha função essencial nos processos metabólicos do organismo como os processos nutricionais, comportamentais, reprodutivos, funções cardiovasculares, renais e intestinais. A toxicidade endócrina, ou desregulação endócrina é um efeito adverso que interfere com uma ou mais das diversas funções desempenhadas pelo sistema endócrino.

A insulina é um hormônio sintetizado nas células beta do pâncreas que possui diversas funções relacionadas principalmente ao metabolismo energético do organismo, dentre outras: (i) captação, armazenamento e utilização da glicose pelas células; (ii) síntese de ácidos graxos; (iii) armazenamento do excesso de gordura no tecido adiposo; (iv) armazenamento de proteínas nos tecidos. (HARRISON, 2009; MASHARANI; KARAM, 2001).

Um estudo epidemiológico de caso controle realizado no período 1993-97 investigou a ocorrência de diabetes mellitus gestacional entre 11.273 mulheres residentes em Iowa e na Carolina do Norte e sua associação com exposição a agrotóxicos no primeiro trimestre da gravidez. As exposições foram classificadas como

indireta (plantio, poda, colheita), residencial (uso doméstico ou no jardim) e na agricultura (preparação, aplicação ou manutenção dos equipamentos de aplicação de agrotóxicos). Para estudar a exposição a agrotóxicos específicos foram levantados aqueles mais frequentemente utilizados na agricultura como os organofosforados (diazonona, malationa, terbufós e forato), carbamatos (carbaril e carbofurano) e herbicidas (2,4-D, 2,4,5-T, 2,4,5-TP, alacloro, atrazina, cianazina, dicamba, glifosato, pendimetalina, trifuralina). Na análise multivariada identificou-se associação estatisticamente significativa de 2,4,5-T, 2,4,5-TP, atrazina, diazinona, carbofurano e forato com o aumento no risco de ocorrência de diabetes mellitus gestacional (SALDANA ET AL, 2007).

A diminuição de insulina ocorre na diabetes mellitus tipo 1 e também já foi observada após a exposição a substâncias químicas que desencadeiam eventos "diabetes-semelhantes" (LUKIĆ; STOSIĆ-GRUJICIĆ; SHAHIN, 1998; SZKUDELSKI, 2001; LENZEN, 2008). Agrotóxicos organofosforados também podem desencadear efeitos adversos associados à diabetes (MEGGS; BREWER, 2007; ABDOLLAHI *et al*, 2004) mesmo após a exposição durante a gestação e lactação (LASSITER et al, 2008; LASSITER; BRIMIJOIN, 2008; MEYER; SEIDLER, F.J.; SLOTKIN, 2004; SLOTKIN; BROWN; SEIDLER, 2005). Nessas condições de hipoinsulinemia, diversos efeitos graves são desencadeados que, não só prejudicam a qualidade de vida do indivíduo, mas podem levar à morte (FORD, 2005; LEE *et al*, 2008).

Durante a hipoinsulinemia ocorre a mobilização de gordura para o sangue circulante que deveria ter sido armazenada no tecido adiposo. O acúmulo de triglicerídeos e colesterol no sangue pode desencadear a aterosclerose, ataques cardíacos, acidentes vasculares cerebrais (GUYTON; HALL, 1996). Além disso, o acúmulo de ácidos graxos no fígado leva à formação de corpos cetônicos (ácido acetoacético, acetona e ácido β-hidroxibutírico), que podem levar ao coma e à morte (NELSON; COX, 2004). A depleção protéica, que também ocorre quando a insulina encontra-se diminuída, provoca fraqueza e comprometimento de diversas funções orgânicas (GUYTON; HALL, 1996; NELSON; COX, 2004).

4.5.2. Toxicidade reprodutiva

A toxicidade reprodutiva pode ser definida como a ocorrência de efeitos adversos no sistema reprodutivo após a exposição a uma substância química. A toxicidade pode ser direcionada aos órgãos reprodutivos e/ou ao sistema endócrino. A manifestação da toxicidade pode ser identificada como alterações no comportamento sexual, fertilidade, desfechos da gravidez ou modificações em outras funções que dependem da integridade do sistema reprodutivo de maneira geral (AGENCY FOR TOXIC SUBSTANCES AND DISEASE REGISTRY, 2001).

Estudo 1 *

Ano: 1965

Espécie: Camundongos

Nº de animais: 8♂ e 16♀

Doses: 0,6; 1,5 e 3 ppm - equivalente a 0,09; 0,23 e 0,45 mg/kg p.c./dia

Via: Oral (dieta)

Concentração do ingrediente ativo (pureza): Não informada

Referência: American Cyanamid Co. (1965) Report on Thimet systemic insecticide: successive generation studies with mice. Unpublished report from Central Medical Department Cyanamid. Submitted to WHO by American Cynamid Co., Wayne, NJ, USA. *Apud* IPCS (1994)

* Estudo multigeração (3 gerações, com 2 ninhadas/geração)

Não foram observados efeitos sobre os índices de fertilidade, gestação ou viabilidade, mas o índice de lactação foi levemente reduzido no grupo que recebeu 3 ppm de forato na dieta. As avaliações microscópicas e patológicas dos tecidos não revelaram anomalias relacionadas ao tratamento. O NOAEL foi fixado em 1,5 ppm, equivalente a **0,23 mg/kg p.c./dia**, baseado na diminuição do índice de lactação (American Cyanamid Co., 1965).

Estudo 2

Ano: 1991

Espécie: Ratos - COBS CD(SD)

Nº de animais: 25♂ e 25♀

Doses: 1, 2, 4 e 6 ppm - equivalente a 0,09, 0,17, 0,35 e 0,52 mg/kg p.c./dia em machos e 0,10, 0,20, 0,40 e 0,62 mg/kg p.c./dia em fêmeas

Via: Oral (dieta)

Concentração do ingrediente ativo (pureza): 92,1%

Referência: Schroeder, R.E. & Daly, I.W. (1991) A two-generation (two litters) reproduction study with AC 35,024 to rats. Final report, Project ID No. 88-3350. Unpublished report dated 23 September 1991 from Bio/dynamics Inc., East Millstone, NJ, USA. Submitted to WHO by American Cyanamid Co., Princeton, NJ, USA. *Apud* IPCS (1994)

Os animais foram expostos por no mínimo 60 dias antes do acasalamento, e produziram 2 ninhadas consecutivas (F_{1a} e F_{1b}). Para formar a segunda geração parental de animais, grupos de 25 machos e 25 fêmeas foram selecionados da ninhada F_{1b}. Devido à alta incidência de mortalidade durante o período pós-desmame entre os filhotes F_{1b} que receberam 6 ppm, 30 machos e 30 fêmeas foram selecionados para formar a geração parental F_1. Os animais da segunda geração parental foram tratados durante 100 dias antes do acasalamento e produziram 2 ninhadas consecutivas (F_{2a} e F_{2b}). Os animais foram expostos continuamente ao forato antes do acasalamento e até o desmame dos filhotes em ambas as gerações. O nível de colinesterase foi determinado no plasma, eritrócitos e cérebro de 10 animais (pais da geração F_1) por sexo em cada grupo ao final do estudo (sacrifício).

Foi observado tremores e diminuição do peso corpóreo nos animais da geração parental tratados com concentrções de forato acima de 4 ppm; nos animais expostos a 6 ppm houve aumento no índice de mortalidade e os principais sinais clínicos observados foram movimentos convulsivos e comportamento agressivo. A avaliação clínica feita rotineiramente nos animais revelou aumento na incidência de efeitos oculares, como exoftalmia, protusão dos tecidos da córnea e opacidade nos grupos que receberam 6 ppm da substância teste. A avaliação oftalmológica subsequente mostrou aumento marcante na incidência e severidade de infecções afetando a córnea, trato uveal anterior e segmento posterior dos olhos nos ratos tratados com a maior dose. Os níveis de colinesterase plasmática foram significativamente diminuídos em fêmeas expostas a 4 e 6 ppm e nos machos tratados com 6 ppm de forato. A atividade eritrocitária foi inibida levemente (10-11%) em machos e fêmeas expostos a 6 ppm. Houve significativa

inibição da atividade colinesterásica cerebral nos machos expostos a 6 ppm (40%) e nas fêmeas expostas a 4 ppm (59%) e 6 ppm (83%).

Foi observada significativa redução na sobrevivência dos filhotes em todas as ninhadas provenientes de ambas as gerações parentais expostas a 6ppm e da ninhada F_{2a} tratada com 4 ppm. O peso médio dos filhotes foi reduzido, nas ninhadas F_{1a} e $F_{1b,}$ dos animais que receberam as duas maiores doses. O peso médio dos filhotes da ninhada F_{2a} expostos a 4 ppm foi diminuído, e também, dos filhotes das ninhadas F_{2a} e F_{2b} que foram expostos a 6 ppm. Foi registrada uma mancha anogenital nos filhotes F_{1b} e F_{2b} testados com a maior dose, mas não foi observado no grupo controle. O NOAEL foi fixado em 2 ppm, equivalente a **0,17 mg/kg p.c./dia** (Schroeder & Daly, 1991).

4.5.3. Toxicidade sobre o desenvolvimento embriofetal

A toxicidade do desenvolvimento refere-se aos efeitos adversos no organismo em formação que ocorrem após a exposição a substâncias químicas antes da concepção, durante o desenvolvimento pré-natal, pós-natal até a maturação sexual. Efeitos adversos no desenvolvimento podem ser detectados em qualquer momento da vida de um indivíduo (AGENCY FOR TOXIC SUBSTANCES AND DISEASE REGISTRY, 2001).

Estudo 1

Ano: 1978

Espécie: Ratas Crl:COBS CD (SD)BR

Nº de animais: 25 fêmeas prenhas/grupo

Doses: 0,125; 0,25 ou 0,5 mg/kg p.c./dia

Via: Oral (entubação gástrica)

Tempo de exposição: 6º ao 15º dia de gestação

Dia do sacrifício: 20º dia de gestação

Concentração do ingrediente ativo (pureza): 91,7%

Referência: Litton Bionetics (1978). Teratology study in rats; Thimet$^{(R)}$ phorate. Final unpublished report from Litton Bionetics, Inc. Unpublished report from Central Medical Department, American Cyanamid Co. Submitted to WHO by American Cynamid Co., Wayne, NJ, USA. *Apud* IPCS

Durante o período gestacional foram observadas mortes no grupo de fêmeas expostas a menor dose (1/24) e a maior dose (7/23). Os fetos expostos a 0,5 mg/kg

p.c./dia apresentaram aumento na frequência cardíaca. O NOAEL para teratogenicidade foi fixado em 0,25 mg/kg pc/dia (Litton Bionetics, 1978).

Estudo 2

Ano: 1990

Espécie: Ratas CDBRVAF/Plus (SD)

Nº de animais: 8 fêmeas prenhas/grupo

Doses: 0,25; 0,5; 0,7 e 0,9 mg/kg p.c./dia

Via: Oral (gavage)

Tempo de exposição: 6º ao 15º dia de gestação

Dia do sacrifício: não informado

Concentração do ingrediente ativo (pureza): 92,1%

Referência: Lochry, E.A. (1990a). An oral developmental toxicity (embryo-fetal toxicity/teratogenicity) pilot study with AC 35,024 in rats. Final report, Project ID No. 101P-012. Unpublished report dated 19 June 1990 from Argus Research Laboratories Inc., Horsham, PA, USA. Submitted to WHO by American Cyanamid Co., Princeton, NJ, USA. Apud IPCS

Doses superiores a 0,5 mg/kg p.c./dia foram letais para as mães e nenhuma fêmea sobreviveu após o 12º dia de gestação. Os sinais clínicos de toxicidade, antes da morte, incluiram tremores, espasmos, salivação excessiva, exoftalmia, ataxia, convulsão clônica, diminuição do peso corporal e da ingestão de alimentos. A avaliação dos animais que morreram revelou glândulas adrenais obstruídas e aumentadas. O NOAEL foi fixado em 0,25 mg/kg pc/dia para toxicidade maternal e do desenvolvimento (Lochry, 1990a).

Estudo 3

Ano: 1990

Espécie: Ratas CDBRVAF/Plus (Sprague-Dawley)

Nº de animais: 25 (grupo controle e grupo tratado com 0,4 mg/kg/dia) e 24 nos demais grupos tratados.

Doses: 0,1; 0,2; 0,3 e 0,4 mg/kg/dia

Via: Oral (gavage)

Tempo de exposição: 6º ao 15º dia de gestação presumida

Dia do sacrifício: 20º dia de gestação

Concentração do ingrediente ativo (pureza): 92,1%

Referência: Lochry, E. A. et al (1990). An oral developmental toxicity (embrio-fetal toxicity/teratogenicity) definitive study with AC 35.024 in rats. Dossiê de registro submetido à ANVISA

Seis ratas que receberam 0,4 mg/kg/dia de forato morreram após o tratamento com 5 a 10 doses (entre o 11º e 16º dias de gestação presumida). Uma rata tratada com a maior dose foi encontrada morta no 15º dia de gestação presumida e não estava grávida. Todas as outras ratas foram encontradas mortas no 11º (duas ratas), 15º (uma rata) ou 16º dia (duas ratas) de gestação (e estavam grávidas realmente grávidas). Essas mortes foram consideradas um efeito da substância teste em virtude dos seguintes fatos: 1) As ratas apresentaram alterações associadas à substância teste (em vistude das seguintes observações clínicas: decréscimo do ganho de peso e/ou perda de peso corpóreo, redução no consumo de alimento e lesões observadas na necropsia) e 2) Todos (8/8) animais tratados com as doses de 0,5; 0,7 e 0,9 mg/kg/dia morreram durante a realização de um estudo piloto.

O grupo tratado com a maior dose apresentou signiticativo aumento no número de animais que exibiram tremores, cromodacriorréia, pele abdominal com mancha de urina, decréscimo da atividade motora, cromorrinorréia, salivação excessiva, reflexos prejudicados, substância vermelha em torno do nariz e dificuldade respiratória. No grupo exposto a 0,4 mg/kg/dia foi observado, em relação ao grupo controle, aumento no número de ratas que exibiram substância vermelha na vagina, substância avermelhada ou "manchada" na região oral e postura arqueada.

Os dados de necropsia revelaram aumento significativo ($p \leq 0,01$) no número de animais tratados com a maior dose que apresentaram pele abdominal com mancha de urina, cromodacriorréia, decréscimo da atividade motora, presença de substância vermelha, amarela ou "manchada" em torno dos olhos, nariz e/ou boca, em comparação ao grupo controle. Nos grupo exposto a 0,4 mg/kg/dia , foi observado aumento no número de fêmeas que apresentaram postura arqueada e substância vermelha ou amarela em torno da região anal e vaginal, em relação ao grupo não tratado. As fêmeas que receberam a maior dose apresentaram, ainda, aumento das glândulas adrenais.

A administração de 0,4 mg/kg/dia da substância teste provocou significativa perda de peso ($p \leq 0,01$), decréscimo significativo ($p \leq 0,01$) na média do ganho de peso

materno durante todo o período do estudo e significativa redução no peso materno entre o 12º e 20º dias de gestação. O peso do corpo materno durante o período gestacional foi reduzido ($p \leq 0,01$) no grupo tratado com a maior dose.

Foi observado decréscimo significativo ($p \leq 0,01$) no consumo de alimento no grupo tratado com a maior dose, essa alteração ocorreu durante todo o estudo e persistiu durante o período pós-dosagem quando comparado ao grupo controle.

No grupo tratado com 0,4 mg/kg/dia foi observado aumento significativo na incidência ($p \leq 0,05$ a $p \leq 0,01$) de fetos e/ou ninhadas com variações na ossificação do esqueleto (retardo na ossificação das esternébras, púbis não calcificado ou com calcificação incompleta e ísquio pobremente calcificado) e significativa ($p \leq 0,05$) redução no peso corporeo do feto. As variações quanto ao atraso na ossificação do esterno e pélvis foram reversíveis, esperadas e inter-relacionadas com o decréscimo significativo ($p \leq 0,05$) no peso do corpo fetal nesse grupo de dose.

Baseado na mortalidade, nos sinais clínicos de toxicidade, no significativo decréscimo no consumo de alimentos e no peso corpóreo materno, assim como na redução do peso corpóreo e retardo na ossificação do esqueleto fetal na maior dose, o NOAEL estabelecido foi **0,3 mg/kg/dia.**

Estudo 4

Ano: 1986

Espécie: Coelhas (New Zealand)

Nº de animais: 5 fêmeas prenhas/grupo

Doses: 0,3; 0,6; 0,9; 1,2 e 1,5 mg/kg/dia

Via: Oral (gavage)

Tempo de exposição: 6º ao 18º dia de gestação

Dia do sacrifício: Não informado

Concentração do ingrediente ativo (pureza): 92,1%

Referência: Schroeder, R.E. & Daly, I.W. (1986) A range-finding teratology study with phorate in rabbits. Final report, project No. 86-3038. Unpublished report dated 5 August 1986 from Bio/dynamics Inc., East Millstone, NJ, USA. Submitted to WHO by American Cyanamid Co., Princeton, NJ, USA. *Apud* IPCS

A incidência de mortalidade nos seis grupos foi de 0/5, 1/5, 1/5, 1/5, 2/5 e 4/5, nos grupos tratados com 0,3; 0,6; 0,9; 1,2 e 1,5 mg/kg/dia de forato, respectivamente.

Foi observada perda de peso corporal nas fêmeas expostas a 1,2 mg/kg p.c./dia. No grupo exposto a maior dose, somente uma fêmea sobreviveu até o dia do sacrifício. Houve redução na ingestão de alimento nos grupos expostos a 0,3; 0,6; 0,9 e 1,2 mg/kg/dia, embora sem clara relação dose-resposta. Foi observado aumento no número de reabsorções e perdas pós-implantação nos animais expostos a doses superiores a 0,6 mg/kg/dia e redução do peso corporal fetal nos animais expostos a 1,2 mg/kg/dia. Baseado na redução no consumo de alimentos e na mortalidade materna, o LOAEL estabelecido foi 0,3 mg/kg/dia.

Estudo 5

Ano: 1987

Espécie: Coelhas (New Zealand)

Nº de animais: 20 fêmeas prenhas/grupo

Doses: 0,15; 0,5; 0,9 e 1,2 mg/kg/dia

Via: Oral (gavage)

Tempo de exposição: 6º ao 18º dia de gestação

Dia do sacrifício: Não informado

Concentração do ingrediente ativo (pureza): 92,1%

Referência: Schroeder, R.E. & Daly, I.W. (1987) A teratology study with phorate in rabbits. Final report, project No. 86-3039. Unpublished report dated 6 April 1987 from Bio/dynamics Inc., East Millstone, NJ, USA. Submitted to WHO by American Cyanamid Co., Princeton, NJ, USA. *Apud* IPCS

Oito fêmeas expostas à maior dose morreram entre 14º e 19º dia de gestação, uma fêmea abortou e outra pariu prematuramente no 28º dia de gestação, portanto, somente 10 fêmeas sobreviveram até o dia do sacrifício. A morte de duas fêmeas tratadas com 0,9 e uma com 0,5 mg/kg também foi atribuída à administração da substância teste. Foi observada redução no peso corporal e no ganho de peso nos animais que receberam doses maiores ou iguais a 0,5 mg/kg. As fêmeas expostas a 1,2 mg/kg apresentaram diminuição no consumo de alimentos. Não foram observados efeitos em relação à perdas pós-implantação, ao número de reabsorções, ao número de fetos vivos, ao peso corporal fetal e à razão de sexo dos fetos. No grupo tratado com a maior dose, todos os três fetos de uma única ninhada (cinco dos oito sítios de implantação estavam com reabsorção precoce) apresentaram pálpebras abertas, escápula

curvada, ausência do processo supraorbital, margem irregular do osso frontal e moleira anterior deslocada. Como algumas alterações também foram observadas no controle histórico, as evidências não foram suficientes para indicar efeito teratogênico nos coelhos expostos ao forato. O NOAEL para toxicidade materna foi fixado em 0,15 mg/kg/dia, baseado na alta incidência de mortalidade e redução do peso corporal nas doses maiores ou iguais a 0,5 mg/kg/dia. O NOAEL para toxicidade do desenvolvimento foi em 0,9 mg/kg p.c./dia.

4.6. Imunotoxicidade

A imunotoxicidade é definida como a interação de substâncias químicas com o sistema imunológico, desencadeando efeitos adversos (Descotes, 1994; Berlin et al, 1987).

O sistema imunológico desempenha sua função de imunovigilância, contra patógenos e células potencialmente neoplásicas, utilizando seus componentes celulares - como os linfócitos e fagócitos- e componentes humorais (anticorpos) (Abbas et al, 2000).

Alterações histopatológicas de tecidos e órgãos do sistema imunológico influenciam na maturação e nas subpopulações de linfócitos e nas alterações funcionais das células imunocompetentes foram descritas em diversos estudos (CASALE et al, 1993; SELGRADE et al, 1984; BARNETT; MCGOWAN; GENTRY; 1980; VOCCIA et al, 1999).

Os agrotóxicos organofosforados podem desregular o sistema imune e afetar mecanismos imunológicos específicos (humorais) e não específicos (celulares). A exposição crônica a baixas doses durante períodos prolongados pode reduzir as respostas imunes humorais. Barnett, 1994 apud Repetto; Baliga, 1996 demonstraram que a exposição ao forato reduz a contagem de linfócitos.

Camundongos suíços albinos adultos foram expostos por via inalatória ao forato. Os animais foram expostos na mesma dose recomendada pelos fabricantes para aplicação no campo (20 kg/hectare) por agricultores. A exposição ocorreu por 3 horas e meia, seis dias por semana, durante 3 meses (MOROWATI, 1998). Na semana 2 de exposição, foi observado aumento de leucócitos totais, particularmente neutrófilos e monócitos. O aumento de monócitos também foi registrado nas semanas 8, 10 e 12. Na semana 4, foi observada apenas a diminuição de linfócitos, mas posteriormente (semanas 6, 8, 10 e 12) as contagens de leucócitos totais, neutrófilos e linfócitos

também diminuíram. Os resultados indicam que o forato causou efeitos imunotóxicos, levando à imunosupressão nos animais expostos por via inalatória em doses correspondentes a exposição humana ocupacional (MOROWATI, 1998).

4.7. Neurotoxicidade

Neurotoxicidade é uma alteração adversa na estrutura ou função do sistema nervoso central e/ou periférico após a exposição a um agente físico, químico ou biológico (TILSON, 1990). Efeitos neurotóxicos funcionais incluem alterações adversas nas funções somáticas/autonômicas, sensoriais, motoras e/ou cognitivas. Efeitos neurotóxicos estruturais são definidos como alterações neuro-anatômicas que ocorrem em qualquer nível da organização do sistema nervoso; alterações funcionais são definidas como efeitos neuroquímicos, neurofisiológicos ou comportamental.

Os químicos podem ser categorizados dentro de quatro classes: aqueles que atuam sobre o sistema nervoso central, fibras nervosas periféricas, ou músculos ou outros tecidos (ALBERT, 1973). Alterações na função podem resultar da toxicidade de outros sistemas órgãos específicos, e essa alteração indireta pode ser considerada adversa. Por exemplo, exposição à alta dose de um químico pode causar dano no fígado, resultando em mal-estar geral e diminuição de um desfecho funcional tal como atividade motora. Nesse caso a alteração motora pode ser considerada como adversa, mas não necessariamente neurotóxica.

Efeitos neurotóxicos podem ser observados em vários níveis de organização do sistema nervoso, incluindo neuro-quimico, anatômico, fisiológico ou comportamental. No nível neuro-quimico, por exemplo, o agente neurotóxico pode inibir a síntese de transmissores ou macromoléculas, alterar o fluxo de íons através da membrana celular, ou impedir a liberação de neurotransmissores no terminal nervoso. Alterações anatômicas podem incluir alterações no corpo celular, o axônio, ou na bainha de mielina. No nível fisiológico o químico pode alterar o limiar para ativação neural ou reduzir a velocidade de neurotransmissão. Alterações de comportamento podem incluir alterações significativas nas sensações da visão, audição, ou tato; alterações de reflexos simples e complexos e funções motoras; alterações nas funções cognitivas tal como aprendizado, memória ou atenção; e alterações no humor, tal como medo ou raiva, desorientação como pessoa, tempo ou espaço, ou distorções de pensamentos e sentimentos, tal como delírio e alucinações.

4.7.1. Mecanismo de ação

O principal efeito prejudicial associado à exposição a OP envolve efeitos no sistema nervoso e suas conseqüências. O neurotransmissor acetilcolina está presente no sistema nervoso autonômico periférico, no sistema nervoso motor somáticos e em algumas porções do sistema nervoso central (SNC). Após a sua liberação no nervo sináptico ou na junção neuromuscular, o transmissor é rapidamente hidrolisado pela acetilcolinesterase.

Os músculos, glândulas e fibras nervosas são estimulados ou inibidos pela constante descarga de sinais através das sinapses. Essa é uma reação que acontece rapidamente com a acetilcolina causando estimulação e a acetilcolinesterase é responsável pela finalização do sinal. O funcionamento do sistema nervoso necessita da enzima chamada colinestersase (ChE), que facilita a transmissão do impulso nervoso. Quando um agrotóxico inibidor da colinesterase entra no sistema, essa função é negativamente afetada.

O mecanismo de toxicidade dos organofosforados (OP) envolve o processo de fosforilação do grupo hiroxil-serina e a ligação irreversível desativando, desse modo, a esterase o que resulta no acúmulo da acetilcolina na placa terminal (CECCHINE et al, 2000; DYRO, 2003). Como resultado da acumulação da acetilcolina na junção neuromuscular, ocorre a persistente despolarização do músculo esquelético e isso resulta em fraqueza e fasciculações, e desregulação da transmissão neural no SNC (SLAPPER, 1999).

Dessa maneira, os organofosforados agem inibindo a atividade da acetilcolinesterase (AChE), enzima responsável por mediar a hidrólise da acetilcolina em ácido acético e colina. Através da fosforilação da enzima, os organofosforados bloqueiam a atividade catalítica da AChE, interrompendo a transmissão do impulso nervoso nas sinapses colinérgicas do sistema nervoso central (SNC), sistema nervoso autônomo (SNA) e da junção neuromuscular. A inativação da AChE provoca uma hiper-estimulação colinérgica pelo acúmulo de acetilcolina na fenda sináptica (TAFURI; ROBERTS, 1987; PRUETT et al, 1992; KECIK et al, 1993; BEACH et al, 1996; SHEETS et al, 1997; RAY, 1998; ESKENAZI; BRADMAN; CASTORINA, 1999; RAY; RICHARDS, 2001; MOSER et al, 2004; COSTA, 2006; KELLAR, 2006; JAMESON; SEIDLER; SLOTKIN, 2007; NARAVANENI; JAMIL, 2007; SLOTKIN; SEIDLER; FUMAGALLI, 2007; ALON et al, 2008; BJØRLING-POULSEN;

ANDERSEN; GRANDJEAN, 2008; DAVIES; EDDLESTON; BUCKLEY, 2008; LAETZ et al, 2009).

Tanto o forato quanto vários de seus metabólitos agem inibindo a AChE. O forato é um inseticida extremamente tóxico (KASHYAP et al, 1984; WORLD HEALTH ORGANIZATION/FOOD AND AGRICULTURE ORGANIZATION, 1988) que apresenta elevada neurotoxicidade em mamíferos (BOSHOFF; PRETORIUS, 1979; GRANDJEAN; LANDRIGAN, 2006). Seus efeitos neurotóxicos em humanos são conhecidos e foram descritos em vários estudos (YOUNG; JUNG; AYER, 1979; KASHYAP et al, 1984; WORLD HEALTH ORGANIZATION/FOOD AND AGRICULTURE ORGANIZATION, 1988; KUSIC et al, 1991; DOBOZY, 1998; ANDERSEN; NIELSEN; GRANDJEAN, 2000; THANAL, 2001; JAYAKUMAR, 2002; MISSION, 2006; PETER; PRABHAKAR; PICHAMUTHU, 2008a; 2008b).

A exposição crônica a agrotóxicos organofosforados, ainda que em baixas doses, pode produzir efeitos neurotóxicos (RAY; RICHARDS, 2001; SLOTKIN; LEVIN; SEIDLER, 2006; BJØRLING-POULSEN; ANDERSEN; GRANDJEAN, 2008; SLOTKIN et al, 2008). A exposição a baixas doses durante o desenvolvimento fetal também pode produzir neurotoxicidade (HARNLY et al, 2005; SLOTKIN; LEVIN; SEIDLER, 2006; JAMESON; SEIDLER; SLOTKIN, 2007; SLOTKIN; SEIDLER, 2007). A exposição pré-natal a organofosforados foi demonstrada através da detecção desses compostos e de seus metabólitos no mecônio, o conteúdo intestinal do recém-nascido, em decorrência da absorção através do cordão umbilical, difusão através da superfície da placenta e ou deglutição do líquido amniótico pelo feto (WHYATT et al, 2001 apud BURATTI; LEONI; TESTAI, 2007).

Estudos demonstram que a exposição contínua de animais ainda em fase de desenvolvimento a baixas doses de organofosforados pode afetar adversamente o crescimento e a maturação neurocomportamental (AHLBOM; FREDRIKSSON; ERIKSSON, 1995; COSTA, 2006; ESKENAZI; BRADMAN; CASTORINA, 1999). A exposição a baixas doses de organofosforados *in utero* ou em recém-nascidos pode levar à deficiência nas habilidades cognitivas dos bebês (BERKOWITZ et al, 2004 apud BURATTI; LEONI; TESTAI, 2007). O fato da exposição aos organofosforados provocar alterações durante o desenvolvimento cerebral, mesmo sem haver inibição da AChE, comprova esse argumento, reforçando ainda a incapacidade desse marcador para a avaliação da exposição ou dos efeitos relacionados à neurotoxicidade (SLOTKIN; LEVIN; SEIDLER, 2006; SLOTKIN et al, 2008). Também foi demonstrado que

crianças em geral são mais suscetíveis a organofosforados devido aos altos níveis de exposição e ou à imaturidade do metabolismo (MILLER et al, 1996 apud BURATTI; LEONI; TESTAI, 2007; COLE et al, 2003 apud BURATTI; LEONI; TESTAI, 2007).

Os danos neurológicos induzidos por organofosforados podem durar muito tempo, podendo persistir por mais de dez anos após o desaparecimento dos sintomas de intoxicação aguda, o que sugere dano residual permanente (KAMEL; HOPPIN, 2004; KAMEL et al, 2005). Mesmo exposições moderadas podem resultar em sequelas neurológicas de longo prazo (WESSELING et al, 2002; KAMEL; HOPPIN, 2004).

Estudos epidemiológicos também sugerem que a exposição a organofosforados está associada à desordens psiquiátricas, particularmente depressão e suicídio. A exposição a estes compostos pode levar ao desenvolvimento de depressão, um fator importante nos suicídios (STEENLAND et al, 1994; STEPHENS et al, 1995; AMR et al, 1997; FIEDLER et al, 1997; LONDON et al, 1997; VAN WIJNGAARDEN, 2003; LONDON et al, 2005; JAGA; DHARMANI, 2007; BESELER et al, 2008). Há evidências de que pacientes cronicamente expostos a organofosforados podem manifestar depressão e déficit cognitivo, sugerindo um incremento no risco de suicídio entre os expostos a esses compostos (PARRÓN; HERNÀNDEZ; VILLANUEVA, 1996; PELEGRINO et al, 2006).

4.7.2. Manifestações clínicas

Os inibidores de colinesterase causam três quadros clínicos de intoxicação no homem e em animais: toxicidade aguda; síndrome intermediária e polineuropatia retardada. Os efeitos decorrentes da exposição aos organofosforados variam de acordo com fatores que podem modificar a toxicidade a esses agrotóxicos, incluindo o tipo de organofosforado utilizado, a dose, duração da exposição, via de absorção, o órgão atingido, fatores sócio-econômicos e culturais e condições ambientais (RAY, 1998).

O bloqueio irreversível da AChE pelos organofosforados desencadeia um quadro neurotóxico agudo em decorrência da hiper-estimulação colinérgica (KAUSHIK; ROSENFELD; SULTATOS, 2007; BJØRLING-POULSEN; ANDERSEN; GRANDJEAN, 2008). O acúmulo de organofosforados no organismo devido à inibição da atividade colinesterásica provoca efeitos subagudos e crônicos. Em casos brandos, ou quando o composto é prontamente eliminado, os sintomas podem desaparecer rapidamente, porém a AChE pode levar meses para retornar aos níveis normais (CARVALHO, 1993).

4.7.3. Neurotoxicidade aguda

A inibição da AChE por compostos organofosforados desencadeia um quadro neurotóxico agudo conhecido como síndrome colinérgica. Essa síndrome é caracterizada por uma ampla gama de sinais e sintomas resultantes da exacerbação da função colinérgica (KAUSHIK; ROSENFELD; SULTATOS, 2007; BJØRLING-POULSEN; ANDERSEN; GRANDJEAN, 2008).

A intoxicação aguda por anticolinesterases produz uma mistura complexa de sinais muscarínicos e nicotínicos. Sinais e sintomas nicotínicos resultam da acumulação da acetilcolina nas terminações nervosas da musculatura esquelética e gânglios autônomos. Os receptores muscarínicos para a acetilcolina são encontrados primariamente nos músculos lisos, coração e glândulas exócrinas, e suas manifestações clínicas ocorrem nos sistemas circulatório, ocular, urinário e nos aparelhos digestivo e respiratório (CARVALHO, 1993; STOKES et al, 1995; BEACH et al, 1996; KELLAR, 2006).

Sintomas precoces de intoxicação aguda por organofosforados dependem da via de exposição e geralmente ocorrem nas primeiras 12 horas. Quando inalados, os primeiros efeitos geralmente são respiratórios e frequentemente incluem sangramento nasal ou rinorréia, tosse, dor torácica e dificuldade respiratória, além de dor de cabeça. Se ingerido, os sinais precoces mais comuns incluem náusea, vômitos, diarréia e câimbras. Sudorese e contração muscular são observadas na exposição através da pele. O contato com a mucosa ocular pode causar dor, lacrimejamento, visão embaçada, miose e sangramentos (INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY, 1993; FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS, 1996).

A crise colinérgica aguda causada pela inibição da AChE pode levar à morte em minutos (HSIEH et al, 2001). A causa imediata de morte em síndromes colinérgicas por organofosforados resulta da falência respiratória (DAVIES; EDDLESTON; BUCKLEY, 2008). Contribuem para este fato a ação muscarínica de broncoconstrição e de aumento das secreções bronquiais, a ação nicotínica de paralisia dos músculos respiratórios e a ação do SNC de paralisia do centro respiratório (CARVALHO, 1993; PELEGRINO et al, 2006).

A intoxicação aguda por organofosforados tem grande importância epidemiológica para humanos e animais. Diversos casos de envenenamento por forato têm sido observados em humanos.

Peter, Prabhakar e Pichamuthu (2008b) relataram um caso onde uma mulher de 28 anos de idade ingeriu 50 ml de forato após uma tentativa de suicídio. Os sintomas iniciais observados foram vômito, tontura, dor abdominal e perda de consciência. Seguiram-se a esses sintomas hipotensão e dificuldade de respirar, havendo necessidade de ventilação mecânica. Quatro dias após a tentativa de suicídio a paciente apresentou espasmos e tremores, além do aumento do tônus muscular dos membros e redução dos níveis séricos da AChE. A paciente entrou em coma profundo e os achados durante esse período foram consistentes com morte cerebral, incluindo ausência de reflexos corneanos, oculoencefálicos, pupilares e musculares, ausência de reações a estímulos de dor ou calor e ausência de respiração espontânea. O eletroencefalograma mostrou supressão global da atividade cortical. Entretanto, quinze dias após o coma a paciente recobrou completamente a consciência.

Em 1991, Kusic et al descreveram 03 casos de envenenamento em decorrência da exposição ao forato. Dois dos casos foram representados por ingestão intencional (tentativa de suicídio) e em um caso a exposição foi acidental e a via de exposição foi a inalatória. Em todos os casos houve severa intoxicação com a manifestação de sintomas colinérgicos e ainda redução dos níveis de colinesterase eritrocitária.

Em 21 de julho de 2006, 20 moradores do vilarejo de Salkiana, distrito de Jalandhar, Índia, necessitaram de cuidados médicos ao apresentar sintomas neurotóxicos agudos após exposição ao forato. O produto foi aplicado em uma plantação de cana-de-açúcar, e a dispersão do forato pelo ar intoxicou moradores do distrito de Salkiana. Foram atendidos em um hospital público 18 casos de envenenamento, sendo 10 crianças (05 do sexo masculino e 05 do sexo feminino) e 08 adultos (07 mulheres e 01 homem). Outras duas pacientes (14 e 18 anos) foram atendidas em hospitais particulares da região. Os casos mais graves foram registrados em uma escola elementar, onde alunos e professores relataram sentir um cheiro estranho, asfixia e dificuldade respiratória. Em poucos minutos 16 alunos perderam a consciência. Além dos casos registrados na escola, moradores do vilarejo também relataram dificuldade respiratória e perda de consciência. Além da dificuldade respiratória, os sintomas mais frequentes foram mal estar, cefaléia, irritação ocular, tontura, náusea, vômito, lacrimejamento, salivação excessiva, dor muscular e cãibras. Algumas crianças com dificuldade respiratória severa

necessitaram da administração de oxigênio. Seis dias após a exposição ao forato, vários pacientes ainda apresentavam sintomas como irritação ocular, reações dérmicas, mal estar, distúrbios gastrointestinais e problemas urinários. Todos relataram perda de apetite, mesmo após o sexto dia (MISSION, 2006).

Vinte e dois casos de intoxicação após a exposição ao forato foram registrados durante os anos de 1982 e 1993 pelo Programa californiano de vigilância a agravos provocados por agrotóxicos. Em um dos casos registrados em 1982, uma criança de 22 meses morreu após se expor ao forato enquanto brincava no quintal do avô. O produto estava armazenado em uma lata de café, e após a exposição, a criança apresentou náuseas e desfaleceu. Apesar de ter sido levada rapidamente ao hospital, a criança entrou em coma, desenvolveu um quadro de pneumonia e edema cerebral. Quadro dias após a exposição, a criança veio a óbito. Em outro caso registrado no período, um estudante entrou sem camisa em um campo que havia sido tratado com um produto formulado com o ingrediente ativo forato. Ele apresentou cefaléia, dores musculares, náusea, diarréia, irritação dérmica e tontura. Em um caso de exposição ocupacional observado no período, um trabalhador inalou o forato e apresentou sintomas como cefaléia, náuseas e manifestações gastrointestinais (DOBOZY, 1998).

Young, Jung e Ayer (1979) relataram dois casos de envenenamento por exposição ocupacional ao forato. No primeiro caso um trabalhador de 34 anos deu entrada em um hospital após apresentar sintomas como confusão, tontura, náusea, vômito, miose e perda de consciência. No segundo caso um homem de 18 anos de idade necessitou de cuidados médicos após apresentar náuseas, transpiração excessiva, vômito, tontura e perda de coordenação muscular. A esses sintomas seguiu-se um quadro severo de dificuldade respiratória e arritmia cardíaca (taquicardia), salivação excessiva, fasciculação muscular e miose. A concentração de forato no ar da indústria onde trabalhavam os dois homens variou entre 0,07 a 14,6 µg/l.

Um jovem de 16 anos adoeceu após trabalhar com sementes de algodão tratadas com forato. Os sintomas incluíram coma, pressão arterial indetectável, miose, saliva espumosa e sanguinolenta e convulsões. Houve redução da atividade das colinesterases eritrocitária e plasmática (WORLD HEALTH ORGANIZATION/FOOD AND AGRICULTURE ORGANIZATION, 1988).

Em outro caso, um homem que trabalhava manipulando forato apresentou sintomas neurológicos após exposição ocupacional ao produto. Houve redução de 50% da AChE plasmática e da eritrocitária (comparação com valores determinados

previamente à exposição). Um metabólito do forato, o dietil fosfato, foi detectado na urina (WORLD HEALTH ORGANIZATION/FOOD AND AGRICULTURE ORGANIZATION, 1988).

Durante duas semanas, quarenta homens entre 19 e 45 anos que trabalharam na formulação de grânulos de forato (10%) foram acompanhados e tiveram a atividade anticolinesterásica e as manifestações clínicas monitoradas. Os achados durante o período do estudo foram comparados com exames clínicos e laboratoriais realizados antes da exposição. Nenhum dos trabalhadores foi exposto ao forato por pelo menos um mês antes do início do estudo. Durante as duas semanas do estudo cada homem trabalhou 8 horas por dia e a produção foi mantida 07 dias por semana. 60% dos trabalhadores desenvolveram quadro de intoxicação aguda com manifestações neurológicas como cefaléia (45%), fadiga (27,5%) e tontura (37,5%), manifestações gastrointestinais como náuseas (42,5%), vômitos (35%) e dores abdominais (52%), além de bradicardia (48%), irritação ocular (40%) e lacrimejamento (15%). Foi observada redução significativa dos níveis de AChE no fim da primeira semana (55%) e da segunda semana (71%) quando comparados a valores estabelecidos antes da exposição.As manifestações clínicas e alterações laboratoriais aconteceram mesmo diante da adoção de boas praticas de higiene e da utilização de EPI, ratificando a toxicidade do composto e a incapacidade dos EPI em proteger trabalhadores repetidamente expostos ao forato (KASHYAP et al, 1984).

Em 26 de junho de 2001, no estado de Kerala, Índia, um adolescente de 16 anos que trabalhava aplicando forato em plantações de cardamomo apresentou sintomas de intoxicação após inalar o referido agrotóxico. O adolescente apresentou sintomas como mal-estar, vômito e salivação excessiva, indo a óbito poucas horas após a exposição. Também em 26 de junho de 2001, em Kerala, uma mulher apresentou sintomas como tonturas, visão embaçada e vômitos meia hora após trabalhar coletando folhas utilizadas para o preparo de chá que estavam contaminadas com forato. No total 41 trabalhadores de Kerala expostos ocupacionalmente ao forato apresentaram sintomas semelhantes e foram hospitalizados na ocasião (THANAL, 2001).

Em uma comunidade agrícola do distrito de Wyanad, estado de Kerala, Índia, 32 crianças menores de 12 anos (16 do sexo feminino e 15 do sexo masculino) apresentaram sintomas severos de intoxicação por organofosforado. As crianças estavam na Escola Primária de Kottathara durante o incidente no qual os afetados apresentaram sintomas como tontura, náusea, vômitos, cefaléia, visão embaçada, dores

no peito, dificuldade respiratória, dores abdominais, cãibras e, em alguns casos, perda de consciência. A escola localizava-se próximo uma plantação de bananas tratadas no dia 10 de julho de 2002 com 300 g de forato (forato 10%), valor 12 vezes acima da dose recomendada (25 g). O produto então se dispersou através do ar, atingindo a escola e afetando as crianças. Sete dias após o episódio, as crianças retornaram ao hospital apresentando os mesmos sintomas (JAYAKUMAR, 2002).

Em um estudo observacional prospectivo, 35 pacientes adultos admitidos na UTI de um hospital universitário em decorrência de envenenamento por organofosforados foram observados durante o período de 01 ano (maio de 2006 a abril de 2007) com o objetivo de observar as características clínicas e os desfechos decorrentes do envenenamento por esses compostos. Todos os pacientes apresentaram intoxicação com manifestações colinérgicas e nicotínicas e redução dos níveis de pseudocolinesterase (<1000 UI/l). Os pacientes apresentaram miose, salivação excessiva, lacrimejamento, transpiração excessiva e bradicardia. Destes, 04 foram intoxicados por forato, 06 por monocrotofós, 06 por quinalfós, 03 por clorpirifós, 01 por parationa metílica, 04 por outros organofosforados e 11 não tiveram o produto identificado. Trinta pacientes (85,7%) necessitaram de ventilação mecânica durante a internação, 03 entraram em coma e outros 03 apresentaram redução na escala de coma de Glasgow, mas sem progressão para o coma. Esses 06 pacientes desenvolveram síndrome intermediária, havendo necessidade de ventilação mecânica. A síndrome intermediária foi provocada pelo forato (01 caso), pela parationa metílica, (01 caso), pelo quinalfós (01 caso) e nos outros 03 casos o organofosforado responsável não foi descriminado (PETER; PRABHAKAR; PICHAMUTHU, 2008a).

Das e Jena (2000) descreveram um caso de envenenamento severo por forato, com complexas manifestações de encefalopatia, síndrome intermediária e polineuropatia retardada. Uma mulher de 20 anos de idade, em uma tentativa de suaicídio, ingeriu 25 g de um produto formulado à base de forato. Ao dar entrada no hospital a paciente estava consciente, apresentava miose, diaforese e salivação excessiva e aumento das secreções broncopulmonares. Dois dias após ingestão do produto e início dos sintomas a paciente perdeu a consciência e desenvolveu hiperreflexia. Seguiram-se a esse quadro síndrome intermediária e polineuropatia retardada.

Diversos estudos descreveram parkinsonismo em indivíduos após exposição aguda a agrotóxicos organofosforados (DAVIS; YESAVAGE; BERGER, 1978; BHATT; ELIAS; MANKODI, 1999; MÜLLER-VAHL; KOLBE; DENGLER, 1999;

ARIMA et al, 2003; KAMEL; HOPPIN, 2004; HANCOCK et al, 2008). A despeito desse fato, a maioria desses estudos não foi capaz de descriminar especificamente qual agrotóxico organofosforado que levou ao desenvolvimento dos sintomas (KAMEL; HOPPIN, 2004).

Hancock e colaboradores (2008) estudaram 319 casos de Parkinson, comparando os pacientes desse grupo com 296 controles, composto por 252 parentes próximos aos casos e os 44 restantes conjugues ou não parentes, a fim de determinar uma possível relação entre a exposição a agrotóxicos organofosforados e a doença de Parkinson. O estudo relacionou positivamente o uso de organofosforados à doença de Parkinson, uma vez que este agravo estava fortemente relacionado à exposição aos agrotóxicos organofosforados.

Arima et al (2003) descreveram um caso de parkinsonismo após severa síndrome colinérgica por exposição a organofosforados em uma mulher de 81 anos.

Bhatt, Elias e Mankodi (1999) descreveram cinco casos onde os pacientes apresentaram parkinsonismo após exposição a agrotóxicos organofosforados, indicando que a síndrome representa um efeito tóxico da exposição a esses compostos.

Müller-Vahl, Kolbe e Dengler (1999) descreveram uma tentativa de suicídio, onde um homem de 56 anos ingeriu uma quantidade desconhecida de um agrotóxico organofosforado, desenvolvendo uma sintomatologia compatível com quadro de síndrome colinérgica, seguida por parkinsonismo severo. O estudo levou à conclusão de que o parkinsonismo deve ser considerado uma seqüela de intoxicação aguda por organofosforados, mesmo após a reversão da síndrome colinérgica.

Em um estudo de caso, Davis, Yesavage e Berger (1978) relataram uma exposição ocupacional de um agricultor que aplicava agrotóxicos organofosforados em diferentes culturas com auxílio de avião. O paciente já havia apresentado inúmeros episódios de intoxicação aguda a organofosforados, estando cronicamente exposto a esses compostos. Tais achados levantaram a hipótese de relação entre o parkinsonismo e organofosforados, onde a exposição ocupacional pode estar relacionada a um maior risco de desenvolvimento da doença.

Tais estudos fortalecem a evidência epidemiológica de que a exposição a agrotóxicos organofosforados deve ser considerada um fator de risco para a doença de Parkinson e o parkinsonismo.

4.7.4. Síndrome intermediária

Outra manifestação da intoxicação por organofosforados é a síndrome intermediária, descrita como uma complicação tardia em alguns casos de severa intoxicação aguda (SENANAYAKE; KARALLIEDE, 1987; RAY; RICHARDS, 2001). Acredita-se que a síndrome intermediária seja resultado da dessensibilização dos receptores colinérgicos em virtude da persistência da acetilcolina na junção neuromuscular (KAMEL; HOPPIN, 2004; JAYAWARDANE et al, 2008).

Os sintomas aparecem entre 24 e 96 horas após o quadro colinérgico desencadeado por organofosforados e duram vários dias. Observações clínicas incluem fraqueza e paralisia muscular que afeta predominantemente os músculos flexores do pescoço, musculatura dos membros e músculos respiratórios, podendo haver falência respiratória aguda (SENANAYAKE; KARALLIEDE, 1987; PELEGRINO et al, 2006).

Peter, Prabhakar e Pichamuthu (2008a) descreveram 06 casos de síndrome intermediária após intoxicação aguda por organofosforados. A síndrome intermediária foi provocada pelo forato (01 caso), pela parationa metílica, (01 caso), pelo quinalfós (01 caso) e nos outros 03 casos o organofosforado responsável não foi descriminado. A paciente intoxicada pelo forato tinha 28 anos de idade e ingeriu aproximadamente 50 ml do produto, tendo ficado internada por 39 dias. 03 dos pacientes entraram em coma e outros 03 apresentaram redução na escala de coma de Glasgow, mas sem progressão para o coma. Todos necessitaram de ventilação mecânica. Os pacientes que entraram em coma manifestaram tremores associados à rigidez muscular, transpiração excessiva, redução da capacidade respiratória, redução da resposta a estímulos dolorosos e perda progressiva da consciência. Durante o coma, os 03 pacientes apresentaram ausência de reflexos oculoencefálicos, pupilares e da córnea e ausência de reflexos tendinosos profundos.

Peter, Prabhakar e Pichamuthu (2008b) relataram um caso onde uma mulher de 28 anos de idade ingeriu 50 ml de forato após uma tentativa de suicídio. Os sintomas iniciais observados foram vômito, tontura, dor abdominal e perda de consciência. Seguiram-se a esses sintomas hipotensão e dificuldade de respirar, havendo necessidade de ventilação mecânica. Quatro dias após a tentativa de suicídio a paciente apresentou espasmos e tremores, além de aumento do tônus muscular dos membros e redução dos níveis séricos da AChE. A paciente entrou em coma profundo e os achados durante esse período foram consistentes com morte cerebral, incluindo ausência de reflexos corneanos, oculoencefálicos, pupilares e musculares, ausência de reações a estímulos de

dor ou calor e ausência de respiração espontânea. O eletroencefalograma revelou mostrou supressão global da atividade cortical. Entretanto, quinze dias após o coma a paciente recobrou completamente a consciência. Apesar do estudo não classificar o caso como síndrome intermediária, o início dos sintomas entre 24-96 horas após episódio de intoxicação aguda, as manifestações clínicas e o quadro auto-limitante revertido com o tratamento adequado foram compatíveis com a síndrome.

Em um caso de envenenamento por forato, Das e Jena (2000) descreveram síndrome intermediária em uma paciente após quadro de intoxicação aguda. Dois dias após ingestão do produto e início dos sintomas a paciente perdeu a consciência e desenvolveu hiperreflexia. No quarto dia a paciente recobrou a consciência, porém apresentava profunda paralisia muscular, com fraqueza dos músculos flexores do pescoço, dos membros superiores e inferiores e dos músculos da laringe e faringe (síndrome intermediária). Apesar de melhora da fraqueza muscular no 8º dia, surgiu uma parálise flácida no final da segunda semana envolvendo os membros inferiores, levando à atrofia e perda de capacidade motora devido à polineuropatia tardia. Atrofia nos músculos das mãos também foi observada.

4.7.5. Polineuropatia retardada

A polineuropatia retardada induzida por organofosforados (OPIDP - organophosphate-induced delayed polyneuropathy) é uma neuropatia motora distal decorrente da exposição a alguns organofosforados e caracterizada pela degeneração de axônios com desmielinização secundária nos sistemas nervosos central e periférico (SHEETS et al, 1997; RAY, 1998; KELLNER; SANBORN; WILSON, 2000; VIDAIR, 2004; LOTTI; MORETTO, 2005; DOHERTY, 2006; PELEGRINO et al, 2006; BJØRLING-POULSEN; ANDERSEN; GRANDJEAN, 2008).

A indução da neuropatia tardia parece estar associada à inibição de uma carboxiesterase neuronal, a esterase neuropática alvo (NTE - neuropathy target esterase) (SHEETS et al, 1997; RAY, 1998; McCONNELL et al, 1999; VIDAIR, 2004; LOTTI; MORETTO, 2005; DOHERTY, 2006; PELEGRINO et al, 2006; BJØRLING-POULSEN; ANDERSEN; GRANDJEAN, 2008). O mecanismo de inibição da NTE pelos compostos organofosforados envolve a fosforilação da NTE seguida pela perda do potencial de reativação da enzima, onde a clivagem de um grupo ligado ao fósforo resulta em um resíduo fosforil ionizado. Acredita-se que esse resíduo fosforil liga-se à membrana neural, causando a neuropatia tardia (LOTTI et al, 1993; JOINT/FAO/WHO

MEETING ON PESTICIDE RESIDUES, 2002). A inibição dessa enzima presente no sistema nervoso pode resultar em danos permanentes (JOHNSON, 1990; MOSER et al, 2004; LOTTI; MORETTO, 2005).

O quadro neurológico subseqüente à inibição da NTE ocorre entre 1 e 4 semanas após uma única exposição a compostos organofosforados, quando os sintomas clássicos da síndrome colinérgica já diminuíram ou desapareceram (FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS, 1996; RAY, 1998; McCONNELL et al, 1999; LOTTI; MORETTO, 2005; PELEGRINO et al, 2006). Casos humanos dessa neuropatia têm sido observados majoritariamente como consequência de severa intoxicação aguda (RAY; RICHARDS, 2001).

Os sintomas clássicos da polineuropatia retardada incluem dor, formigamento de pés e mãos, seguido de perda da sensibilidade, fraqueza muscular progressiva, espasmos, hiperreflexia e ataxia que pode evoluir para uma paralisia flácida, estendendo-se para as extremidades dos membros superiores e inferiores, com perda da coordenação motora. Mesmo quando a lesão nos nervos periféricos se estabiliza, danos à medula espinhal podem persistir com quadros espásticos, ataxia ou quadriplegia. (SENANAYAKE; KARALLIEDE, 1987; CARVALHO, 1993; FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS, 1996; MOSER et al, 2004; LOTTI; MORETTO, 2005; PELEGRINO et al, 2006). A recuperação pode levar anos após o início dos sintomas, podendo haver dano residual permanente (SENANAYAKE; JOHNSON, 1982; SENANAYAKE; KARALLIEDE, 1987; FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS, 1996).

Das e Jena (2000) relataram um caso de polineuropatia tardia após manifestaçõs clínicas agudas e síndrome intermediária em uma mulher de 20 anos que ingeriu 25 g de um produto formulado à base de forato. Aproximandamente duas semanas após a ingestão do composto, a paciente apresentou uma parálise flácida envolvendo os membros inferiores, levando à atrofia e perda de capacidade motora devido à polineuropatia tardia. Atrofia nos músculos das mãos também foi observada. Exames revelaram axonopatia, isto é, redução na velocidade de condução do impulso nervoso. O eletromiograma sugeriu lesão neurogênica.

4.7.6. Estudos experimentais de neurotoxicidade

Seis galinhas adultas por grupo foram alimentadas com 0 ou 40 ppm forato, equivalente a 5 mg/kg, na dieta durante 4 semanas. Foi formado um terceiro grupo,

controle positivo, que recebeu 4000 ppm fosfato de tri- *orto*-tolil. As galinhas foram anestesiadas e imediatamente perfundidas com formol tamponado, e partes do cérebro, cordão torácico e nervo ciático foram preparados para o exame microscópico. O grupo exposto ao fosfato de tri- *orto*-tolil apresentou perda mielina, mas as galinhas expostos ao forato não apresentaram efeitos adversos sobre as fibras nervosas e a bainha de mielina (LEVINSKAS et al, 1965).

Galinhas Leghorn (22-23 meses de vida) receberam forato (89,5% de pureza) dissolvido em óleo de milho por via oral, gavagem. A DL_{50} após dose única foi de 14,2 mg/kg. Cinquenta galinhas receberam 10 mg/kg de sulfato de atropina, via intramuscular, e 1 h depois receberam dose única de 14,2 mg/kg de forato. Um grupo adicional de 15 galinhas recebeu óleo de milho somente e 15 galinhas que não receberam atropina foram expostas a 500mg/kg de fosfatotri-orto-tolil, como controle positivo. Todas as galinhas sobreviventes do grupo teste e do grupo controle veículo receberam as mesmas doses durante 21 dias, exceto a de sulfato de atropina que recebeu 30 mg/kg. Os animais foram observados diariamente para mortalidade, sinais clínicos e evidência de reações neurotóxicas. A cada 3 dias foram registrados o peso corporal e o consumo de alimentos. Todas as galinhas que morreram durante o estudo e todas as galinhas que foram sacrificadas no fim do estudo (42 dias) foram submetidas à necrópsia. Aquelas sacrificadas no final do estudo foram perfundidas com formol tamponado 10% e o cérebro, coluna vertebral e todo o nervo ciatico direito e esquerdo foram retirados e fixados. Cortes histológicos dos tecidos nervosos foram preparados e corados. Os tecidos de 10 galinhas por grupo foram avaliados histologicamente.

Nenhuma das 15 galinhas alimentadas com oleo de milho morreu e todas as 15 que receberam fosfato de tri-orto-tolil foram sacrificadas ao extreme no dia 16 do estudo com sinais clínicos de neuropatia. Esses sinais incluiram fraqueza generalizada, ataxia e paralisia das pernas e asas. Das 50 galinhas tratadas com forato, 27 morreram dentro de 24h após a 1º dose e 13 dentro de 24h após a 2º dose. Dez galinhas sobreviveram até o fim do estudo. Não foram observados sinais clínicos de neuropatia retardada em qualquer animal controle veículo e teste. Em comparação com o controle veículo, a média do ganho de peso corporal das galinhas testes foi maior entre os dias 0-21 e menor entre os dias 21-42. Não foram observados efeitos relacionados ao forato com relação à necropsia. Avaliação histológica do tecido neural das galinhas controle positivo revelou lesões (degeneração axonal, demielinização, hiperplasia das células de Schwann) relacionadas ao tratamento envolvendo cérebro, medula espinhal e/ou nervo

ciático das 10 galinhas. Essas lesões foram compatíveis com a resposta neurotóxica retardada induzida pelo fosfato de tri-orto-tolil. Degeneração axonal focal do nervo ciático foi observada em 3 de 10 galinhas tratadas com o forato; não foi observada essa degeneração no grupo controle. A degeneração axonal observada nas aves tratadas foi associada com infiltração intersticial das céluas linfóides, que também foi observada nas galinhas controle veiculo e em outros testes. O tratamento não induziu sinais histopatológicos e clínicos indicativos de neuropatia retardada (FLETCHER, 1984).

Os estudos experimentais aportados nos dossiês toxicológicos de registro submetidos à ANVISA, com relação à neurotoxicidade, estão descritos a seguir:

Estudo 1

Ano: 1998

Espécie: Rato (Sprague-Dawley)

Nº de animais: 20/sexo/dose

Doses: 0,25; 0,5; ou 1,0 mg/kg p.c.(dose única)*

Via: Oral (gavage)

Tempo de exposição: 14 dias

Concentração do ingrediente ativo (pureza): 91,8%

Referência: Mandella, R. et. al. (1998). An acute neurotoxicity study with AC 35024 in the rat via oral gavage administration. Dossiê de registro submetido à ANVISA.

*O volume administrado foi 5mL/kg de p.c.

Animais tratados com 0,5 (dois machos e duas fêmeas) e com 1 mg/kg/p.c (2 machos e 5 fêmeas) apresentaram miose. Uma fêmea tratada com 0,5 mg/kg/p.c. exibiu tremores e uma fêmea tratada com a maior dose apresentou fasciculações, ligeiro prejuízo na capacidade locomotora, tremores, assim como alterações na locomoção das patas traseiras. A recuperação foi evidente na segunda semana após a exposição, as avaliações da bateria de observações funcionais realizadas no 8º e 15º dia foram normais em todos os grupos tratados.

A inibição da colinesterase foi um achado típico nos animais expostos às duas maiores doses de forato e foi observado no 1º dia durante a avaliação da bateria de observações funcionais. Houve significativa inibição na atividade da colinesterase eritrócitária, plasmática e cerebral nos machos e fêmeas tratados com a maior dose. A atividade da colinesterase plasmática foi inibida em 27,5% e 67,7%, a eritrocitária em

21,4% e 65,1% e a cerebral em 14% e 65,2%, em machos e fêmeas, respectivamente, em comparação ao grupo controle.

A atividade da acetilcolinesterase cerebral foi estatisticamente reduzida nos machos tratados com 0,5 mg/kg/p.c. imediatamente após a administração da substância teste, quando comparado ao controle. Entretanto, segundo o diretor do estudo, baseado na pequena magnitude desse decréscimo (6%) e na ausência de diminuição estatisticamente ou biologicamente significante da atividade da acetilcolibnesterase cerebral nas fêmeas (sexo mais sensível à inibição da colinesterase pelo forato) tratadas com a mesma dose, bem como ausência de inibição da acetilcolinesterase eritrocitária e plasmática nas fêmeas tratadas com 0,5 mg/kg/p.c., essa ligeira redução na atividade da acetilcolinesterase cerebral nos machos tratados com a maior dose não foi considerada biologicamente significante.

No grupo tratado com a maior dose a completa recuperação da atividade da acetilcolinesterase plasmática ocorreu no 8º dia após a administração da substância teste. A atividade da colinesterase eritrocitária e cerebral foi parcialmente recuperada após o 14º dia.

Baseado nas alterações da bateria de observações funcionais (miose), o NOAEL foi fixado em 0,25 mg/kg/p.c.

Estudo 2

Ano: 2004

Espécie: Rato (Wistar)

Nº de animais: 45 fêmeas*

Doses: 0,03; 0,1 e 0,3** mg/kg p. c. (fêmeas) / 0,03 e 0,1 mg/kg p.c. (filhotes)

Via: Oral (gavage)

Tempo de exposição: do 6º dia após o coito ao 10º dia após parto (fêmeas) e do 11º ao 21º dia depois do parto (filhotes)

Concentração do ingrediente ativo (pureza): 91,8%

Referência: Kaufmann, W. et al. (2004). Devolopmental neurotoxicity study in Wistar rats oral administration to the dams and pups (gavage). Dossiê de registro submetido à ANVISA.

* Com prenhez presumida.

** Antes do inicio da lactação houve diminuição da dose, de 0,3 para 0,2 mg/kg/p.c, em virtude do alto índice de mortalidade das ratas grávidas e dos filhotes.

OBSERVAÇÃO: Em virtude da severa toxicidade materna e excessiva mortalidade dos filhotes, a maior dose foi reduzida de 0,3 mg/kg/p.c. para 0,2 mg/kg/p.c., porém o estudo foi completamente cancelado após a primeira semana de lactação em função da extrema toxicidade materna, da elevada quantidade de

natimortos, bem como da excessiva mortalidade dos filhotes no inicio da lactação por causa do insuficiente cuidado materno com as crias, em razão da severa toxicidade exibida pelas mães.

No grupo tratado com a maior dose (0,3/0,2 mg/kg/p.c.) foi observado pronunciada mortalidade e expressiva toxicidade materna culminando no elevado número de filhotes natimortos e excessiva mortalidade dos filhotes durante o início da lactação. Esses achados justificaram o término prematuro do tratamento com essas doses após aproximadamente a primeira semana de lactação. Em relação às fêmeas expostas à maior dose, quatro fêmeas foram encontradas mortas nos dias 21 e 22 depois do coito e duas no 1º dia após o parto. Os sinais clínicos de toxicidade materna foram: tremores (em três fêmeas no 21º dia após o coito e em cinco logo após o parto), salivação e cromodacriorréia após a administração da substância teste (em duas fêmeas nos dias 17 e 18 depois do coito e em duas até o primeiro dia após o parto), severa alteração no modo de andar, no estado geral e dificuldade respiratória foram observadas em seis fêmeas do 1º ao 4º dias pós parto. Houve ainda a formação de crosta vermelha no nariz de uma fêmea exposta à maior dose da substância teste do 2º ao 4º dias depois do parto. Nutrição incorreta dos filhotes foi uma alteração secundária à toxicidade materna e observada em nove fêmeas do grupo que recebeu 0,3/0,2 mg/kg/p.c. de forato durante os 4 primeiros dias após o parto, período em que onze fêmeas tratadas com essa dose perderam os filhotes em virtude da alimentação deficiente (verificada pela ausência ou pela quantidade insuficiente de leite no estômago dos filhotes). O grupo tratado com a maior dose exibiu decréscimo estatisticamente significante, 16% menos em relação ao controle, no consumo de alimentos durante o 1º ao 7º dias após o parto. Também foi observado redução estatisticamente significante, 5% menos em comparação ao grupo controle, na média do peso corpóreo logo após o parto, 2 dias após o parto essa diminuição foi de 6% em relação ao grupo não tratado. O ganho de peso foi prejudicado nas fêmeas expostas a maior dose nos dias 7 e 8 após o coito (39% menos em comparação ao controle), nos dias 9 e 10 depois do coito (28% menor em relação ao controle), nos dias 19 e 20 pós coito (-25%), durante toda a gestação (-10%), bem como nos primeiros dois dias após o parto (perda de 3,8 gramas no grupo que recebeu a maior dose *versus* ganho de 1,0 grama no controle)

Em relação aos filhotes das mães expostas a maior dose, foi observado aumento estatisticamente significante na quantidade de fêmeas com crias natimortas (13 no grupo de fêmeas tratadas com a maior dose *versus* 4 no grupo não tratado) e no número de

filhotes natimortos (39 *versus* 4 no grupo controle). Consequentemente houve redução estatisticamente significante na quantidade de filhotes nascidos vivos (-21% em comparação ao controle). Foi verificado aumento (25%) no número de filhotes mortos durante os quatro primeiros dias da lactação (66 no grupo tratado com a maior dose *versus* 1 no grupo não tratado), adicionalmente 18 filhotes foram sacrificados em virtude de morte materna. A avaliação realizada no 1º dia após o parto revelou que os machos e as fêmeas exibiram diminuição estatisticamente significante do peso corpóreo. Nos primeiros quatro dias depois do parto foi observado que os animais tratados com a maior dose apresentaram redução no ganho de peso corporal (-14% em comparação ao controle).

A avaliação realizada nos filhotes 21 dias após o parto mostrou que os machos tratados com 0,1 mg/kg/p.c. exibiram significativa redução na atividade da colinesterase plasmática (-15%, $p \leq 0,05$), eritrocitária (-16%, $p \leq 0,01$) e cerebral (-22%, $p \leq 0,01$), já as fêmeas que receberam essa dose apresentaram significante diminuição da colinesterase plasmática (-19%, $p \leq 0,01$).

O NOAEL estabelecido para o estudo foi 0,03 mg/kg/p.c.

Estudo 3*

Ano: 2004

Espécie: Rato (Wistar)

Nº de animais: 45 fêmeas**

Dose: 0,2 mg/kg p.c.

Via: Oral (gavage)

Tempo de exposição: do 6º dia após o coito ao 10º dia após parto (fêmeas) e do 11º ao 21º dia depois do parto (filhotes)

Concentração do ingrediente ativo (pureza): 91,8%

Referência: Kaufmann, W. et al. (2004). Devolopmental neurotoxicity study in Wistar rats oral administration to the dams and pups (gavage). Dossiê de registro submetido à ANVISA.

* Em função do estudo anterior (*Estudo 2*) ter sido cancelamento o presente estudo foi conduzido com apenas uma única dose (0,2 mg/kg/p.c.)

** Com prenhez presumida.

A avaliação realizada no 21º dia após o parto revelou que as fêmeas e os filhotes tratados com 0,2 mg/kg/p.c. exibiram redução na atividade da colinesterase plasmática,

eritrocitária e cerebral, essa diminuição foi de cerca de 13 a 21% no caso das fêmeas e entre 40 a 55% no caso dos filhotes.

Não foi possível estabelecer o NOAEL para o estudo, nem mesmo qualquer relação dose-resposta, uma vez que apenas a dose de 0,2 mg/kg/p.c. foi administrada nos animais experimentais.

Estudo 4

Ano: 2004

Espécie: Rato (Wistar)

Nº de animais: 10 animais jovens** /sexo e 20 adultos*** jovens /sexo

Doses*: 0,03; 0,1; ou 0,2 mg/kg p.c./dia

Via: Oral (gavage)

Concentração do ingrediente ativo (pureza): 91,8%

Referência: Schneider, S. et al. (2004). Study of the effects on cholinesterase levels in juvenile and young adult Wistar rats (age sensitivity) oral administration (gavage). Dossiê de registro submetido à ANVISA.

* Foi administrado o volume padrão de 5mL/kg de p.c.

** A substância teste foi administrada no 11º e 21º dia após o parto

*** A substância teste foi administrada do 60º ao 70º dia após o parto

Filhotes (machos) tratados com a menor dose no 11º dia após o parto exibiram diminuição da atividade da colinesterase plasmática (-3%), eritrocitária (-15%) e cerebral (-3%). Na avaliação realizada no 21º dia de tratamento nos filhotes expostos a 0,03 mg/kg/p.c./dia foi observado que as fêmeas apresentaram redução de 7% e 8%, respectivamente, na atividade da colinesterase plasmática e cerebral, já os filhotes machos exibiram decréscimo de 7% na atividade da colinesterase cerebral. Quanto ao 60º dia na qual os adultos "jovens" foram tratados com 0,03 mg/kg/p.c./dia substância teste, o machos exibiram redução de 16% e 5%, respectivamente, na atividade da colinesterase cerebral e plasmática; em relação às fêmeas, a análise mostrou redução de 27% e 7%, respectivamente, na atividade da colinesterase cerebral e plasmática. Após 11 dias de tratamento (70º dia após o parto) com a menor dose, os machos apresentaram aumento de 26% na atividade da colinesterase cerebral e as fêmeas exibiram aumento de 23% na atividade da colinesterase eritrocitária e diminuição de 17% na atividade da colinesterase cerebral.

No 11º dia após o parto, os filhotes machos tratados com dose única de 0,1 mg/kg/p.c./dia da substância teste, apresentaram decréscimo de 6 e 10%, respectivamente, na atividade da colinesterase plasmática e eritrocitária; quanto às fêmeas, houve aumento de 17% na atividade da colinesterase cerebral. No 21º dia após o parto a administração de apenas uma dose de 0,1 mg/kg/p.c./dia da substância teste em filhotes fêmeas provocou redução de 9 e 13%, respectivamente, na atividade da colinesterase plasmática e cerebral. Durante o 60º dia após o parto, as fêmeas expostas a 0,1 mg/kg/p.c./dia de forato apresentaram diminuição de 11 e 26%, respectivamente, na atividade da colinesterase plasmática e cerebral. Após 11 dias de tratamento (70º dia após o parto) com 0,1 mg/kg/p.c./dia da substância teste, os machos exibiram aumento de 16% na atividade da colinesterase cerebral e as fêmeas apresentaram redução de 12% tanto na atividade da colinesterase cerebral como na plasmática.

Inibição estatisticamente significante na atividade da colinesterase plasmática, eritrocitária e cerebral foi observada nos filhotes (machos) expostos a dose única de 0,2 mg/kg/p.c./dia da substância teste no dia 11º após o parto, a diminuição foi de 22, 20 e 18%, respectivamente, na atividade da colinesterase plasmática, eritrocitária e cerebral. Quanto às fêmeas, houve redução de 17, 10 e 1%, respectivamente, das enzimas em questão. No 21º dia após o parto, administração de apenas uma dose de 0,2 mg/kg/p.c./dia da substância teste, em filhotes fêmeas, provocou redução de 14 e 9% respectivamente, na atividade da colinesterase plasmática e cerebral; quanto aos filhotes machos tratados nessas mesmas condições, houve redução de 6, 16 e 6%, respectivamente, na atividade das colinesterases plasmática, cerebral e eritrocitária. Durante o 60º dia após o parto, os machos expostos a maior dose exibiram diminuição de 17% na atividade da colinesterase cerebral, ao passo que as fêmeas mostraram decréscimo de 15 e 34%, respectivamente, na atividade da colinesterase plasmática e cerebral. Após 11 dias de tratamento (70º dia após o parto) com 0,2 mg/kg/p.c./dia da substância teste, os machos apresentaram aumento de 9% na atividade da colinesterase cerebral e as fêmeas exibiram redução de 41 e 15%, respectivamente, na atividade da colinesterase plasmática e eritrocitária..

Baseado nas alterações descritas, não foi possível estabelecer um NOAEL para o estudo.

Estudo 5

Ano: 2004

Espécie: Rato (Wistar)

Nº de animais: 25 animais juvenis** /sexo e 25 adultos/sexo

Doses: 0,2 mg/kg p.c./dia

Via: Oral (gavage)

Concentração do ingrediente ativo (pureza): 91,8%

Referência: Schneider, S. et al. (2004). Study of the effects on cholinesterase activities in juvenile and adult Wistar rats after single administration ("time-to-peak" study) oral administration (gavage). Dossiê de registro submetido à ANVISA.

* Foi administrado o volume padrão de 5mL/kg de p.c.

** Aos 22 dias após o nascimento

O objetivo do estudo foi avaliar a atividade da colinesterase plasmática, eritrocitária e cerebral após 30 minutos, 1, 2, 4 e 8 horas da administração de 0,2 mg/kg/p.c./dia de forato a ratos juvenis e adultos. As tabelas a seguir mostram o comportamento dessa enzima nos animais expostos à substância teste.

Tabela 5 – Avaliação da atividade da enzima colinesterase (plasmática, eritrocitária e cerebral) após a administração de apenas uma dose (0,2 mg/kg p.c./dia) do ingrediente ativo forato a machos adultos de ratos Wistar.

Tempo após a administração da substância teste		**0,5 h**	**1 h**	**2 h**	**4 h**	**8 h**
Colinesterase Plasmática	**Controle**	13,38	12,68	14,79	11,07	10,45
	Tratado	13,60 (+2%)	14,80 (+17%)	13,57 (-8%)	11,96 (+8%)	13,89 (+33%)
Colinesterase Eritrocitária	**Controle**	34,82	31,85	27,70	27,94	29,32
	Tratado	28,98 (-17%)	32,03 (+1%)	32,08 (+16%)	28,33 (+1%)	26,05 (-11%)
Colinesterase Cerebral	**Controle**	1,80	2,61	3,15	1,97	2,61
	Tratado	2,43 (+35%)	2,87 (+10%)	2,07 (-34%)	2,12 (+8%)	2,32 (-11%)

Tabela 6 – Avaliação da atividade da enzima colinesterase (plasmática, eritrocitária e cerebral) após a administração de apenas uma dose (0,2 mg kg/p.c./dia) do ingrediente ativo forato a fêmeas adultas de ratos Wistar.

Tempo após a administração da substância teste		**0,5 h**	**1 h**	**2 h**	**4 h**	**8 h**
Colinesterase Plasmática	**Controle**	69,39	57,72	54,49	52,52	51,47
	Tratado	54,25	55,27	59,43	51,91	48,26

		(-22%)	(-4%)	(+9%)	(-1%)	(-6%)
Colinesterase Eritrocitária	**Controle**	34,71	31,50	29,12	26,49	31,00
	Tratado	36,64 (+6%)	32,59 (+3%)	32,26 (+11%)	30,60 (+16%)	27,80 (-10%)
Colinesterase Cerebral	**Controle**	2,69	3,16	1,77	2,69	2,71
	Tratado	2,46 (-8%)	3,31 (+5%)	2,14 (+20%)	1,99 (-26%)	2,72 (0%)

Tabela 7 – Avaliação da atividade da enzima colinesterase (plasmática, eritrocitária e cerebral) após a administração de apenas uma dose (0,2 mg/kg p.c./dia) do ingrediente ativo forato a filhotes machos de ratos Wistar.

Tempo após a administração da substância teste		**0,5 h**	**1 h**	**2 h**	**4 h**	**8 h**
Colinesterase Plasmática	**Controle**	14,24	14,72	13,91	14,20	13,05
	Tratado	13,68 (-4%)	14,02 (-5%)	12,42 (-11%)	12,85 (-10%)	13,53 (+4%)
Colinesterase Eritrocitária	**Controle**	42,07	47,46	42,24	38,56	43,53
	Tratado	41,79 (-1%)	42,34 (-11%)	40,98 (-3%)	41,14 (+7%)	41,07 (-6%)
Colinesterase Cerebral	**Controle**	1,90	1,88	1,65	2,48	2,64
	Tratado	1,66 (-13%)	2,10 (+12%)	1,80 (+9%)	2,45 (-1%)	2,11 (-20%)

Tabela 8 – Avaliação da atividade da enzima colinesterase (plasmática, eritrocitária e cerebral) após a administração de apenas uma dose (0,2 mg/kg p.c./dia) do ingrediente ativo forato a filhotes fêmeas de ratos Wistar.

Tempo após a administração da substância teste		**0,5 h**	**1 h**	**2 h**	**4 h**	**8 h**
Colinesterase Plasmática	**Controle**	14,07	13,65	13,33	13,46	13,80
	Tratado	13,37 (-5%)	13,11 (-4%)	14,37 (+8%)	13,26 (-1%)	13,23 (-4%)
Colinesterase Eritrocitária	**Controle**	50,18	44,70	44,01	42,04	41,32
	Tratado	40,73 (-19%)	41,86 (-6%)	42,18 (-4%)	41,04 (-2%)	42,12 (+2%)
Colinesterase	**Controle**	1,82	1,83	1,76	2,22	2,98

Cerebral	**Tratado**	1,77 (-2%)	1,82 (-1%)	1,79 (+2%)	2,12 (-4%)	2,40 (-20%)

Os resultados mostraram uma forte tendência à redução da atividade da colinesterase cerebral, em ambos os sexos, no intervalo de 8 horas após a exposição. A inibição da atividade da colinesterase eritrocitária foi mais pronunciada nos machos adultos e nos filhotes fêmeas 30 minutos após o tratamento com a substância teste. Quanto à colinesterase plasmática, a maior oscilação em comparação aos animais não tratados, ocorreu no grupo de fêmeas adultas, o grupo em questão exibiu decréscimo de 20% na atividade da referida enzima 30 minutos após receber o ingrediente ativo forato.

Não foi possível estabelecer o NOAEL para o estudo, nem mesmo qualquer relação dose-resposta, uma vez que apenas a dose de 0,2 mg/kg/p.c. foi administrada nos animais experimentais.

4.8. Nefrotoxicidade

A integridade do sistema renal é fundamental para a manutenção da homeostase. Nos mamíferos, os rins são os principais responsáveis pela excreção, regulação do volume extracelular, composição eletrolítica e equilíbrio ácido-base. Além disso, alguns hormônios são sintetizados e liberados pelos rins, como a renina e a eritropoietina (GUYTON; HALL, 1996; SCHNELLMANN, 2001).

Efeitos tóxicos no sistema renal podem desregular uma ou mais dessas funções prejudicando o metabolismo do organismo. Apesar dos rins possuirem mecanismos compensatórios e capacidade regenerativa, em alguns casos os efeitos tóxicos não são revertidos, causando danos permanentes. O tratamento dos efeitos tóxicos no sistema renal pode envolver diálise ou transplante de rins (SCHNELLMANN, 2001).

Embora existam poucos estudos de toxicidade que avaliem o potencial nefrotóxico de uma substância, o forato provocou alguns efeitos em animais de laboratório.

Camundongos suíços albinos adultos foram expostos ao forato na mesma dose recomendada pelos fabricantes para aplicação no campo (20 kg/hectare). A exposição ocorreu por 3 horas e meia, seis dias por semana, durante 3 meses (MOROWATI, 2001) em uma câmara de inalação de 21 litros de capacidade. A cada duas semanas amostras de sangue foram coletadas e amostras de tecido para análises bioquímicas e

histopatológicas. A partir da quarta semana de exposição foi observado um aumento de creatinina sérica. Esses níveis permaneceram elevados até o fim do tratamento (12ª semana). Trinta dias após o fim do tratamento, os níveis voltaram ao normal (MOROWATI, 2001). A partir da segunda semana foram encontradas degenerações disseminadas nos túbulos corticais, assim como eritrócitos e macrófagos nos espaços intersticiais do córtex e da medula. Os túbulos apresentaram alterações no formato normal. Os autores sugeriram que as doses recomendadas para utilização nas lavouras podem causar danos renais nos trabalhadores expostos. Apesar dos efeitos terem sido revertidos trinta dias após o fim do tratamento, períodos mais longos de exposição e doses mais elevadas podem desencadear danos permanentes (MOROWATI, 2001).

4.9. Toxicidade sobre o sistema respiratório

A exposição a substâncias químicas por via inalatória pode ter dois tipos de efeito: nos tecidos pulmonares ou em órgãos mais distantes. Dessa maneira, toxicidade do sistema respiratório refere-se a alterações no trato respiratório produzidas por substâncias químicas (WITSCHI; JEROLD, 2001).

Diversas doenças pulmonares já foram associadas à exposição a substâncias químicas como silicose, asbestose e câncer de pulmão (HAMILTON; THAKUR; HOLIAN, 2008; MADL et al, 2007; SMITH, 1997; KAMP, 2009). Agrotóxicos organofosforados também já foram associados a alterações respiratórias em seres humanos (FIETERN et al, 2009; HERNANDEZ et al, 2004; BOERS et al, 2008; HERNANDEZ et al, 2008; HERNANDEZ et al, 2006; HOPPIN et al, 2002, HOPPINE et al, 2007; HOPPIN et al, 2008). Alterações nas funções cardíacas, provenientes ou não de disfunções respiratórias também já foram observadas após a exposição a organofosforados (ANAND et al 2009; KARKI, et al, 2004; SAADEH; FARSAKH; AL-ALI, 1997).

Camundongos suíços albinos adultos foram expostos ao forato na mesma dose recomendada pelos fabricantes para aplicação no campo (20 kg/hectare). A exposição ocorreu por 3 horas e meia, seis dias por semana, durante 3 meses (MOROWATI, 1998) em uma câmara de inalação de 21 litros de capacidade. A cada duas semanas amostras de sangue e pulmões foram coletados para análise. A partir da segunda semana de exposição os alvéolos apresentaram congestão por exsudatos de fibrina, monócitos, polimorfos e linfócitos, os bronquíolos apresentaram alterações celulares. O quadro mostrava-se de broncopneumonia devido a lesões causadas por irritação. Na quarta

semana os pulmões apresentaram agressões focais de células inflamatórias, causando a congestão de sacos alveolares e alvéolos. Na sexta semana foi observado enfisema proeminente e descamação bronquial. O quadro geral era de broncopneumonia e enfisema. Os sintomas permanceram os mesmos até a décima segunda semana onde o quadro geral era de broncopneumonia, enfisema e colapso. Trinta dias após o fim do tratamento, as alterações enfisematosas pulmonares ainda estavam presentes (MOROWATI, 1998). Os efeitos pulmonares provocados pelo forato no sistema respiratório podem causar aumento da resistência vascular e hipertensão pulmonar. Esses eventos provocam sobrecarga no coração direito e, frequentemente, ataque cardíaco (MOROWATI, 1998). Os autores concluíram que indivíduos expostos ao forato apresentam elevado risco de danos respiratórios, mesmo depois de cessada a exposição.

Esses achados em animais de laboratório são corroborados por estudos epidemiológicos que descreveram que dificuldade respiratória em aplicadores de agrotóxicos está associada positivamente ao forato (HOPPIN et al, 2006). Em outro estudo, foram estudados pacientes com necessidade de ventilação respiratória assistida após exposição ao forato (SINGH ET AL, 2001). No estudo de Kashyap e colaboradores, foram avaliados indivíduos expostos por duas semanas ao forato. Em 60% dos trabalhadores foram observados diversos sintomas de toxicidade, como gastrointestinais, diminuição da frequência cardíaca e sintomas neurológicos, sendo os dois primeiros, os efeitos mais proeminentes (KASHYAP ET AL, 1984).

6. Aspectos regulatórios – a situação internacional do registro do forato

Tabela 9: Situação Internacional do registro dos produtos a base de forato

País	***Status* Regulatório**
Austrália	Prioridade 2 para ser reavaliado, devido a preocupações quanto aos danos à saúde humana
Canadá	No processo de *phase out* para batata: 2011-2012
Estados Unidos	Medidas mitigatórias tais como uso em sistemas fechados, proibição de aplicação aérea, restrição de culturas autorizadas e regiões, definição de uma única aplicação por safra, entre outras medidas restritivas
Outros	Fora do Anexo I da Diretiva 91/414/EEC da União Européia.

7. Conclusões e recomendações

Os efeitos decorrentes das mudanças globais na produção agrícola e pecuária têm imposto um modelo produtivo cada vez mais dependente dos agrotóxicos que traz para a saúde pública enormes desafios que não podem ser enfrentados apenas por ações restritas ao campo da assistência médico-hospitalar às pessoas intoxicadas por exposições agudas e ou crônicas. Cabe uma ação integrada de proteção da saúde para uma efetiva promoção, proteção da saúde e de prevenção das situações de riscos nos processos produtivos e de consumo de alimentos.

Pela Lei brasileira 7.802/89 um agrotóxico pode ter seu registro banido quando da ausência de métodos para desativação do produto, na ausência de antídoto ou tratamento eficaz, quando provoca distúrbios hormonais ou danos ao aparelho reprodutor, quando são teratogênicos, carcinogênicos ou mutagênicos. Além disso, também quando se apresenta mais perigoso para o homem do que em animais.

Os metabólitos foratoxon, foratoxon sulfóxido e foratoxon sulfona são 100 a 1000 vezes mais potentes como inibidores da acetilcolinesterase.

O forato foi demonstrado ser extremamente tóxico, provocando letalidade em doses baixas, por diferentes vias de exposição.

O forato possui vários efeitos adversos para a saúde humana como associação com diabetes mellitus na gravidez, nefrotoxicidade, toxicidade reprodutiva, toxicidade para o sistema respiratório e neurotoxicidade.

Diversos estudos demonstram que trabalhadores agrícolas expostos ao forato são vítimas de intoxicações e óbitos relacionados às características de toxicidade desse princípio ativo. A exposição torna-se ainda mais perigosa devido às dificuldades relacionadas com a indisponibilidade e/ou ineficiência dos EPI. Além disso, disto, diversas questões de ordem social (baixa escolaridade, baixa renda) e biológica (idade e gênero) são fatores que aumentam a vulnerabilidade e a gravidade das intoxicações por esse organofosforado.

Os estudos experimentais e epidemiológicos envolvendo o trato respiratório demonstram que o forato possui elevada toxicidade para esse sistema. Os estudos experimentais, realizados com doses semelhantes à exposição humana ocupacional, corroboram para a plausibilidade biológica dos achados. Enfisema, broncopneumonia, alterações inflamatórias e dificuldade respiratória foram os principais efeitos encontrados, sendo que alguns se mostraram irreversíveis pelo período de tempo de observação mesmo depois de cessada a exposição. É sabido que esses efeitos podem

causar aumento da resistência vascular pulmonar, sobrecarregar o coração direito e até causar insuficiência cardíaca. Tais efeitos podem não só diminuir a eficiência no trabalho, mas também prejudicar irremediavelmente a qualidade de vida de indivíduos expostos e levar a morte.

A neurotoxicidade do forato também já foi demonstrada em estudos epidemiológicos. Manifestacões neurotóxicas tais como vômito, tontura, dor abdominal, taquicardia, salivação excessiva, miose e hipotensão foram observadas em casos de intoxicação intencional, ocupacional, e acidental por exposição ao forato. Sintomas mais graves como convulsões, espasmos, tremores, perda de coordenação muscular, aumento do tônus muscular dos membros, dificuldade respiratória, edema cerebral, perda de consciência e coma profundo também foram descritos. Achados em alguns pacientes foram consistentes com morte cerebral, incluindo ausência de reflexos corneanos, oculoencefálicos, pupilares e musculares, ausência de reações a estímulos de dor ou calor e ausência de respiração espontânea, com supressão global da atividade cortical. Alguns casos de intoxicação evoluíram para o óbito.

O forato pode provocar complexas manifestações clínicas neurológicas como encefalopatia, síndrome intermediária e polineuropatia retardada em humanos. No entanto, não foram descritos, em animais de laboratório, casos de síndrome intermediária ou polineuropatia tardia, caracterizando-o como mais tóxico para seres humanos do que os testes em animais tenham podido demonstrar.

Embora alguns órgãos internacionais como EPA e IPCS ainda classifiquem o forato como não carcinogênico e não mutagênico, há evidências relatadas em estudos publicados na literatura científica que apontam efeitos clastogênicos e mais recentemente demonstrando que o forato é potencialmente promotor de câncer para indivíduos expostos com histórico familiar positivo para o câncer de próstata.

Por último, cumpre ressaltar a recomendação da OMS para proibição de produtos extremamente tóxicos, com vista a redução de perigos à população exposta à este produtos. (OPAS/OMS, 1996).

Considerando todos os efeitos toxicológicos associados ao ingrediente ativo Forato e a sua inclusão dentre as características proibitivas de registro, especialmente a de “possuir características mais tóxicas para o ser humano do que testes com animais tenham podido demonstrar”, o mesmo deve ter seu uso proibido no Brasil, de maneira a proteger a saúde dos trabalhadores expostos, dos consumidores e da população em geral.

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Use of Agricultural Pesticides and Prostate Cancer Risk in the Agricultural Health Study Cohort

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The authors examined the relation between 45 common agricultural pesticides and prostate cancer incidence in a prospective cohort study of 55,332 male pesticide applicators from Iowa and North Carolina with no prior history of prostate cancer. Data were collected by means of self-administered questionnaires completed at enrollment (1993–1997). Cancer incidence was determined through population-based cancer registries from enrollment through December 31, 1999. A prostate cancer standardized incidence ratio was computed for the cohort. Odds ratios were computed for individual pesticides and for pesticide use patterns identified by means of factor analysis. A prostate cancer standardized incidence ratio of 1.14 (95% confidence interval: 1.05, 1.24) was observed for the Agricultural Health Study cohort. Use of chlorinated pesticides among applicators over 50 years of age and methyl bromide use were significantly associated with prostate cancer risk. Several other pesticides showed a significantly increased risk of prostate cancer among study subjects with a family history of prostate cancer but not among those with no family history. Important family history-pesticide interactions were observed.

agrochemicals; fungicides, industrial; herbicides; insecticides; pesticides; prostatic neoplasms; risk

Abbreviations: CI, confidence interval; DDT, dichlorodiphenyltrichloroethane; EPTC, *S*-ethyl dipropylthiocarbamate; OR, odds ratio; SIR, standardized incidence ratio; 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid; 2,4,5-TP, 2,4,5-trichlorophenoxypropionic acid.

Prostate cancer is the most common malignancy among men in the United States and in most Western countries (other than nonmelanoma skin cancer), and in the United States, it is the second leading cause of cancer death (1, 2). Despite the common occurrence of this tumor, its etiology remains largely unknown.

Age, family history, African-American ethnicity, hormonal factors, and possibly a high consumption of animal fat and red meat are the most consistent risk factors reported (3–10). An inverse association with vegetable and fruit consumption has been suggested (9, 11, 12), while smoking may be related to the occurrence of fatal prostate cancer (13).

Farming has been the most consistent occupational risk factor for prostate cancer (14, 15). Farm-related potential risk factors include exposures to insecticides, fertilizers, herbicides, and other chemicals (16–23). However, the role of specific agricultural chemicals has not been firmly established because of the lack of precise exposure data (20, 21). We examined the exposure-response relation between 45 important agricultural pesticides and prostate cancer incidence in the Agricultural Health Study cohort

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while controlling for known and suspected risk factors for prostate cancer.

MATERIALS AND METHODS

Cohort enrollment

The Agricultural Health Study is a prospective cohort study of 89,658 people, including 52,395 private applicators and 4,916 commercial applicators licensed to apply restricted use pesticides and 32,347 spouses of farmer applicators from Iowa and North Carolina (24). Private applicators were farmers or nursery workers, and "commercial" applicators were persons employed by pest control companies or businesses that use pesticides (e.g., warehouse operators, grain mills). Pesticide applicators were enrolled when they completed an enrollment questionnaire. In Iowa, both commercial and farmer applicators attend the same pesticide certification testing sessions, and both were invited to participate in the study. In North Carolina, because private and commercial applicators attend separate training, only private applicators were enrolled. Private and commercial applicators were also asked to complete "take-home" questionnaires that sought more extensive information on occupational activities. Recruitment of applicators and their spouses began in December 1993 and continued until December 1997. Male spouses are too few for meaningful analysis at this time.

Questionnaires

The enrollment questionnaire sought information on the use of 50 pesticides (ever/never), crops grown and livestock raised, personal protective equipment used, pesticide application methods used, other agricultural activities and exposures, nonfarm occupational exposures, smoking, alcohol consumption, fruit and vegetable intake, multiple vitamin use, medical conditions, medical conditions in first-degree relatives including a history of prostate cancer, and basic demographic data (all questionnaires are at http://www.aghealth.org). For 22 of the 50 pesticides in the enrollment questionnaire, we also obtained information on the duration of use (years) and frequency of use (days per year). Information on application methods and protective equipment was used to compute an exposure "intensity index I" (25). For the remaining 28 pesticides listed in the enrollment questionnaire, exposure information was limited to ever versus never used. The enrollment questionnaire also included two activities (painting and engine repair) that frequently result in exposure to solvents. The take-home questionnaires included the following: detailed use information on the 28 pesticides reported as ever/never use in the enrollment questionnaire, more detailed information on personal protective equipment use, dietary and cooking practices, supplemental vitamin use, height and weight (used for body mass index), occupational exposures to welding and solvents, nonfarm jobs, and hours spent in strenuous physical activity.

Cohort follow-up

Cohort members were matched to cancer registry files in Iowa and North Carolina for case identification and to the state death registries and to the National Death Index to ascertain vital status; prostate cancer cases diagnosed prior to enrollment were excluded from the analyses. Incident cases were identified from enrollment (i.e., 1993–1997) through December 31, 1999. Study subjects alive but no longer residing in Iowa or North Carolina were identified through personal contacts with the study subject, motor vehicle records, pesticide registration records, and the Internal Revenue Service address database (which has current address information on all Americans filing a tax return). This includes over 98 percent of the Agricultural Health Study cohort. Fewer than 0.4 percent of the cohort were lost to mortality or cancer incidence follow-up (n = 319).

Analysis

A standardized incidence ratio for prostate cancer was computed to compare prostate cancer incidence among male cohort members with incidence in the male populations of Iowa and North Carolina. Expected numbers for the standardized incidence ratio were developed from 5-year age and calendar-time (i.e., 1994–1998), race-specific cancer incidence rates from the population-based cancer registries in Iowa and North Carolina. The statistical significance of the standardized incidence ratios and 95 percent confidence intervals was based on standard methods (26, 27).

Because the follow-up period for case ascertainment was less than 5 years (i.e., an average of 4.3 years) and the prostate cancer incidence rate did not vary appreciably, multivariate logistic regression (28) was used to compare prostate cancer cases with noncases on a number of factors possibly associated with prostate cancer risk. In this analysis, we examined 50 pesticides, crops grown and livestock raised, personal protective equipment used, pesticide application methods used, other agricultural activities and exposures, nonfarm occupational exposures, regular recreational physical activity, smoking, alcohol consumption, red meat consumption, fruit and vegetable intakes, multiple vitamin use, medical conditions, medical conditions in first-degree relatives including a history of prostate cancer, "high pesticide exposure events" (29), age, race, state of residence, license type, education, and basic demographic data. All analyses excluded both female applicators and 414 prevalent prostate cancer cases.

Factor analysis was used to examine the interrelations among ever/never use of 50 pesticides, state (Iowa, North Carolina), and age (≤50 and >50 years) (30). Only variables that shared at least 15 percent of the variance with the factor, corresponding to a factor-loading score of 0.40 or higher, were considered when interpreting the factors. Factor scores were computed for each subject and then divided into tertiles based on the factor scores for cases. The upper tertile was divided in half, and the upper half was then divided in half again to examine more extreme exposure scores (resulting in categories at ≤33.3 percent, 33.4–66.7 percent, 66.8–83.3 percent, 83.4–91.6 percent, >91.6 percent). Logistic regres-

TABLE 1. Characteristics of licensed pesticide applicators in the Agricultural Health Study, 1993–1997

Characteristics*	Prostate cancer		Cohort member		Adjusted odds ratio†	95% confidence interval	*p* value
	Cases	%	Noncases	%			
Total (all)	566		54,766				
Age (years)							
<55	67	11.8	38,860	70.9	1.0‡		<0.0001§
55–59	78	13.8	5,374	9.8	5.2	3.1, 8.7	
60–64	139	24.6	4,581	8.4	12.8	8.1, 20.2	
65–69	159	28.1	3,165	5.8	22.4	14.2, 35.3	
70–74	77	13.6	1,804	3.3	19.6	11.4, 33.6	
≥75	46	8.1	980	1.8	25.6	13.5, 48.6	
Race							
White	546	96.5	53,425	97.6	1‡		0.50
Black and other races	20	3.5	1,341	2.4	1.55	0.5, 4.4	
Residence							
Iowa	326	57.6	35,560	64.9	1‡		0.29
North Carolina	240	42.4	19,206	35.1	0.82	0.6, 1.1	
Education (years)							
<12	97	18.6	4,669	9.1	1‡		0.36§
12	279	53.4	24,631	48.1	1.41	0.9, 2.2	
>12	147	28.1	21,958	42.8	1.35	0.8, 2.2	
License type							
Private	541	95.6	50,090	91.5	1‡		0.41
Commercial	25	4.4	4,676	8.5	1.10	0.6, 2.0	
Smoker							
Never	195	39.8	25,159	51.1	1‡		0.06§
Former	243	49.6	15,423	31.4	1.30	0.9, 1.7	
Current	52	10.6	8,629	17.5	1.42	0.9, 2.2	
Family history of prostate cancer							
No	391	81.1	45,342	91.4	1‡		0.0001
Yes	91	18.9	4,271	8.6	1.90	1.4, 2.7	

Table continues

sion analysis was performed to evaluate the association between factor scores and the risk of prostate cancer, controlling for the same potentially confounding variables as above.

Unconditional logistic regression analysis was also used to evaluate risks associated with a reported history of mixing or applying specific pesticides. We used the "never used the specific pesticide" category as the reference group and the five percentile categories described above as the exposed groups. Exposure variables for the 22 pesticides included in the enrollment questionnaire, evaluated on the entire Agricultural Health Study male cohort, included the following: 1) application days per year; 2) total years of exposure; 3) an exposure "intensity index I," which includes information about the application method, a score for whether the applicator repaired his own pesticide application equipment, and a score for the use of protective equipment (25); and 4) a cumulative pesticide exposure score: (application days per year) × (total years of exposure) × (exposure intensity index I). We omitted pesticides from this analysis if a total of five or fewer applicators were exposed to the chemical.

For the subset of male applicators (n = 24,034) who also completed the take-home questionnaires, exposure variables (for 28 additional pesticides) included the following: 1) application days per year; 2) total years of exposure; 3) an exposure "intensity index II," which included information about mixing methods, an application methods score, whether an enclosed tractor was used in applying pesticides, whether the applicator repaired his own pesticide application equipment, whether the applicator washed his pesticide equipment, a score for the use of protective equipment, personal hygiene information, whether the applicator changed clothes after a chemical spill, and the frequency of replacing gloves (25); and 4) a cumulative pesticide expo-

TABLE 1. Continued

Characteristics	Prostate cancer		Cohort member		Adjusted odds ratio†	95% confidence interval	*p* value
	Cases	%	Noncases	%			
Vegetable							
<5 times/week	156	32.0	17,001	34.0	1‡		0.76§
5–7 times/week	169	34.6	18,250	36.5	0.75	0.5, 1.0	
>1/day	163	33.4	14,808	29.5	0.93	0.7, 1.3	
Red meat							
0–<2 times/week	115	35.2	7,150	30.2	1‡		0.70§
2 times/week	84	25.7	6,612	27.9	0.96	0.7, 1.4	
≥3/week	128	39.1	9,942	41.9	0.94	0.7, 1.3	
Supplemental vitamin use							
No	218	69.0	15,771	67.6	1‡		0.40§
Not regularly	38	12.0	3,556	15.2	0.92	0.6, 1.4	
Regularly	60	19.0	4,004	17.2	0.87	0.6, 1.2	
Hours of exercise/week (leisure time)							
None	120	37.5	5,678	24.2	1‡		0.23§
<1	53	16.6	4,148	17.7	0.68	0.5, 1.0	
1–1.5	46	14.4	3,978	17.0	0.80	0.5, 1.2	
1.6–4	46	14.4	4,557	19.4	0.64	0.4, 1.0	
4.1–8	32	10.0	2,792	11.9	0.86	0.5, 1.4	
>8	23	7.2	2,312	9.9	0.57	0.3, 1.0	
Body mass index							
Quartile 1 (lowest)	69	23.8	5,838	25.2	1.0‡		0.44§
Quartile 2	83	26.2	5,742	24.8	1.34	0.9, 2.0	
Quartile 3	86	27.1	5,798	25.1	1.23	0.8, 1.8	
Quartile 4 (highest)	79	24.9	5,761	24.9	1.31	0.9, 2.0	
High pesticide exposure event							
No	276	87.6	19,825	85.0	1‡		0.48
Yes	39	12.4	3,510	15.0	1.11	0.8, 1.6	

* Information on age, race, state of residence, education, license type, smoking history, family history of prostate cancer, and vegetable intake was taken from the enrollment questionnaire completed by 54,766 non-prostate cancer cohort members and 566 new prostate cancer cohort members; 414 cohort members had prostate cancer before enrollment into the study and were not included in this analysis. Information on high pesticide exposure events, supplemental vitamin use, hours of leisure exercise per week, body mass index, and red meat intake was taken from the farmer applicator and commercial applicator questionnaire completed by 24,034 non-prostate cancer cohort members and 331 prostate cancer cohort members. Data reflect cohort characteristics as of December 31,1999. Missing data for some questions are responsible for differences in total cell counts.

† Odds ratios of prostate cancer adjusted for age, race, state of residence, education, license type, smoking history, family history of prostate cancer, vegetable intake, supplemental vitamin use, body mass index, high pesticide exposure events, exercise per week, and red meat intake.

‡ Reference group.

§ *p* value for trend test.

sure score: (application days per year) × (total years of exposure) × (exposure intensity index II). For both algorithms, exposure-response was assessed by a linear trend test, treating the cumulative score as a continuous variable, and also by selecting the median cumulative score of each exposure category and treating the cumulative score as a categorical variable. Analyses of prostate cancer risk were conducted by state and by license type in Iowa (i.e., private vs. commercial) to evaluate the consistency of findings within the cohort. All odds ratios were adjusted for age as a categorical variable (<55, 55–59, 60–64, 65–69, 70–74, and ≥75 years). Institutional review boards approved the study

TABLE 2. Risk from occupational exposures to licensed pesticide applicators off the farm and from painting and welding on the farm, Agricultural Health Study, 1993–1997

Exposure	Prostate cancer		Cohort member		Adjusted odds ratio*	95% confidence interval*	*p* value*
	Cases	%	Noncases	%			
			Off-the-farm jobs†				
Pesticides‡							
No	278	95.2	20,103	90.8	1		0.27
Yes	14	4.8	2,028	9.2	0.74	0.4, 1.3	
Solvents‡							
No	267	91.4	18,138	82.0	1		0.02
Yes	25	8.6	3,993	18.0	0.60	0.4, 0.9	
Gasoline‡							
No	268	91.8	18,128	81.9	1		0.003
Yes	24	8.2	4,003	18.1	0.53	0.3, 0.8	
Asbestos‡							
No	278	95.2	20,833	94.1	1		0.50
Yes	14	4.8	1,298	5.9	0.8	0.5, 1.4	
Grain dust‡							
No	276	94.5	19,768	89.3	1		0.36
Yes	16	5.5	2,363	10.7	0.79	0.5, 1.3	
Wood dust‡							
No	275	94.2	19,725	89.1	1		0.12
Yes	17	5.8	2,406	10.9	0.68	0.4, 1.1	
Silica/sand dust‡							
No	281	96.2	21,090	95.3	1		0.76
Yes	11	3.8	1,041	4.7	1.10	0.6, 2.0	
Engine exhaust‡							
No	257	88.0	17,048	77.0	1		0.58
Yes	35	12.0	5,083	23.0	0.88	0.6, 1.4	

Table continues

proposal and the manner in which informed consent was obtained from study participants.

RESULTS

This analysis was restricted to the 55,332 male private and commercial applicators with no history of prostate cancer at enrollment. A total of 1,197 deaths occurred among male applicators during the mean follow-up period of 4.3 years. A total of 566 incident prostate cancers were observed between enrollment and December 31, 1999. Based on age-adjusted state incidence rates, 494.5 prostate cancer cases were expected, yielding a standardized incidence ratio of 1.14 (95 percent confidence interval (CI): 1.05, 1.24). For the same period, cancer incidence from all sites was significantly less than expected, with an overall standardized incidence ratio of 0.80 (95 percent CI: 0.76, 0.83). The prostate cancer standardized incidence ratio (SIR) appeared higher among commercial applicators (SIR = 1.41, 95 percent CI: 0.89, 2.11) than among private applicators (SIR = 1.13, 95 percent CI: 1.04, 1.24) and higher among Iowa Whites (SIR = 1.27, 95 percent CI: 1.13, 1.27) than among North Carolina Whites (SIR = 1.10, 95 percent CI: 0.99, 1.21). There were too few prostate cancer cases among non-Whites in North Carolina (n = 19) and Iowa (n = 0) for meaningful calculation of standardized incidence ratios at this time. For the subset of the male applicator cohort (n = 24,034) who completed the take-home questionnaire, the prostate cancer standardized incidence ratio of 1.22 (95 percent CI: 1.09, 1.36) and the overall cancer standardized incidence ratio of 0.81 (95 percent CI: 0.75, 0.87) were similar to those for the entire cohort.

Odds ratios for prostate cancer increased sharply with age, and cases were more likely to have a family history of prostate cancer (table 1). Nineteen percent of prostate cancer cases reported a family history of prostate cancer among first-degree relatives, compared with 8.6 percent of noncases. No other characteristic in table 1 was statistically significant after adjustment for the other characteristics shown. A nearly significant positive association was observed for cigarette smoking.

TABLE 2. Continued

Exposure	Prostate cancer		Cohort member		Adjusted odds ratio	95% confidence interval	*p* value
	Cases	%	Noncases	%			
Lead solder‡							
No	281	96.2	21,172	95.7	1		0.57
Yes	11	3.8	959	4.3	0.84	0.5, 1.5	
Welding fumes‡							
No	260	89.0	18,147	82.0	1		0.25
Yes	32	11.0	3,984	18.0	0.80	0.6, 1.2	
Other metals‡							
No	281	96.2	21,340	96.4	1		0.34
Yes	11	3.8	791	3.6	1.36	0.7, 2.5	
Pneumatic drill‡							
No	284	97.3	20,550	92.9	1		0.10
Yes	8	2.7	1,581	7.1	0.55	0.3, 1.1	
No exposure off the farm reported‡							
No	232	79.5	18,541	83.8	1		0.10
Yes	60	20.5	3,590	16.2	1.27	0.9, 1.7	
			On farm				
Painting on farm§							
No	254	44.8	19,485	35.6	1		0.22
Yes	312	55.2	35,281	64.4	1.13	0.9, 1.4	
Welding on farm§							
No	1	51.4	19,209	35.1	1		0.33
Yes	275	48.6	35,559	64.9	0.91	0.8, 1.1	

* Odds ratios, 95% confidence intervals, and *p* values adjusted for age and family history of prostate cancer; the "no" exposure was always used as the reference category.

† Eight occupational exposures occurring off the farm including x-rays, cotton dust, mineral dust, electroplating fumes, lead, mercury, cadmium, and mixing herbicides in the military were omitted from the table because fewer than five exposed cases were observed.

‡ Information on all off-the-farm jobs/activities completed by 24,034 non-prostate cancer cohort members and 331 prostate cancer cohort members.

§ Information on age and on family history of prostate cancer, painting (on-farm activity), and welding (on-farm activity) taken from the enrollment questionnaire completed by 54,766 non-prostate cancer cohort members and 566 prostate cancer cohort members; 414 cohort members had prostate cancer before enrollment into the study and were not included in this analysis. Missing data for some questions are responsible for the differences in total cell counts.

Table 2 lists odds ratios for prostate cancer by selected occupational exposures on and off the farm. No characteristic in table 2 was significantly associated with prostate cancer after adjustment for age and family history of prostate cancer.

Table 3 lists the 50 herbicides, insecticides, fungicides, and fumigants for which information concerning the frequency, duration, intensity, and cumulative exposure score was available in this study.

Results of the factor analysis showed a tendency for the use of certain pesticides to group together (table 4). Three factors explained almost 90 percent of the variance in pesticide usage in the observed data (appendix table 1). Factor 1 showed significant loading scores (i.e., correlations) with the herbicides atrazine, dicamba, cyanazine, metolachlor, *S*-ethyl dipropylthiocarbamate (EPTC), alachlor, imazethapyr, 2,4-dichlorophenoxyacetic acid (2,4-D), trifluralin, chlorimuron ethyl, metribuzin, petroleum oil, pendimethalin, and butylate and with the insecticide terbufos. These are pesticides used primarily on corn, soybeans, and other grain crops, which are especially important in Iowa. Factor 2 showed significant loading scores for North Carolina residence (i.e., –70 for Iowa). Pesticides descriptive of this factor include one herbicide (paraquat), three insecticides (parathion, carbaryl, aldicarb), one fumigant (methyl bromide), and four fungicides (benomyl, chlorothalonil, maneb/mancozeb, and metylaxyl). These pesticides are used on cotton, tobacco, vegetables, and fruit crops raised mostly in North Carolina that require intensive treatment for insects, nematodes, and fungi. Factor 3 loaded heavily on study

TABLE 3. Pesticides evaluated in this study for an association with prostate cancer by frequency of use,* duration of use,† intensity of use,‡ and cumulative use,§ Agricultural Health Study, 1993–1997

Herbicides	Insecticides	Fungicides	Fumigants
Alachlor	Aldicarb	Benomyl	Aluminum phosphide
Atrazine	Aldrin	Captan	Ethylene dibromide
Butylate	Carbofuran	Chlorothanil	Carbon tetrachloride/carbon disulfide
Chlorimuron-ethyl	Carbaryl	Maneb/macozeb	Methyl bromide
Cyanazine	Chlordane	Metalaxyl	
Dicamba	Chlorpyrifos	Ziram	
2,4-D¶	Coumaphos		
EPTC¶	Dichlorvos¶		
Glyphosate	Diazinon		
Imazethypyr	Dieldrin		
Metolachlor	DDT¶		
Metribuzin	Fonofos		
Paraquat	Heptachlor		
Pendimethalin	Lindane		
Petroleum oil as herbicide	Malathion		
2,4,5-T¶	Parathion		
2,4,5-TP¶	Permethrin (for crops)		
Trifluralin	Permethrin (for animals)		
	Phorate		
	Terbufos		
	Toxaphine		
	Trichlorofon		

* Frequency as application days/year.
† Duration as years of application.
‡ Intensity as the algorithm score.
§ Cumulative exposure as the product of frequency × duration × intensity.
¶ 2,4-D, 2,4-dichlorophenoxyacetic acid; EPTC, *S*-ethyl dipropylthiocarbamate; dichlorvos, 2,2-dichloroethenyl dimethylphosphate; DDT, dichlorodiphenyltrichloroethane; 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid; 2,4,5-TP, 2,4,5-trichlorophenoxypropionic acid.

subjects over 50 years of age; on chlorinated insecticides no longer registered for use in the United States, including aldrin, chlordane, dieldrin, dichlorodiphenyltrichloroethane (DDT), heptachlor, and toxaphene; and on two chlorinated phenoxy herbicides, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 2,4,5-trichlorophenoxypropionic acid (2,4,5-TP).

Table 4 shows odds ratios for categories of factor scores and tests of linear trends adjusted for age and family history of prostate cancer. Factor 3 was significantly associated with an excess risk of prostate cancer, while factor 1 and factor 2 were not.

Table 5 displays odds ratios for the 10 pesticides for which ever versus never use data and cumulative exposure scores were available from the enrollment questionnaires. For 35 additional pesticides for which similar cumulative exposure data were available (listed in table 3), no exposure-response association with prostate cancer was observed, and they were omitted from table 5 to save space (five pesticides were excluded from the analysis because five or fewer cases were exposed (i.e., trichlorofon, ziram, aluminum phosphide, ethylene dibromide, and carbon tetrachloride/carbon disulfide)). No meaningful differences were found in the exposure-response when analyzed as either a continuous or a categorical variable, so only the categorical analysis results are presented. We computed odds ratios adjusted for age and family history (reduced model) and for all the variables listed in table 1 (full model). Because the full model did not substantially change the odds ratio estimates for any pesticide, we provide the results from the reduced model in table 5. Among the pesticides listed in the enrollment questionnaire, only methyl bromide, a fumigant used by approximately 12 percent of the cohort, showed a significant linear trend ($p = 0.008$) with prostate cancer risk. This trend is almost entirely due to the elevated risk in the two highest exposure categories. Odds ratios were 1 (reference, no exposure), 1.01 (95 percent CI: 0.66, 1.56), 0.76 (95 percent CI: 0.47, 1.25), 0.70 (95 percent CI: 0.38, 1.28), 2.73 (95 percent CI: 1.18, 6.33), and 3.47 (95 percent CI: 1.37, 8.76). The trend in prostate cancer risk with methyl bromide did not differ by tumor grade; that is,

TABLE 4. Odds ratios, confidence intervals, and number of prostate cancer cases for factor scores, based on factor analysis of 50 pesticides, family history of prostate cancer, and age,* Agricultural Health Study, 1993–1997

Factor	Level†					*p* value, linear trend
	I (lowest exposure)	II	III	IV	V (highest exposure)	
Factor 1 (herbicides)						
Odds ratio	1.0	0.99	1.18	1.10	1.25	0.53
95% confidence interval		0.78, 1.26	0.89, 1.56	0.78, 1.55	0.88, 1.76	
No. of cases	188	189	94	48	47	
Factor 2 (fumigants/fungicides, North Carolina)						
Odds ratio	1.0	1.04	0.97	0.94	0.84	0.82
95% confidence interval		0.83, 1.30	0.74, 1.28	0.66, 1.34	0.59, 1.18	
No. of cases	188	189	95	46	48	
Factor 3 (older age, chlorinated pesticides)						
Odds ratio	1.0	1.29	1.51	1.37	1.39	0.005
95% confidence interval		1.02, 1.63	1.15, 2.00	0.96, 1.97	0.99, 1.97	
No. of cases	188	189	95	47	47	

* Adjusted for age and family history of prostate cancer.

† Levels = tertiles, with the upper tertile divided in half, and the resulting half divided in half again (levels IV and V) (i.e., level I, 0–33.3; level II, 33.4–66.6; level III, 66.7–83.3; level IV, 83.4–91.6; and level V, 91.7–100.0).

both well-differentiated tumors and poorly differentiated tumors were observed to have a significant linear trend with methyl bromide exposure ($p = 0.03$ and $p = 0.04$, respectively) (data not shown). Methyl bromide was also associated with a significantly increased risk of prostate cancer among private applicators in both states, with a linear trend p of 0.05 in North Carolina (odds ratios (ORs) for previously defined categories = 1 (reference), 0.9, 0.8, 0.7, 2.8, and 3.8) and a linear trend p of 0.04 in Iowa (ORs for previously defined exposure categories = 1 (reference), 1.7, 1.2, and 4.4; no cases in higher exposure categories), and among commercial applicators in Iowa, with a linear trend p of 0.01 (ORs for previously defined exposure categories = 1 (reference), 1.1, 3.1, 8.9, and 14.0; no cases in the highest exposure category). Similarly, significantly elevated exposure-response trends were observed for frequency of use, with $p = 0.02$ (ORs = 1 (reference), 0.93, 0.76, 1.31, 1.44, and 4.39), and lifetime application days, with $p = 0.02$ (ORs = 0.87, 0.78, 0.97, 2.09, and 2.63). The odds ratio for ever versus never use of methyl bromide data was elevated but not significantly (OR = 1.10, 95 percent CI: 0.85, 1.36).

Few differences were found between the cohort members who completed the take-home questionnaire (i.e., 40 percent applicators) and those that did not (31). These take-home questionnaires sought more detailed information on 28 pesticides (including 18 currently used pesticides and 10 pesticides no longer currently registered for use in the United States). Applicators who ever used any one of five insecticides, including three chlorinated insecticides associated with factor 3 (i.e., aldrin, DDT, and heptachlor), were at a significantly elevated risk of prostate cancer: carbofuran (OR = 1.25, 95 percent CI: 1.03, 1.52), permethrin for animal use (OR = 1.38, 95 percent CI: 1.01, 1.89), aldrin (OR = 1.32, 95 percent CI: 1.09, 1.60), DDT (OR = 1.37, 95 percent CI: 1.12, 1.67), and heptachlor (OR = 1.20, 95 percent CI: 1.00, 1.47). Little evidence was found, however, to support an exposure-response trend for prostate cancer with the use of any pesticide other than methyl bromide (table 5), and this significant association was unchanged when other pesticides were added to the logistic model (data not shown).

To assess the possible influence of a family history of prostate cancer on pesticide-associated risks (table 6), we assessed effect modification by including a cross-product term in the logistic model, that is, age + family history + pesticide exposure + (family history × pesticide exposure). Significant interaction odds ratios occurred among persons who used butylate (OR = 1.93, 95 percent CI: 1.19, 3.11), a widely used thiocarbamate herbicide; four commonly used organophosphorothioate insecticides including coumaphos (OR = 2.58, 95 percent CI: 1.29, 5.18), fonofos (OR = 2.04, 95 percent CI: 1.21, 3.44), chlorpyrifos (OR = 1.65, 95 percent CI: 1.02, 2.66), and phorate (OR = 1.64, 95 percent CI: 1.02, 2.63); and a pyrethroid, permethrin (for animal use) (OR = 2.31, 95 percent CI: 1.17, 4.56). Similar results were found in North Carolina and Iowa (results not shown). These associations did not change when other pesticides were added to the logistic model. Several other pesticides had nonsignificant but elevated interaction odds ratios ($p < 0.10$), including EPTC (OR = 1.68, 95 percent CI: 0.96, 2.94) (thiocarbamate herbicide), terbufos (OR = 1.52, 95 percent CI: 0.94, 2.45) (organophosphorothioate), dicamba (OR = 1.51, 95 percent CI: 0.95, 2.43) (benzoic herbicide), 2,2-dichloroethenyl dimethylphosphate (dichlorvos) (OR = 1.92, 95 percent CI: 0.98, 3.75) (organophosphate), aldicarb (OR = 2.01, 95 percent CI:

TABLE 5. Odds ratios,* confidence intervals, and number of exposed cases of prostate cancer by ever/never exposed and cumulative exposure score for methyl bromide and selected pesticides with no observed exposure-response association with prostate cancer,† Agricultural Health Study, 1993–1997

Pesticide	Ever/never use‡	Cumulative exposure score categories§ from enrollment questionnaire¶ and the farmer applicator and commercial applicator questionnaire#						*p* value, linear trend
		0 (no exposure, reference category)	I (lowest exposure)	II	III	IV	V (highest exposure)	
			Herbicides					
Alachlor¶								
Odds ratio	1.00	1	0.91	1.11	1.35	0.70	0.77	0.52
95% confidence interval	0.83, 1.20		0.70, 1.18	0.85, 1.45	0.95, 1.92	0.44, 1.12	0.48, 1.26	
No. of cases	263/303	303	81	82	40	20	20	
Atrazine¶								
Odds ratio	0.94	1	1.02	0.91	0.89	0.82	0.97	0.34
95% confidence interval	0.78, 1.14		0.79, 1.31	0.71, 1.18	0.65, 1.23	0.54, 1.25	0.63, 1.48	
No. of cases	364/202	202	113	114	57	27	28	
			Insecticides					
Carbofuran¶								
Odds ratio	1.25	1	1.29	1.93	1.00	0.68	1.01	0.23
95% confidence interval	1.03, 1.52		0.95, 1.74	1.42, 2.62	0.66, 1.51	0.38, 1.23	0.58, 1.77	
No. of cases	166/400	400	54	50	26	12	13	
Chlorpyrifos¶								
Odds ratio	0.90	1	0.95	1.04	0.89	0.64	0.73	0.23
95% confidence interval	0.74, 1.09		0.70, 1.30	0.75, 1.42	0.58, 1.36	0.35, 1.18	0.41, 1.31	
No. of cases	174/392	392	49	48	24	12	12	
Permethrin¶ (animal, animal confinement area application)								
Odds ratio	1.38	1	1.30	2.31	1.11	1.73	0.74	0.63
95% confidence interval	1.01, 1.89		0.76, 2.24	1.38, 3.87	0.54, 2.25	0.63, 4.75	0.24, 2.33	
No. of cases	48/518	518	16	16	8	4	4	
Aldrin#								
Odds ratio	1.32	1	1.44	1.12	1.56	0.87	1.38	0.70
95% confidence interval	1.09, 1.60		0.98, 2.11	0.76, 1.66	0.92, 2.64	0.38, 1.99	0.60, 3.19	
No. of cases	207/359	226	33	34	17	7	8	
DDT#,**								
Odds ratio	1.37	1	1.18	1.17	0.76	1.38	1.14	0.89
95% confidence interval	1.12, 1.67		0.84, 1.66	0.81, 1.69	0.46, 1.27	0.71, 2.68	0.59, 2.21	
No. of cases	323/243	178	50	45	23	11	11	

Table continues

0.95, 4.23) (carbamate insecticide), and carbofuran (OR = 1.58, 95 percent CI: 0.98, 2.55) (carbamate insecticide). No fungicide or fumigant, no chlorinated or inorganic insecticides, and no herbicides of the following chemical classes—acetamides, triazines, pyrimidines, phosphinic acids, imidazolines, bipyridyls, chlorinated phenoxies, dinitroanilines, or aliphatic hydrocarbons—had elevated ($p < 0.10$) interaction odds ratios.

To examine the specificity of these pesticide associations with family history, we examined the risk of prostate cancer from exposure to the same 45 pesticides, stratified by those with and without a family history of any cancer other than prostate cancer in a first-degree relative (data not shown). Only butylate (OR = 1.52, 95 percent CI: 1.13, 2.02) had a significantly elevated risk of prostate cancer in the group with a family history of cancer (other than prostate cancer), and only butylate showed significant effect modification, although a number of other nonsignificant interactions were observed. Permethrin for animal use (OR = 1.59, 95 percent CI: 1.07, 2.36) and phorate (OR = 1.31,

TABLE 5. Continued

Pesticide	Ever/never use	Cumulative exposure score categories from enrollment questionnaire and the farmer applicator and commercial applicator questionnaire						*p* value, linear trend
		0 (no exposure, reference category)	I (lowest exposure)	II	III	IV	V (highest exposure)	
Heptachlor#								
Odds ratio	1.20	1	1.08	0.86	1.00	0.64	0.66	0.41
95% confidence interval	0.99, 1.47		0.67, 1.74	0.53, 1.41	0.51, 1.98	0.20, 2.03	0.21, 2.09	
No. of cases	165/401	273	20	19	10	6	3	
			Fumigants					
Methyl bromide¶								
Odds ratio	1.10	1	1.01	0.76	0.70	2.73	3.47	0.004
95% confidence interval	0.77, 1.36		0.66, 1.56	0.47, 1.25	0.38, 1.28	1.18, 6.33	1.37, 8.76	
No. of cases	84/482	482	23	22	11	6	5	
			Fungicides					
Captan¶								
Odds ratio	1.05	1	1.07	1.09	1.89	0.95	2.79	0.11
95% confidence interval	0.78, 1.43		0.50, 2.30	0.48, 2.48	0.58, 6.12	0.23, 3.93	0.35, 22.1	
No. of cases	48/518	518	7	6	3	2	1	

* Odds ratios adjusted for age and family history of prostate cancer.

† Five pesticides (i.e., trichlorofon, ziram, aluminum phosphide, ethylene dibromide, carbon tetrachloride/carbon disulfide) were not included in this table because we observed five or fewer exposed cases. Thirty-five other pesticides (i.e., cyanazine, dicamba, 2,4-dichlorophenoxyacetic acid, thiocarbamate, glyphosate, imazethapyr, metachlor, trifluralin, coumaphos, 2,2-dichloroethenyl dimethylphosphate, fonofos, permethrin for crop use, turbufos, chlorothalonil, butylate, chlorimuron-ethyl, metribuzin, paraquat, pendimehalin, petroleum oil used as herbicide, 2,4,5-trichlorophenoxyacetic acid, 2,4,5-trichlorophenoxypropionic acid, aldicarb, carbaryl, chlordane, diazinon, dieldrin, lindane, malathion, parathion, phorate, toxaphene, benomyl, maneb/macozeb, methylaxyl) were not included in this table because they did not demonstrate a significant exposure-response association with prostate cancer.

‡ Study subjects in the ever/never analysis equal or exceed the number in the exposure-response analysis because of occasional missing data for the exposure algorithm.

§ Categories: 0 (no use), I (0.1–33.3 percentile of use), II (33.4–66.7 percentile of use), III (66.8–83.3 percentile of use), IV (83.4–91.6 percentile of use), and V (>91.6 percentile of use).

¶ Information on age, family history of prostate, ever/never use of 50 pesticides, and cumulative use of 22 pesticides taken from the enrollment questionnaire completed by 54,766 non-prostate cancer cohort members and 566 prostate cancer cohort members.

Information on cumulative pesticide use of 28 pesticides from farmer applicator and commercial applicator questionnaire completed by 24,034 non-prostate cancer cohort members and 331 prostate cancer cohort members.

** DDT, dichlorodiphenyltrichloroethane.

95 percent CI: 1.03, 1.67) were the only chemicals observed to have a significant excess risk among those with no family history of cancer, but no significant effect modification was observed (data not shown). We also examined the risk of any cancer other than prostate cancer (n = 816 other cancers) among those exposed to each of the 45 pesticides, stratified by a family history of any cancer (other than prostate cancer), and found little evidence of effect modification (data not shown).

DISCUSSION

The literature suggests that prostate cancer may be elevated among farmers (14, 16, 18–22, 32, 33). Consistent with these earlier reports, we found that farmers in the Agricultural Health Study cohort experienced a small but statistically significant excess of prostate cancer compared with the general population in Iowa and North Carolina (SIR = 1.14). It is challenging to relate cancer risks to specific lifestyle or agricultural exposures. We used four approaches in this paper. First, we evaluated a broad range of factors including demographic characteristics, lifestyle factors, agricultural factors, and nonfarm occupational factors to identify associations with prostate cancer. Second, factor analysis was used to identify groupings of pesticide exposures that might be related to prostate cancer. Third, analyses of individual pesticides were conducted. Finally, effect modification was assessed between individual pesticide use and a family history of prostate cancer.

In the factor analysis, three temporally and geographically distinct factors of pesticide use were identified. Only one of these factors (factor 3) was significantly related to prostate cancer. This factor included ever use of the chlorinated pesticides aldrin, chlordane, dieldrin, DDT, heptachlor, and

TABLE 6. Odds ratios, confidence intervals, and number of prostate cancer cases by exposure status to 15 of 45* evaluated pesticides with and without a first-degree family history of prostate cancer, Agricultural Health Study, 1993–1997

Pesticide (chemical class)	Prostate cancer risk for those with exposure to pesticide but no family history of prostate cancer†			Prostate cancer risk for those with exposure to pesticide and a family history of prostate cancer‡			Statistical interaction between family history of prostate cancer and exposure to pesticide§		
	Odds ratio	95% confidence interval	No. of prostate cancer cases	Odds ratio	95% confidence interval	No. of prostate cancer cases	Interaction odds ratio	95% confidence interval	*p* value
Herbicides									
Alachlor (acetamide)	0.93	0.76, 1.14	190	1.36	0.88, 2.10	56	1.50	0.93, 2.41	0.10
Atrazine (triazine)	0.88	0.72, 1.09	253	1.28	0.77, 2.12	70	1.52	0.88, 2.62	0.13
Butylate (thiocarbamate)	0.96	0.77, 1.20	110	1.78	1.16, 2.73	44	1.93	1.19, 3.11	0.007
Dicamba (benzoic)	0.95	0.77, 1.17	163	1.35	0.88, 2.08	50	1.51	0.95, 2.43	0.09
EPTC¶	0.90	0.67, 1.20	55	1.44	0.89, 2.34	24	1.68	0.96, 2.94	0.07
Insecticides									
Aldicarb (carbamate)	0.81	0.57, 1.16	35	1.60	0.83, 3.09	11	2.01	0.95, 4.23	0.07
Carbofuran (carbamate)	1.14	0.92, 1.42	118	1.81	1.18, 2.77	43	1.58	0.98, 2.55	0.06
Chlorpyrifos (organophosphorothioate)	0.82	0.66, 1.02	121	1.29	0.84, 1.98	40	1.65	1.02, 2.66	0.04
Coumaphos (organophosphorothioate)	0.86	0.57, 1.28	26	2.17	1.24, 3.82	16	2.58	1.29, 5.18	0.008
2,2-Dichloroethenyl dimethylphosphate (organophosphate)	0.95	0.66, 1.37	32	1.75	1.00, 3.06	16	1.92	0.98, 3.75	0.06
Fonofos (organophosphonodithioate)	0.92	0.71, 1.19	71	1.80	1.14, 2.84	30	2.04	1.21, 3.44	0.008
Permethrin, animal use (pyrethriod)	1.13	0.77, 1.66	30	2.38	1.34, 4.25	16	2.31	1.17, 4.56	0.02
Phorate (organophosphorodithioate)	1.05	0.85, 1.30	140	1.67	1.09, 2.56	48	1.64	1.02, 2.63	0.04
Terbufos (organophosphorodithioate)	0.99	0.80, 1.23	126	1.45	0.95, 2.23	40	1.52	0.94, 2.45	0.09
Fumigants									
Methyl bromide (halogenated hydrocarbon)	0.93	0.70, 1.23	58	1.31	0.75, 2.29	16	1.36	0.73, 2.54	0.34

* Five pesticides (i.e., trichlorofon, ziram, aluminum phosphide, ethylene dibromide, carbon tetrachloride/carbon disulfide) were not included in this table because we observed five or fewer exposed cases. Thirty other pesticides (i.e., chlorimuron-ethyl, cyanazine, 2,4-dichlorophenoxyacetic acid, glyphosate, imazethapyr, metachlor, trifluralin, permethrin for crop use, chlorothalonil, metribuzin, paraquat, pendimehalin, petroleum oil used as herbicide, 2,4,5-trichlorophenoxyacetic acid, 2,4,5-trichlorophenoxypropionic acid, aldrin, carbaryl, chlordane, diazinon, dieldrin, dichlorodiphenyltrichloroethane, heptachlor, lindane, malathion, parathion, toxaphene, benomyl, captan, maneb/macozeb, methylaxyl) were not included in this table because they did not demonstrate a significant exposure-response association with prostate cancer.

† Reference group, no family history of prostate cancer and no pesticide exposure.

‡ Reference group, family history of prostate cancer and no pesticide exposure.

§ Adjusted for age and family history of prostate cancer.

¶ EPTC, *S*-ethyl dipropylthiocarbamate.

toxaphene; ever use of two chlorinated phenoxy herbicides (2,4,5-T and 2,4,5-TP); and farmers over the age of 50 years. Three of the chlorinated insecticides in this factor, that is, aldrin, DDT, and heptachlor, were associated with a significant excess risk of prostate cancer in ever/never analyses, although no exposure-response pattern was observed for these chemicals. Because the factors in this analysis are based on ever versus never use (pesticide) data, they would be more apt to show statistical significance if several chemicals in the factor had the same association with prostate cancer. Lacking an exposure-response pattern with individual pesticides suggests that the relation with chlorinated pesticides could be due to other exposures not identified in this analysis.

Among the 45 specific pesticides evaluated, the only statistically significant exposure-response trend observed occurred with methyl bromide. This could be a chance observation because we evaluated a large number of pesticides. However, methyl bromide was significantly associated with prostate cancer risk among both North Carolina and Iowa pesticide applicators and among both private and commercial applicators. The association was also found

when we used other measures of exposure, including frequency of use (days per year) and total days of use in a lifetime. Moreover, the pattern of risk was not substantially changed when other pesticides were added to the logisitic model with methyl bromide. Methyl bromide is an alkylating agent (34), and the National Institute for Occupational Safety and Health considers it to be a potential occupational carcinogen (35). Additionally, evidence of genotoxicity was observed in a small cross-sectional study of nonsmoking methyl bromide fumigation workers, with excesses of micronuclei and gene mutations (i.e., *HPRT* mutations) observed in the lymphocytes and oropharyngeal cells of exposed workers (36). Field testing by the National Institute for Occupational Safety and Health demonstrates that concentrations of methyl bromide in the breathing zones of agricultural workers conducting soil fumigation under tarpaulins (a common soil fumigation procedure used by many farmers in North Carolina but not in Iowa) frequently exceeded the recommended occupational limits set by the Institute (37). Approximately 27,000 tons of methyl bromide were used in 1997 in the United States for soil fumigation (87 percent), commodity and quarantine treatment (8 percent), and structural fumigation (5 percent) (38). Our data would suggest that, if methyl bromide is responsible for an elevated prostate cancer risk, it may be among only those with relatively frequent use. Because we had no specific a priori hypothesis linking methyl bromide to prostate cancer, we cannot rule out the possibility that our observation occurred by chance alone; however, the consistency of the findings argues against this.

A family history of prostate cancer among first-degree relatives conferred a twofold excess risk of prostate cancer on these subjects, consistent with other reports (8). Furthermore, significant associations between specific pesticides and prostate cancer risk were observed largely among those with a family history of prostate cancer. Although a family history of cancer other than prostate cancer seemed to have a similar pattern of prostate cancer risk with some pesticides, only butylate had a statistically significant positive association. No pattern of effect modification was seen when we evaluated all cancers, other than prostate, and a history of cancer other than prostate cancer. These findings tend to mitigate the possibility of a family history-driven case-recall bias in these data. The specificity for family history of prostate cancer suggests the possibilities of familial genes that enhance susceptibility or of shared environmental risk factors for prostate cancer among family members. The significant effect modification in selected chemical classes (e.g., thiocarbamates, organophosphorothioates, and pyrethoid) lends further support to this hypothesis.

This study does have limitations. First, the exposure weightings used in our algorithm are based on a literature review and not on direct measurements of exposure made within the study cohort. An exposure-monitoring effort within the study cohort is under way and will help to refine our estimates of exposure in the future. Second, some subjects in this study were asked to recall pesticide use from years ago. For the oldest members of the cohort, this was decades earlier. Although recall can be faulty after many years, previous evaluation of this issue has shown that recall of pesticide use by the Agricultural Health Study cohort is comparable with the recall of other variables, such as diet and alcohol consumption, which have been used by epidemiologists in other studies as a standard procedure (39). Third, follow-up of this cohort is relatively short, and it is not possible to evaluate time-dependent exposures and risk.

The Agricultural Health Study has five principal strengths. First, the data collection prior to the diagnosis of cancer precludes the possibility of case-ascertainment bias. Second, detailed information on exposure for each pesticide included days of use per year, years of use, application methods, and protective equipment use, adding specificity to the analysis. Third, ascertainment of and statistical adjustment for other occupational, demographic, and lifestyle factors previously suggested as prostate cancer risk factors mitigate the possibility of uncontrolled confounding. Fourth, the large size of the study gives sufficient statistical power to examine the risk of exposure to a number of specific chemical exposures. Fifth, the outcome is cancer incidence obtained from population-based tumor registries, which eliminates survival problems.

In conclusion, farmers and commercial pesticide applicators have a small but significantly higher rate of prostate cancer than the general population of Iowa and North Carolina. Occupational use of a widely used halogenated fumigant, methyl bromide, was shown to be significantly associated with a risk of prostate cancer in the Agricultural Health Study cohort among those with the highest exposure. A pattern of chlorinated pesticide use may also be related to prostate cancer risk. A family history of prostate cancer appeared to significantly modify the prostate cancer risks among those using several widely used insecticides, including chlorpyrifos, coumaphos, fonofos, phorate, and permethrin for animal use, and a herbicide, butylate. The methyl bromide and family history findings are novel and unexpected and need to be confirmed in later follow-up periods in this cohort and in other studies of prostate cancer in farmers.

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(*Appendix follows*)

APPENDIX

APPENDIX TABLE 1. Results of factor analysis for pesticide use, age, and state (*n* = 42,948), Agricultural Health Study, 1993–1997*

Variable	Factor I	Factor II	Factor III
Herbicides			
Atrazine	58†	0	1
Dicamba	54†	–23	3
Cyanazine	55†	–12	4
Metolachlor	59†	8	–11
EPTC‡	48†	–8	–2
Alachlor	49†	10	3
Imazethapyr	60†	–22	–11
Glyphosate	31	27	–5
Trifluralin	60†	–2	–2
2,4-D‡	47†	0	8
Chlorimuron ethyl	54†	17	–13
Metribuzin	65†	–1	3
Paraquat	19	51†	4
Petrolium oil	44†	12	12
Pendimethalin	50†	30	–15
Butylate	52†	9	8
2,4,5-TP‡	6	10	44†
2,4,5-T‡	6	0	56†
Insecticides			
Permethrin (crop)	32	30	–8
Terbufos	40†	–1	4
Fonofos	30	–9	12
Trichlorfon	2	10	2
Carbofuran	30	16	16
Chlorpyrifos	31	22	0
Coumaphos	9	1	16
Permethrin (animal)		23	–58†
Dichlorvos‡	18	–5	21
Lindane	14	6	36
Malathion	34	16	16
Parathion	6	40†	25
Carbaryl	11	44†	17
Diazinon	7	39	25
Aldicarb	5	61†	–5
Phorate	36	–2	19
Aldrin	9	–7	65†
Chlordane	0	18	53†
Dieldrin	–1	0	59†
DDT‡	–11	11	62†
Heptachlor	10	–13	65†
Toxaphene	6	27	43†

Table continues

APPENDIX TABLE 1. Continued

Variable	Factor I	Factor II	Factor III
Fumigants			
Methyl bromide	–11	59†	–3
Aluminum phosphide	14	16	15
80/20 mix	2	11	38
Ethylene dibromide	–2	31	25
Fungicides			
Chlorothalonil	2	53†	–11
Captan	14	16	9
Ziram	–5	23	22
Benomyl	–3	61†	6
Mancozeb	–8	58†	10
Metylaxyl	–1	62†	–3
State of Iowa	36	–70†	10
Age of ≥50 years	–21	–11	52†
% of variance explained	0.44	0.30	0.15
% of cumulative variance	0.44	0.74	0.89

* Factor loadings are multiplied by 100 and rounded to the nearest integer.

† Indicates a factor loading score of greater than or equal to ±0.40.

‡ EPTC, *S*-ethyl dipropylthiocarbamate; 2,4-D, 2,4-dichlorophenoxyacetic acid; 2,4,5-TP, 2,4,5-trichlorophenoxypropionic acid; 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid; dichlorvos, 2,2-dichloroethenyl dimethyl-phosphate; DDT, dichlorodiphenyltrichloroethane.

A-SM

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11-29-98

MEMORANDUM

SUBJECT: Phorate - Review of Pesticide Poisoning Incident Data

FROM: Virginia A. Dobozy, V.M.D., M.P.H., Veterinary Medical Officer
Registration and Special Review Section
Occupational and Residential Exposure Branch

THRU: Jerome Blondell, Ph.D., M.P.H.
Registration and Special Review Section
Occupational and Residential Exposure Branch

and

Francis B. Suhre, Acting Section Head
Registration and Special Review Section
Occupational and Residential Exposure Branch

TO:
Registration and Special Review Section
Occupational and Residential Exposure Branch

The following data bases have been consulted for the poisoning incident data on the active ingredient phorate (PC Code: 057201):

1) OPP Incident Data System (IDS) - reports of incidents from various sources, including registrants, other federal and state health and environmental agencies and individual consumers, submitted to OPP since 1992.

2) Poison Control Centers - as the result of Data-Call-Ins issued in 1993, OPP received poison control center data covering the years 1985 through 1992 for 28 organophosphate and carbamate chemicals. Most of the national Poison Control Centers (PCCs) participate in a national data collection system, the Toxic Exposure Surveillance which obtains data from 70 centers at hospitals or universities. PCCs provide telephone consultation for individuals and health care providers on suspected poisonings, involving drugs, household products, pesticides, etc.

3) California Department of Food and Agriculture (replaced by the Department of Pesticide Regulation in 1991) - California has

collected uniform data on suspected pesticide poisonings since 1982. Physicians are required, by statute, to report to their local health officer all occurrences of illness suspected of being related to exposure to pesticides. The majority of the incidents involve workers. Information on exposure (worker activity), type of illness (systemic, eye, skin, eye/skin and respiratory), likelihood of a causal relationship,and number of days off work and in hospital are provided.

4) National Pesticide Telecommunications Network (NPTN) - NPTN is a toll-free information service supported by OPP. A ranking of the top 200 active ingredients for which telephone calls were received during calendar years 1984-1991, inclusive has been prepared. The total number of calls was tabulated for the categories humans, animals, calls, incidents and others.

PHORATE REVIEW

IDS

There were 18 separate incidents reported to the IDS as of December 12, 1995. The vast majority of these involved wildlife and ecological adverse effects which will be reviewed by the Environmental Fate and Effects Division. One report of agricultural exposure involved a Brazilian worker who spooned phorate granules from a container for application around coffee trees. He did not wear safety equipment and after 10 days at work, developed nausea and headache. He recovered with hospitalization and treatment.

Poison Control Center Data

Phorate was one of the 28 chemicals for which poison control center data were requested. The following statistics are taken from an analysis of these data; see December 5, 1994 memo from Jerome Blondell to Joshua First.

Occupational and Non-occupational Exposure

There were a total of 109 cases of occupational exposure to phorate reported to the Poison Control Centers; 85 (78%) involved exposure to phorate alone and 24 (22%) involved exposure to multiple chemicals, including phorate. There were a total of 82 non-occupational exposures; 74 (90%) involved this chemical alone and 8 (10%) were attributed to multiple chemicals.[1] (Phorate is registered mainly for agricultural uses.)

In this analysis, four measures of hazard were developed based on the Poison Control Center data, as listed below.

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[1] Workers who were indirectly exposed (not handlers) were classified as non-occupational cases.

(2)

1. Percent of all accidental cases that were seen in or referred to a health care facility (HCF).

2. Percent of these cases (seen in or referred to HCF) that were admitted for medical care.

3. Percent of cases reporting symptoms based on just those cases where the medical outcome could be determined.

4. Percent of those cases that had a major medical outcome which could be defined as life-threatening or resulting in permanent disability.

Exposure to phorate alone or in combination with other chemicals was evaluated for each of these categories, giving a total of 8 measures.

The following table presents the analyses for occupational and non-occupational exposures. The number in parenthesis is the median score for that category.

	Occupational Exposure	Non-occupational Exposure
Percent Seen in HCF		
Single chemical exposure	68.2 (68.2)	66.2* (44.0)
Multiple chemical exposure	66.1 (69.8)	64.6* (46.1)
Percent Hospitalized		
Single chemical exposure	15.5 (12.2)	16.3 (9.9)
Multiple chemical exposure	15.3 (14.3)	15.1 (12.6)
Percent with Symptoms		
Single chemical exposure	94.3* (85.8)	74.5 (74.0)
Multiple chemical exposure	95.3* (85.8)	76.9 (75.2)
Percent with Life-threatening Symptoms		
Single chemical exposure	3.8* (0.0)	0.0 (0.0)
Multiple chemical exposure	3.1* (0.5)	0.0 (0.05)

* Included in the top 25% of insecticides.

A ranking of the 28 chemicals was done based on the above eight measures, with the lowest number being the most frequently

implicated in adverse effects. Phorate ranked number 6 for occupational exposure and number 7 for non-occupational exposure (in spite of being registered for mostly agricultural uses). Phorate was one of eight chemicals in the top 10 rankings for both occupational and non-occupational exposure.

Exposure in Children

A separate analysis of the number of exposures in children five years of age and under from 1985-1992 was conducted. For phorate, there were a total of 26 reports; 25 involved exposure to this chemical alone while 1 was attributed to multiple chemicals. Further analysis showed that 36.0% with exposure to phorate alone were seen at a health care facility[2]. The percentage was 38.5% when phorate was used in combination with other chemicals[3]. Of these cases, the percentage hospitalized was 11.1% and 10.0% with single and multiple chemical exposures, respectively.

Ratios of Poisoning Per Reported Use - California Data

California data on the number of systemic poisonings (1982 through 1989) and number of applications per year were used to calculate a ratio of the poisonings/1,000 applications. The data on phorate are presented below. The median for 29 insecticides is presented for comparison.

Number of Applications	Systemic Poisonings/1,000 Applications (Number) Primary Pesticide Only		
	Handlers	Field Workers	Total
12,984	.46 (6)	.31 (4)	.77 (10)
Median	.21	.20	.41

Ratios of Poisonings - U.S. Poison Control Center Data

The poison control center data for those pesticides used almost exclusively in agriculture and data on 15 insecticides used in U.S. crop production[4] were used to calculate the following ratios: exposure per use, poisonings per use, health care referral per use and hospital admitted cases per use. The ratios for phorate are presented in the table below.

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[2] This percentage was the second highest of the 17 chemicals with 25 or more incident reports. Only carbofuran exposure resulted in a higher percentage of referrals to a HCF.

[3] This percentage was the third highest of the 17 chemicals with 25 or more incident reports. Only carbofuran and aldicarb were higher.

[4] Gianessi. L.P., Puffer, C.A. 1992. Insecticide Use in U.S. Crop Production. Resource for the Future, Washington, D.C.

	Exposure per Use	Poisonings per Use	Health Care Referral per Use	Hospital Admitted Cases per Use
Phorate	.023	.013	.015	.002
Median	.033	.013	.027	.004

California Detail Data - 1982-1993 - Circumstances of Poisoning

There were 22 case reports of adverse reactions received by the California Pesticide Illness Surveillance Program from 1982 to 1993; 20 involved use of phorate alone while in 2 cases other chemicals were also used. The following table presents the categories of illness by year[5].

	Systemic	Skin	Eye	Respiratory
1982	6			
1983	2			
1984	1			
1985	1			
1987	1			
1988	1		1	
1989	3	1		1
1990	1			
1991	1			
1992	1	1		
Total	18	2	1	1

One incident reported both systemic and skin illnesses; another systemic and respiratory illness (not used until 1989).

Phorate application (5 cases) and mixing/loading (4 cases) were the most frequently cited activity classifications. Many of the reports did not list crop treated; sugar beets (4 cases) was the most frequently listed.

One fatality was reported in 1982 in a 22-month old child who was playing in his grandparent's yard where a can of Thimet was in a coffee can. The child became nauseated and collapsed. He was rushed to the hospital but died four days later. Symptoms listed on the report are nausea, coma, pneumonia, cerebral edema and death. Three

[5] There were no reports for 1986.

emergency response personnel were exposed to the pesticide under similar conditions in 1982. They inhaled the chemical while assisting a patient with a coffee can of Thimet. Symptoms included headache, blurred vision and nausea.

Seven incidents involved accidents or failure to use protective equipment. In one case, a student who was barechested entered a field which had been treated with Thimet a day earlier to set up irrigation and check flow. He experienced headache, muscle aches, nausea, diarrhea, mild rash and dizziness. Two workers were exposed on two separate occasions while carrying bags of phorate with holes. A mixer/loader did not wear a respirator and developed headache, nausea and heartburn. A fifth worker was not wearing goggles when pesticide dust blew into his eye. Two UPS drivers were exposed to the fumes of phorate from a spill during a delivery. It is also interesting to note from the comments section of the reports that several workers did not feel ill until working with the pesticide for multiple days.

NPTN

A total of 116 calls on phorate were handled by NPTN from 1984 to 1991, inclusively. A total of 39 incidents, involving 29 humans and 5 animals, were reported.

CONCLUSIONS

The following conclusions can be drawn from the above analyses of the Poison Control Center data from 1985 through 1992.

1. The percent of occupational exposures to phorate alone or in combination with other chemicals which resulted in both symptoms and life-threatening symptoms exceeded the median score for the 28 chemicals analyzed. Four of the four calculations were in the top 25% of chemicals most frequently associated with adverse effects that had symptomatic or life-threatening outcomes. (See page 3.)

2. Non-occupational exposure to phorate, whether alone or in combination with other chemicals, exceeded the median score for the number of cases referred to a health care facility (HCF). (The Poison Control Centers classified workers indirectly exposed, i.e., non-handlers, as non-occupational exposures.) (See page 3.)

3. Of the 28 chemicals, pnorate ranked 6 for occupational exposure and 7 for non-occupational exposure, with number 1 being most frequently associated with adverse effects. This suggests that phorate is above average in its ability to cause adverse effects. Therefore, regulatory restrictions to prevent acute poisoning should be in accordance with other organophosphates that are above average.

4. When using the California data and calculating ratios for the number of systemic poisonings per 1,000 applications, the

calculations are for phorate are higher than the median score for the 28 chemicals. Note, however, that California calculations were based on a relatively small number of cases. (See page 4, Ratios of Poisonings per Reported Use - California.) When using U.S. data, the ratios for exposure per use, poisonings per use, health care referral per use and hospital admitted cases per use were below the median scores. (See page 4, Ratios of Poisonings - U.S. Poison Control Center Data.) However, it should be remembered that these 28 chemicals were selected for a Data-Call-In because of concerns about the incidence of poisonings in California agricultural workers.

5. Approximately one-third of children exposed to phorate, whether alone or in combination with other chemicals, were referred to a HCF.

The following conclusions can be drawn from the detailed California Incident Data from 1982-1993.

1. Symptoms of a systemic illness are more likely reported after phorate exposure as compared to ocular and dermal effects.

2. Applicators and mixer/loaders are the most frequently affected activity categories.

3. Phorate is currently only used in granular formulations. Some of the above average ratios or measures of hazard (described above) suggest that handlers may not fully observe precautions because of the perception that poisoning is much less likely with a granular than liquid formulation. A similar pattern, with even greater hazard measures, has been found for granular aldicarb. Label requirements for these products need to be as stringent as for liquids. A prominent label warning that failure to follow precautions may be expected to result in serious or even life-threatening poisoning requiring immediate medical care should be considered. Also, the following may be added, "This granular formulation is soluble and is readily absorbed across skin to cause poisoning."

In-laws, insecticide—and a mimic of brain death

John Victor Peter, Appaswamy T Prabhakar, Kishore Pichamuthu

Lancet 2008; 371: 622

See Comment page 538

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In December, 2006, a 28-year-old woman from Andhra Pradesh, India, impulsively swallowed 50 mL of phorate (a diethyl organophosphorus insecticide) after quarrelling with her husband's family, with whom she lived. Her in-laws saw her vomit and briefly lose consciousness—and, suspecting what she had done, took her by moped to a local hospital. The patient was given gastric lavage, before being transferred by ambulance to the emergency department at our hospital, 400 km away. The patient arrived 9 h 30 min after her suicide attempt. Her giddiness and vomiting had persisted, and she now also had abdominal pain; but she was stable.

5 h after arrival, the patient became increasingly breathless; her arterial oxygen saturation decreased to 77%, necessitating intubation and ventilation. She was transferred to our intensive-care unit. We prescribed atropine, at 4 mg/h, to counteract the effects of organophosphate. Although the chest radiograph was clear, we suspected that the breathlessness was caused by aspiration, and prescribed penicillin and levofloxacin. We also prescribed morphine and lorazepam, to keep the patient comfortable but easily arousable. We titrated the dose of atropine to the patient's heart rate, but aimed also for quiet bowel sounds, pupils that were neither contracted nor dilated, a clear-sounding chest, and a systolic blood pressure higher than 90 mm Hg. The patient recovered steadily until her 4th day in hospital, when her score on the Glasgow coma scale (GCS) decreased to 8T (figure), prompting us to discontinue sedation. Over the next 12 h, the patient's limbs trembled and jerked, although she did not have seizures; we noted that the muscular tone of the limbs had increased. The GCS score then decreased to 2T over the next 12–24 h. We could not find any cause for the coma, other than organophosphate poisoning, despite doing blood tests (including arterial blood gases), CT of the head, a lumbar puncture, and monitoring the patient's arterial oxygen saturations. The serum concentration of pseudocholinesterase was 254 IU/mL (normal range 3000–6000 IU/mL). During the coma, findings on examination were largely consistent with brain death: oculocephalic, pupillary, corneal, and deep-tendon reflexes were absent; the patient did not react to painful stimuli or caloric stimulation; she did not breathe spontaneously. Unlike in brain death, however, the pupils remained constricted. An electroencephalogram showed global suppression of cortical activity. We continued to prescribe atropine. After 5 days of deep coma, the patient started to recover, and was fully conscious by day 15. She later developed pneumonia, but was discharged from the hospital, in good health, after a 39-day stay. When last seen, in March, 2007, she was well.

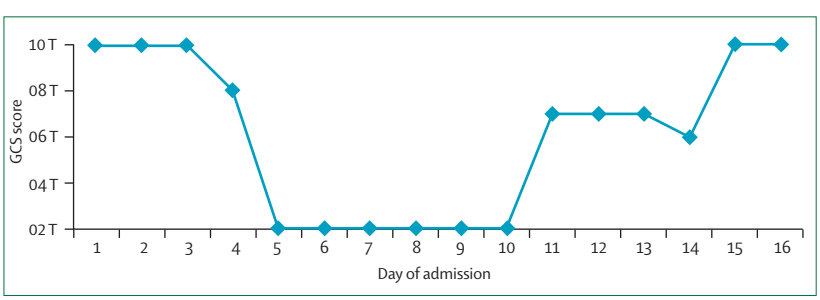

Figure: **Delayed-onset coma in organophosphate poisoning**
Since the verbal component of the GCS cannot be accurately assessed in patients who are intubated, the GCS is given on a scale of 2T–10T, where 2T is equivalent to 3, and 10T is equivalent to 15.

Organophosphates inhibit acetylcholinesterase, causing overstimulation of nicotinic, muscarinic, and central acetylcholine receptors. Neurological manifestations of organophosphate poisoning range from anxiety, restlessness, and tremors to seizures, central respiratory depression, and coma.[1] Although neurological manifestations are usually observed shortly after poisoning occurs, they can be delayed.[2,3] Recognition of delayed symptoms and signs can avert unfortunate misdiagnoses, such as brain death. Phorate is lipid-soluble: we conclude that much of the swallowed insecticide was absorbed by the patient's body fat, and released several days into her hospital stay.[2] Patients who have swallowed lipid-soluble organophosphates may benefit from treatment with oximes, which separate organophosphate from acetylcholinesterase, for longer than patients who have swallowed other organophosphates. By contrast, gastric lavage may not be helpful,[4] although patients' relatives may demand it, as a sign that all possible efforts are being made. Most hospitals in rural India are able to provide gastric lavage and atropine—however, few are able to intubate and ventilate the patient, and many prefer to avoid the legal and administrative complications of suicide attempts. Banning the most toxic pesticides in China and India could save more than 150 000 lives a year.[5]

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Cancer Research

Pesticide Use Modifies the Association Between Genetic Variants on Chromosome 8q24 and Prostate Cancer

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Abstract

Genome-wide association studies have identified 8q24 region variants as risk factors for prostate cancer. In the Agricultural Health Study, a prospective study of licensed pesticide applicators, we observed increased prostate cancer risk with specific pesticide use among those with a family history of prostate cancer. Thus, we evaluated the interaction among pesticide use, 8q24 variants, and prostate cancer risk. The authors estimated odds ratios (OR) and 95% confidence intervals (95% CI) for interactions among 211 8q24 variants, 49 pesticides, and prostate cancer risk in 776 cases and 1,444 controls. The ORs for a previously identified variant, rs4242382, and prostate cancer increased significantly ($P < 0.05$) with exposure to the organophosphate insecticide fonofos, after correction for multiple testing, with per allele $OR_{nonexposed}$ of 1.17 (95% CI, 0.93–1.48), per allele OR_{low} of 1.30 (95% CI, 0.75–2.27), and per allele OR_{high} of 4.46 (95% CI, 2.17–9.17; *P*-interaction = 0.002, adjusted *P*-interaction = 0.02). A similar effect modification was observed for three other organophosphate insecticides (coumaphos, terbufos, and phorate) and one pyrethroid insecticide (permethrin). Among ever users of fonofos, subjects with three or four risk alleles at rs7837328 and rs4242382 had approximately three times the risk of prostate cancer (OR, 3.14; 95% CI, 1.41–7.00) compared with subjects who had zero risk alleles and never used fonofos. We observed a significant interaction among variants on chromosome 8q24, pesticide use, and risk of prostate cancer. Insecticides, particularly organophosphates, were the strongest modifiers of risk, although the biological mechanism is unclear. This is the first report of effect modification between 8q24 and an environmental exposure on prostate cancer risk. *Cancer Res; 70(22); OF1–10. ©2010 AACR.*

Introduction

Until recently, only increasing age, race/ethnicity, and family history have been established as risk factors for prostate cancer (1). Genome-wide association studies (GWAS) have identified several independent loci on chromosome 8q24 as additional risk factors for prostate cancer. Single nucleotide polymorphisms (SNP) in four regions of 8q24 (2, 3), including several SNPs in region 2, rs45114 and rs620861 in region 4, rs6983267 in region 3, and rs1447295 and rs4242382 in region 1, were associated with prostate cancer (3–11). These variants are located in a region with no known protein-coding genes, and thus the mechanism by which they confer greater prostate cancer susceptibility is unclear. Approximately 200 kb from the nearest prostate GWAS locus is the c-myc oncogene (*MYC*), which has a well-established role in carcinogenesis (12, 13). It is still unclear, however, whether 8q24 variants act to influence *MYC* expression or other yet undetermined biological processes (14–16). Additional clues to the genetic susceptibility of prostate cancer may be gained from evaluating the interaction of the 8q24 region with environmental exposures and risk for prostate cancer.

The Agricultural Health Study (AHS) is a prospective cohort of licensed private and commercial pesticide applicators in Iowa and North Carolina, with in-depth characterization of lifetime mixing, loading, and application of pesticides. We had previously reported that men in this cohort of pesticide applicators have a higher risk of prostate cancer than men in the general population of Iowa and North Carolina [private applicators standardized incidence ratio (SIR), 1.24; 95% confidence interval (95% CI), 1.18–1.33; commercial applicators SIR, 1.37; 95% CI, 0.98–1.86; refs. 17, 18]. Previous analyses within the cohort had shown that the use of chlorinated pesticides and the use of the fumigant methyl bromide were associated with increased risk of prostate cancer (19).

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Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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Further, among those pesticide applicators with a family history of prostate cancer, we have observed significant ($P < 0.05$) exposure-response associations with four widely used organophosphate insecticides (chlorpyrifos, fonofos, phorate, and coumaphos), one pyrethroid insecticide (permethrin), and a thiocarbamate herbicide (butylate). These associations were not observed among those without a family history of disease (19–23). Thus, these findings suggest that genetic determinants may interact with pesticide exposures experienced by agricultural workers to alter prostate cancer risk.

In this study we evaluated the interaction among pesticide use, genetic variation on chromosome 8q24, and risk of prostate cancer in 2,220 AHS subjects.

Materials and Methods

Genotyping and SNP selection

Genotyping of prostate cancer cases and controls using buccal cell DNA was performed at the National Cancer Institute Core Genotyping Facility, using the Custom Infinium BeadChip Assays (iSelect) from Illumina Inc. as part of an array of 26,512 SNPs. For the 8q24 region encompassing >660 kb including chr8: 128,232,156 to 128,816,653, tag SNPs were selected from HapMap CEU using common SNPs (minor allele frequency ≥5%) and an r^2 threshold > 0.80. Tag SNPs were determined using a modified version of the method described by Carlson and colleagues (24) as implemented in the GLU software package (25). The tagged region includes SNPs telomeric to the prostate cancer–associated region 2 through the region associated with bladder cancer (26) to ~1 kb 5′ of *MYC* for a total of 211 SNPs. Blinded duplicate samples (2%) were also included and concordance of these samples ranged from 96% to 100%. The overall genotyping rate was between 97.5% and 100% in the 8q24 region SNPs.

Study population

The AHS is a prospective cohort study that includes 57,310 licensed pesticide applicators in Iowa and North Carolina. Applicators were recruited from 1993 through 1997; a detailed description of this cohort has been previously published (27). During a follow-up telephone interview conducted in 1999–2003, applicators were asked to provide a mouthwash rinse sample for extraction of DNA from buccal cells. Informed consent was obtained and the study protocol was reviewed by all relevant institutional review boards. Approximately 72% of responding applicators returned a sample. Subjects diagnosed with incident prostate cancer between 1993 and 2004 who also provided a buccal cell sample were included in the current nested case-control study. In addition, applicators with incident prostate cancer that did not return a sample at follow-up and met inclusion criteria for the current study were asked separately to provide a mouthwash rinse sample, with 307 of 561 (55%) returning a sample. Cancer cases were coded using the International Classification of Diseases for Oncology, 2nd edition, and stage (local, regional, distant, unstaged) and grade (well differentiated, moderately differentiated, poorly differentiated, undifferentiated, missing) were abstracted by the state cancer registries in Iowa and North Carolina. Controls were male applicators who provided buccal cell material, were alive at the time of case diagnosis, and had no previous cancer diagnosis except nonmelanoma skin cancer. Eligible controls were frequency matched 2:1 to cases by date of birth (±1 year) and were not lost to follow-up at the time of case diagnosis. All subjects for the nested case-control study were Caucasian. Based on these inclusion criteria, 841 cases (66% of total Caucasian cases as of 2004) and 1,659 controls (total of 2,500) were identified. Due to genotyping space limitations 164 controls, with the lowest DNA mass, were excluded. Of the remaining samples, 108 were removed due to insufficient or poor DNA quality ($n = 20$; 14 cases, 6 controls) or <90% completion rate (>10% of the SNP assays failed for a given sample, $n = 88$; 47 cases, 41 controls).

To explore the underlying genetic structure of the population, we identified 2,563 autosomal SNPs from the genotyping panel with low local background linkage disequilibrium (pairwise $r^2 < 0.01$ measured in the AHS samples for any pair <500 kb apart) using the algorithm described by Yu and colleagues (28). This set of SNPs was used for population substructure evaluation. Using STRUCTURE software (29), we identified three subjects with substantial non-European ancestry (with <80% European admixture coefficients). Using principal component analysis (30) we further identified five additional subjects that were outliers in the first two axes of variation (i.e., >10 SD from the mean on either of top two axes). After removing those subjects, we had a final sample size of 776 cases and 1,444 controls. We conducted another principal component analysis on these 2,220 subjects and found that case/control status was distributed evenly (the Wilcoxon rank-sum test P value >0.1) on each of the top five major principal components. Thus, there was no evidence of strong population stratification, and we did not adjust for population stratification in our analysis.

Statistical analysis

Unconditional logistic regression was used to estimate odds ratios (OR) and 95% CI for the association between 8q24 SNPs and prostate cancer and the interaction among SNPs in 8q24, pesticide use, and prostate cancer risk. Genotypes were coded as counts of the risk allele assuming a log-additive model. Exposure to pesticides was classified from responses to two self-administered questionnaires that were administered at enrollment. These questionnaires collected comprehensive data on lifetime use of 49 pesticides (18 herbicides, 21 insecticides, 4 fumigants, and 6 fungicides). Participants were asked how many years they applied each chemical (≤1, 2–5, 6–10, 11–20, 21–30, or >30 years) and how many days it was personally used in an average year (<5, 5–9, 10–19, 20–39, 40–59, 60–150, or >150 days). Pesticides were categorized as either ever/never use and by lifetime exposure days (years of use × days per year) in this analysis from AHS data release version P1REL0712.04. Lifetime exposure days were categorized as nonexposed, low exposed, and high exposed to a given chemical using the

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Table 1. Characteristics of prostate cases and controls in the Agricultural Health Study nested case-control study compared with the entire study cohort

Characteristic	Nested case-control*		AHS cohort*	
	Cases (n = 776) No. (%)	Controls (n = 1,444) No. (%)	Prostate cancer (n = 1,275) No. (%)	Noncancer (n = 48,286) No. (%)
Age at enrollment, years				
<40	3 (0.4)	5 (0.4)	9 (0.7)	17,801 (36.9)
40–49	74 (9.5)	144 (10.0)	111 (8.7)	13,592 (28.2)
50–59	259 (33.4)	491 (34.0)	409 (32.1)	9,515 (19.7)
60–69	355 (45.8)	634 (43.9)	573 (44.9)	5,657 (11.7)
≥70	85 (11.0)	170 (11.8)	173 (13.6)	1,721 (3.6)
State of residence				
Iowa	520 (67.0)	991(68.6)	789 (61.9)	32,740 (67.8)
North Carolina	256 (33.0)	453 (31.4)	486 (38.1)	15,546 (32.2)
Applicator type				
Private	741 (95.5)	1,363 (94.4)	1,219 (95.6)	43,895 (90.9)
Commercial	35 (4.5)	81 (5.6)	56 (4.4)	4,391 (9.1)
Family history of prostate cancer†				
No	575 (74.1)	1,193 (82.6)	924 (72.5)	41,365 (85.7)
Yes	130 (16.8)	145 (10.0)	212 (16.6)	3,748 (7.8)
Prostate cancer stage				
I - local	447 (57.6)	—	741 (58.1)	—
II - regional	102 (13.1)	—	167 (13.1)	—
III - distant	6 (0.8)	—	26 (2.0)	—
IV - not staged	221(28.5)	—	341 (26.7)	—
Prostate cancer grade				
Well differentiated	38 (4.9)	—	60 (4.7)	—
Moderately differentiated	547 (70.5)	—	855 (67.1)	—
Poorly differentiated	168 (21.6)	—	302 (23.7)	—
Undifferentiated	4 (0.5)	—	6 (0.5)	—
Not graded	19 (2.4)	—	52 (4.1)	—

*Excludes females, nonwhite subjects, and subjects with any previous cancer.
†Family history of prostate cancer in first-degree relative.

median cut point based on distribution of lifetime days among cases and controls together for each chemical. Previously reported GWAS SNPs and those with a main effect of $P < 0.01$ were evaluated for interaction. SNP-pesticide interactions were examined using a multiplicative model. The P value for each SNP-pesticide interaction was computed by comparing nested models with and without the cross-product terms using a likelihood ratio test. SNP-pesticide (ever/never) interactions with P-interaction <0.20 were carried further for exploration with expanded pesticide categories (nonexposed, low, high). All models were adjusted for age (10-year intervals) and state of residence (Iowa or North Carolina). Because GWAS have identified variants in regions 3 and 1 to have independent effects on prostate cancer, we also decided to explore whether a combination of risk alleles in these two independent regions further increased risk of prostate cancer in the presence of exposure. Additional factors that were examined but ultimately not considered in the modeling because they did not change point estimates by >10% were family history of prostate cancer in first-degree relative (no, yes, missing), type of applicator (private or commercial), and other pesticide adjustment based on the correlations between selected pesticides. We were not able to explore aggressive prostate cancer (distant and poorly differentiated) alone due to small numbers.

To take into account the large number of tests performed, the number of effective tests, M_{eff}, was calculated for the 15 8q24 SNPs carried forward for interaction analyses by use of the SNP spectral decomposition approach (31) and P values adjusted for multiple testing were calculated using the region M_{eff} value of 11. This correction is similar to a Bonferroni correction (assumes independence) but takes into account the correlation between SNPs, which are not all independent. We considered each chemical as independent. All statistical tests were two-sided, and interactions were considered to be significant if the adjusted P-interaction values were <0.05 (after correction for multiple tests). All P values presented represent uncorrected P values unless otherwise stated.

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Results

Similar to the characteristics of the whole cohort, applicators in this nested case-control study tended to be private applicators and were predominantly from Iowa (Table 1). Cases selected for the nested case-control study were similar to all prostate cancer cases from the cohort in age, state of residence, applicator type, presence of familial prostate cancer, and prostate cancer disease characteristics. Cases tended to have a higher proportion of first-degree relatives with a family history of prostate cancer compared with controls (16.7% versus 10.0%), and 57.8% of prostate cancers were diagnosed while the cancer was still confined to the primary site (local). A list of all 49 pesticides, their prevalence, and the median level of lifetime days of exposure to each chemical is presented in Supplementary Table S1.

Of the 211 SNPs evaluated, 12 were associated with prostate cancer at the $P < 0.01$ level, and three SNPs previously reported by GWAS of prostate cancer were associated with prostate cancer at the $P < 0.05$ level (Table 2). GWAS SNPs in region 3 (rs6983267) and region 1 (rs4242382) were associated with prostate cancer (OR, 1.23; 95% CI, 1.09–1.40; P-trend = 1.02×10^{-3}; and OR, 1.35; 95% CI, 1.11–1.64; P-trend = 2.28×10^{-3}, respectively). Several other SNPs in region 1 showed similar associations and were highly correlated (r^2 = 0.74–0.99) with rs4242382. Five additional SNPs in region 3 were associated with risk; however, not all were strongly correlated with the GWAS SNP rs6983267 (r^2 = 0.06–0.94). SNPs rs445114 and rs620861 in region 4 and rs1447295 in region 1 were significant only at the $P < 0.05$ level. One additional SNP, rs12547643, telomeric to region 1 and about 30 kb upstream of *MYC*, was also associated with prostate cancer. Main effect P values for all 211 SNPs in 8q24 are shown in Supplementary Table S2.

All 15 8q24 SNPs associated with risk in Table 2 were examined for potential effect modification by pesticide use. Those interactions with a P for interaction <0.20 (based on pesticide defined as ever/never) and which showed an increasing trend in risk across strata are presented in Table 3; seven SNPs from Table 2 showed interactive effects. All other interactions with $P < 0.20$ are presented in Supplementary Table S3.

In region 1, the association between rs4242382 and prostate cancer was statistically significantly increased across strata of fonofos exposure ($OR_{nonexposed}$, 1.17; 95% CI, 0.93–1.48; OR_{low}, 1.30; 95% CI, 0.75–2.27; OR_{high}, 4.46; 95% CI, 2.17–9.17; P-interaction = 0.002; adjusted P-interaction = 0.02; Table 3). A similar exposure-response association was observed for terbufos ($OR_{nonexposed}$, 1.13; 95% CI, 0.87–1.47; OR_{low}, 1.71; 95% CI, 1.07–2.74; OR_{high}, 2.15; 95% CI, 1.32–3.52; P-interaction = 0.02) and to a lesser degree for phorate (P-interaction = 0.26). A similar pattern was observed for the association between rs4242382 and prostate cancer across strata of permethrin exposure ($OR_{nonexposed}$, 1.18; 95% CI, 0.94–1.47; OR_{low},1.66; 95% CI, 0.87–3.18; OR_{high}, 2.73; 95% CI, 1.31–5.69; P-interaction = 0.03). Comparable associations were observed for other SNPs in region 1, which were highly correlated with rs4242382. After correction for multiple tests only those interactions with fonofos and region 1 variants remained statistically significant.

Table 2. Risk of prostate cancer in the AHS with previously reported GWAS SNPs and SNPs with a P value <0.01 in the chromosome 8q24 region

SNP*	Risk allele	Position	Region	Pairwise r^2 within region	RAF†	OR‡ (95% CI)	P-trend
rs445114 T>C	T	128392363	Region 4	0.98	0.37	1.14 (1.01–1.31)	0.0323
rs620861 G>A	G	128404855	Region 4	Ref	0.37	1.14 (1.00–1.30)	0.0461
rs10505477 A>G	A	128476625	Region 3	0.94	0.50	1.26 (1.11–1.43)	2.80E-04
rs10505476 C>T	T	128477298	Region 3	0.28	0.23	1.32 (1.15–1.51)	1.01E-04
rs17467139 A>G	G	128481192	Region 3	0.06	0.06	1.44 (1.13–1.83)	3.42E-03
rs6983267 G>T	G	128482487	Region 3	Ref	0.49	1.23 (1.09–1.40)	1.02E-03
rs10505474 C>T	T	128486686	Region 3	0.66	0.41	1.21 (1.07–1.37)	2.87E-03
rs7837328 G>A	A	128492309	Region 3	0.65	0.41	1.21 (1.07–1.37)	3.11E-03
rs1447295 C>A	A	128554220	Region 1	0.91	0.11	1.22 (1.00–1.48)	0.0485
rs4242382 G>A	A	128586755	Region 1	Ref	0.10	1.35 (1.11–1.64)	2.28E-03
rs4242384 A>C	C	128587736	Region 1	0.99	0.10	1.35 (1.11–1.64)	2.67E-03
rs9656967 A>T	T	128603769	Region 1	0.74	0.12	1.30 (1.08–1.55)	5.27E-03
rs9656816 A>G	G	128603836	Region 1	0.78	0.09	1.35 (1.10–1.65)	4.25E-03
rs7837688 G>T	T	128608542	Region 1	0.89	0.11	1.33 (1.10–1.61)	3.75E-03
rs12547643 G>A	G	128782355	Upstream of *MYC*	—	0.36	1.20 (1.06–1.37)	5.62E-03

Abbreviation: RAF, risk allele frequency.

*Bold SNPs are independent susceptibility loci described by genome-wide association studies of prostate cancer.

†RAF among controls.

‡OR per risk allele assuming a log-additive model. Adjusted for age and state of residence.

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Several insecticides also modified the association between region 3 SNPs and prostate cancer risk. The association between rs7837328 and prostate cancer increased across strata of coumaphos exposure ($OR_{nonexposed}$, 1.16; 95% CI, 1.01–1.33; OR_{low}, 1.26; 95% CI, 0.66–2.42; OR_{high}, 3.02; 95% CI, 1.48–6.16; *P*-interaction = 0.02). Fonofos and terbufos exposure also seemed to modify the association between rs7837328 and prostate cancer, although these interactions were not statistically significant (*P*-interaction = 0.30 and 0.16, respectively). The association between several region 3 SNPs and prostate cancer increased across strata of methyl bromide and heptachlor exposure, although these interactions were also not statistically significant (smallest *P*-interaction = 0.27 and 0.24, respectively; Table 3). The interaction between toxaphene and rs10505476 was not significant (*P*-interaction = 0.07), although an interesting trend across strata was evident.

Six chemicals (diazinon, parathion, captan, chlordane, dieldrin, and metolachlor) seemed to modify the associations between rs12547643, which is telomeric to regions 3 and 1, and prostate cancer risk, although even those for captan and diazinon were not statistically significant after correction for multiple testing (*P*-interaction = 0.01 and 0.007, respectively; Table 3). Additional adjustment for family history did not alter the risk estimates across strata for any chemical-SNP combination, and analyses among subjects with no family history of prostate cancer yielded similar results (data not shown). The correlations between the pesticides presented in Table 1 were modest (Pearson r^2 range, 0.01–0.42); adjustment for other pesticides in interaction models did not alter the risk estimates across strata for any chemical-SNP combination (data not shown).

Because rs7837328 and rs4242382 showed the most consistent trends across exposure strata, the associations between joint categories of fonofos or terbufos exposure and the combination of rs7837328 and rs4242382 genotypes and prostate cancer risk were assessed. The results are presented in Table 4. Among ever users of fonofos, subjects with three or four risk alleles had 3.14 times the risk of prostate cancer (95% CI, 1.41–7.00) compared with subjects who had zero risk alleles and had never used fonofos. Among ever users of terbufos, subjects with three or four risk alleles had 3.15 times the risk of prostate cancer (95% CI, 1.65–6.02), compared with subjects who had zero risk alleles and had never used terbufos.

Discussion

In this nested case-control study the association between 8q24 SNPs and prostate cancer is modified by pesticide use. In particular, the association between rs4242382 and prostate cancer is increased across strata of fonofos, terbufos, and permethrin exposure. Furthermore, the joint effect of fonofos exposure and the carrying of three or four risk alleles in rs7837328 and rs4242382 resulted in a 3-fold increased risk of prostate cancer compared with subjects who had zero risk alleles and had never used fonofos. The association between several region 3 SNPs and prostate cancer also seemed to be modified by several pesticides, including coumaphos and permethrin, and increased risks associated with rs12547643 (telomeric to regions 3 and 1) across strata of other chemicals are also evident.

The observed associations for prostate cancer among 8q24 variants in our study are consistent with GWAS reports. Similar to several reports, we observed a stronger association for rs4242382 compared with rs1447295 in region 1 and risk of prostate cancer (5, 6, 10). The independent risk marker rs6983267 in region 3 (9, 11) was also associated with prostate cancer, although other correlated variants in region 3 showed stronger signals in our study. Additionally, the reported prostate cancer susceptibility loci rs445114 and rs620861 were also associated with prostate cancer risk, but these were not among the strongest signals. Another locus, rs12547643, telomeric to regions 3 and 1, was found to be associated with prostate cancer in our study. This marker lies in a separate block of linkage disequilibrium from the other regions and is close to a marker, rs9642880, which has been associated with susceptibility to bladder cancer (26). Although the association between rs9642880 and prostate cancer was not significant in our study (*P*-trend = 0.153), the pairwise correlation between rs9642880 and rs12547643 is not negligible (r^2 = 0.20). This variant, however, has not been shown to be associated with prostate cancer in GWAS (*P* = 0.77; ref. 11), and thus the observed association in our study may be due to chance. GWAS SNPs from region 2 were not genotyped in this study.

In the AHS we have observed the association between prostate cancer and pesticides to be modified by family history of prostate cancer (19), which could be a marker of genetic susceptibility. Some of the chemicals that are shown here to modify the association between 8q24 variants and prostate cancer have also been associated with prostate cancer among those with a family history in pesticide-specific analyses within the AHS (19–22), and include fonofos, phorate, coumaphos, and permethrin. Thus, we also explored whether family history of prostate cancer was related to 8q24 variants. Two studies have reported a potential association between region 1 SNPs, rs4242382 and rs1447295, and family history of prostate cancer, although both studies are noted to be underpowered due to the limited number of cases and controls with a family history of prostate cancer (32, 33). Larger GWAS reports have reported no significant interaction between rs1447295 or rs6983267 and family history of prostate cancer (9, 34), although not all GWAS loci have been evaluated. We did not observe an association between region 1 variants and family history of cancer. We did observe a greater proportion of subjects with risk alleles in region 3 variants among those with a family history (data not shown), but the proportions among cases and controls were similar, and adjustment for family history in all interaction models did not change the risk estimates. When we restricted analyses to those with no family history of prostate cancer all results persisted. Thus, the previously observed effect modification of family history on the association between pesticides and prostate cancer does not seem to be explained by variants on chromosome 8q24. It is possible that other genes, multiple genes, or nongenetic factors that track in families might account for this previously observed association.

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Table 3. Pesticide-8q24 SNP interactions with increased trends across strata of lifetime exposure days and risk of prostate cancer in the AHS

SNP	Pesticide	Region	Nonexposed	
			Cases/Controls	OR* (95% CI)
rs10505477	Coumaphos	Region 3	610/1142	1.21 (1.05–1.39)
rs7837328	Coumaphos	Region 3	609/1134	1.16 (1.01–1.33)
rs1447295	Coumaphos	Region 1	610/1144	1.14 (0.91–1.42)
rs12547643	Diazinon	*MYC*	513/961	1.09 (0.93–1.28)
rs7837328	Fonofos	Region 3	510/982	1.14 (0.98–1.32)
rs1447295	Fonofos	Region 1	511/992	1.05 (0.83–1.34)
rs4242382	Fonofos	Region 1	511/991	1.17 (0.93–1.48)
rs1447295	Malathion	Region 1	225/399	0.96 (0.67–1.38)
rs12547643	Parathion	*MYC*	627/1173	1.16 (1.00–1.34)
rs1447295	Phorate	Region 1	462/846	1.02 (0.79–1.32)
rs4242382	Phorate	Region 1	462/846	1.13 (0.87–1.46)
rs7837328	Terbufos	Region 3	405/795	1.10 (0.93–1.30)
rs1447295	Terbufos	Region 1	406/803	1.03 (0.79–1.34)
rs4242382	Terbufos	Region 1	406/802	1.13 (0.87–1.47)
rs17467139	Permethrin	Region 3	575/1096	1.13 (0.85–1.49)
rs1447295	Permethrin	Region 1	575/1100	1.04 (0.83–1.31)
rs4242382	Permethrin	Region 1	575/1100	1.18 (0.94–1.47)
rs12547643	Metolachlor	*MYC*	369/711	1.05 (0.87–1.27)
rs12547643	Captan	*MYC*	623/1141	1.12 (0.96–1.29)
rs12547643	Chlordane	*MYC*	505/885	1.08 (0.92–1.28)
rs12547643	Dieldrin	*MYC*	660/1199	1.13 (0.98–1.31)
rs7837328	Heptachlor	Region 3	544/996	1.14 (0.99–1.33)
rs10505476	Toxaphene	Region 3	585/1084	1.25 (1.06– 1.47)
rs10505477	Methyl bromide	Region 3	637/1213	1.21 (1.06–1.39)

(*Continued on the following page*)

Several pesticides of similar chemical structure were observed to modify the association between 8q24 SNPs and prostate cancer. The organophosphate insecticides coumaphos, fonofos, phorate, and terbufos consistently modified the association between regions 3 and 1 SNPs and prostate cancer. The strongest association was for fonofos where the risk of prostate cancer was over three times as likely per rs4242382 risk allele among high users. Among ever users of fonofos, subjects with three or four risk alleles in rs7837328 and rs4242382 had 3.14 times the risk of prostate cancer compared with subjects who had zero risk alleles and had never used fonofos. Previous analyses revealed the strongest effect modification for any particular pesticide and family history was for fonofos (21). Thus, the risk of prostate cancer among fonofos users may be especially important among genetically susceptible subgroups. Ten organophosphate chemicals were examined and the results for coumaphos, phorate, and terbufos were similar to those observed for fonofos although not statistically significant. Fonofos, coumaphos, phorate, and terbufos are all classified by the U.S. Environmental Protection Agency as group E for carcinogenicity (evidence of noncarcinogenicity for humans) based on the absence of mutagenic, genotoxic, and carcinogenic observations in limited experimental and animal tests (35). In 1998, the registrant of fonofos voluntarily cancelled its registration (36) whereas the other organophosphates are still in widespread agricultural use.

Although interactions were not statistically significant after correction for multiple testing, several other pesticides seemed to modify the association between 8q24 SNPs and prostate cancer. The increasing pattern of risk across permethrin exposure strata was similar to that observed for the four organophosphates, possibly due to the fact that it is also an insecticide, although not all insecticides examined modified risk. Several other compounds with chlorine substituents, including captan, chlordane, dieldrin, and metolachlor, seemed to modify risk with rs12547643 (telomeric to regions 3 and 1). We had previously reported that AHS applicators over the age of 50 who used chlorinated pesticides had an increased risk of prostate cancer (19). It is unclear whether the chlorinated components of these chemicals are relevant to risk or whether this SNP is truly associated with prostate cancer. Thus, it is possible that these trends across strata may be due to chance.

Although some similar chemicals were observed to interact with the 8q24 region, suggesting a common biological

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Table 3. Pesticide-8q24 SNP interactions with increased trends across strata of lifetime exposure days and risk of prostate cancer in the AHS (Cont'd)

Low exposed		High exposed		*P*-interaction
Cases/Controls	OR* (95% CI)	Cases/Controls	OR* (95% CI)	
36/66	1.13 (0.58–2.21)	30/65	2.70 (1.34–5.45)	0.07
36/65	1.26 (0.66–2.42)	30/66	3.02 (1.48–6.16)	0.02
36/66	1.78 (0.74–4.27)	30/66	2.27 (0.81–6.36)	0.09
68/117	1.30 (0.81–2.10)	47/122	2.35 (1.34–4.12)	0.01
104/180	1.31 (0.91–1.90)	74/132	1.55 (1.04–2.32)	0.12
104/181	1.16 (0.66–2.07)	74/132	3.84 (1.89–7.77)	0.003†
104/181	1.30 (0.75–2.27)	74/132	4.46 (2.17–9.17)	0.002†
173/351	1.33 (0.89–1.99)	142/310	1.71 (1.09–2.68)	0.05
28/36	1.51 (0.65–3.50)	24/49	2.68 (1.09–6.60)	0.08
79/155	1.18 (0.61–2.28)	76/195	1.60 (0.90–2.85)	0.23
79/155	1.49 (0.80–2.78)	76/195	1.69 (0.95–3.01)	0.26
156/255	1.40 (1.04–1.90)	124/245	1.50 (1.10–2.05)	0.05
156/256	1.33 (0.83–2.15)	124/246	2.08 (1.27–3.42)	0.02
156/256	1.71 (1.07–2.74)	124/246	2.15 (1.32–3.52)	0.02
78/117	2.23 (0.95–5.23)	55/128	3.37 (1.40–8.07)	0.009
78/117	1.50 (0.79–2.87)	55/128	2.67 (1.26–5.64)	0.01
78/117	1.66 (0.87–3.18)	55/128	2.73 (1.31–5.69)	0.03
179/302	1.15 (0.87–1.53)	133/302	1.47 (1.08–2.00)	0.05
30/68	1.97 (1.03–3.77)	31/62	2.62 (1.19–5.76)	0.007
77/190	1.28 (0.84–1.97)	52/111	1.45 (0.86–2.42)	0.24
21/52	2.31 (1.02–5.20)	6/26	2.25 (0.53–9.49)	0.11
48/106	1.24 (0.77–2.01)	52/122	1.73 (1.02–2.93)	0.12
36/77	2.05 (1.11– 3.79)	43/86	2.28 (1.25–4.16)	0.03
52/108	1.54 (0.96–2.49)	56/96	1.59 (0.99–2.53)	0.16

*OR per risk allele assuming a log-additive model. Adjusted for age and state of residence.
†Significant after adjustment for multiple tests. rs1447295-fonfos adjusted *P*-interaction = 0.03; rs4242382-fonfos adjusted *P*-interaction = 0.02.

mechanism of action, the precise mechanism is unclear. Little is known overall about whether or how pesticides may be associated with cancer; however, substantial literature documents the principal involvement of phase I and phase II enzymes in the metabolism of specific xenobiotic substrates, including pesticides (37, 38). Most organophosphate insecticides (including coumaphos, terbufos, fonofos, and phorate) must be activated to their oxon (potent cholinesterase inhibitors) to be excreted. The oxon form of these compounds has been associated with a number of biological end points including neurotoxicity, the generation of reactive oxygen species, and DNA damage (39). Currently, however, there is no evidence to suggest that variation in the 8q24 region impacts xenobiotic metabolism. Other evidence indicates that at least one of the 8q24 variants resides in an androgen receptor transcriptional enhancer site, suggesting a potential hormone-related mechanism (40).

Recent studies have explored whether 8q24 variants might be related to the nearest coding region, the *MYC* gene and its expression. Two studies show that rs6983267 is located in a transcriptional enhancer and affects a binding site for TCF4, a transcription factor that interacts with β-catenin to activate transcription of Wnt target genes (15, 16). It was also shown that a DNA restriction fragment containing rs6983267 physically interacts with the *MYC* promoter in colorectal cancer cell lines (16). Imbalances in Wnt-mediated regulation of *MYC* are associated with altered cellular adhesion, proliferation, and differentiation (12, 13, 41). In addition, experimental animal studies have also shown that Wnt signaling can be altered upon exposure to organophosphates (42) and chlorinated pesticides (43, 44), and that pesticides can influence MYC expression as well (45–47). Thus, this may be a potential mechanism by which genetic variation in 8q24 modifies the risk associated with pesticide use and suggests a role for enhanced alterations in important global cancer signaling pathways.

Several strengths and limitations of our study should be recognized. High-quality genotype and pesticide information is available in the AHS. For many gene-exposure studies, the key limitation is the quality of the exposure information. The information on pesticide use among AHS participants is superior to any other epidemiologic studies of cancer. This is because farmers are aware of the type and duration of pesticides they use as these are often restricted use chemicals

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Table 4. Joint effects of fonofos and terbufos exposure and increasing number of risk alleles in GWAS loci and risk of prostate cancer in the AHS

No. of risk alleles	Combinations rs7837328/rs4242382	Fonofos exposure			
		No		Yes	
		Cases/Controls	OR* (95% CI)	Cases/Controls	OR* (95% CI)
0	GG/GG	127/268	Ref	38/111	0.73 (0.48–1.13)
1	GG/AG, AG/GG	212/439	1.02 (0.78–1.33)	78/141	1.19 (0.84–1.70)
2	AG/AG, AA/GG, GG/AA	137/221	1.31 (0.97–1.77)	51/68	1.61 (1.05–2.47)
3 or 4	AG/AA, AA/AG, AA/AA	34/54	1.33 (0.82–2.15)	16/11	3.14 (1.41–7.00)
P-trend					0.02
P-interaction					0.009
		Terbufos exposure			
		No		Yes	
		Cases/Controls	OR* (95% CI)	Cases/Controls	OR* (95% CI)
0	GG/GG	105/222	Ref	61/156	0.84 (0.58–1.23)
1	GG/AG, AG/GG	164/345	1.01 (0.75–1.36)	127/237	1.16 (0.84–1.60)
2	AG/AG, AA/GG, GG/AA	112/181	1.31 (0.94–1.83)	77/109	1.53 (1.05–2.23)
3 or 4	AG/AA, AA/AG, AA/AA	24/47	1.07 (0.62–1.85)	26/18	3.15 (1.65–6.02)
P-trend					0.01
P-interaction					0.004

*Adjusted for age and state of residence.

for which a license is required; self-reported pesticide use information has been found to be reliable in this cohort (48, 49). Furthermore, the ability of the AHS to look at individual pesticides rather than at groups (herbicides or insecticides or chemical classes) is critical because observed cancer risks seem to be chemical specific. Under this premise, we chose to treat each pesticide as independent in the multiple comparison correction that results in a less conservative correction. Thus, the highlighted results for fonofos interactions, like all the interactions presented in this study, would need to be confirmed to see if they are truly important for prostate cancer. In addition, the numbers within some pesticide use strata were small, but to our knowledge there are no other studies with more power to examine this interaction. Moreover, we were not able to examine disease aggressiveness due to small numbers. Finally, subjects in this study were all Caucasian, which limits the generalizability of the results to other racial/ethnic groups.

In conclusion, we observed an interaction among variants on chromosome 8q24, pesticide use, and risk of prostate cancer. Insecticides, particularly several organophosphates, seem to be the strongest modifiers of risk. This is the first report of effect modification between an 8q24 region variant and environmental exposure on prostate cancer risk. These results need to be subjected to replication due to the high probability of false-positive results from the multiple tests of interaction. Opportunities for replication, however, are currently limited because of the lack of comparable agricultural populations with sufficient sample size, detailed information on pesticide use, as well as DNA for genetic analyses. We continue, however, to look for possible studies in which to replicate these findings. Further research should continue to explore the possible mechanism or mechanisms for pesticide-induced prostate carcinogenesis because if the current interactions are indeed true, this could provide critical new information about a previously unsuspected biological pathway through which prostate carcinogenesis occurs. Similarly, mechanistic studies that help identify the role of the 8q24 region on prostate cancer risk may also offer critical new clues to help explain prostate carcinogenesis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Pesticide Use Modifies the Association Between Genetic Variants on Chromosome 8q24 and Prostate Cancer

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Phorate Exposure and Incidence of Cancer in the Agricultural Health Study

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Background: We recently reported a link between use of the organophosphate pesticide phorate and risk of prostate cancer among applicators with a family history of prostate cancer in the Agricultural Health Study (AHS).

Objective: This finding, together with findings of associations between other organophosphate pesticides and cancer more broadly, prompted us to examine phorate exposure and overall cancer incidence in the AHS. Adding 3 years of follow-up and using more detailed exposure information allowed us to see whether the prostate cancer finding held.

Methods: The AHS is a prospective study of licensed restricted-use pesticide applicators from North Carolina and Iowa. To our knowledge, this is the largest examination of workers occupationally exposed to phorate. Pesticide exposure and other information was collected using two self-administered questionnaires completed from 1993 to 1997. Poisson regression was used to calculate rate ratios (RR) and 95% confidence intervals (CI), adjusting for potential confounders.

Results: Phorate use was not related to the incidence of all cancers combined or to any individual cancer, although we had insufficient numbers to study non-Hodgkin lymphoma or leukemia, which have been linked to organophosphates in other studies. Although prostate cancer risk was not significantly related to phorate use overall or among those without a family history, the risk tended to increase among applicators with a family history of prostate cancer. The interaction RR was 1.53 (95% CI, 0.99–2.37).

Conclusion: The observed statistical interaction suggests a gene–environment interaction between family history and phorate exposure in the incidence of prostate cancer, but other explanations are also possible.

Key words: agriculture, insecticides, neoplasms, occupational exposure, organophosphorus compounds, organothiophosphorus compounds, pesticides, prostate, phorate. *Environ Health Perspect* 114:1205–1209 (2006). doi:10.1289/ehp.8911 available via *http://dx.doi.org/* [Online 18 April 2006]

Phorate {*O*,*O*-diethyl *S*-[(ethylthio)methyl] phosphorothioate; trade name: thimet} is an organophosphate compound used agriculturally, primarily in the production of corn, cotton, and potatoes, to control sap-feeding insects including various beetles, mites, grubs, and worms. There are no registered residential uses. Phorate was first registered for use in the United States in 1959 [U.S. Environmental Protection Agency (EPA) 2001]. In the United States, almost 2.5 million acres are treated annually with 2–3 million pounds of phorate, making it the sixth most common organophosphate used (Donaldson et al. 2002).

The currently limited body of literature does not provide evidence to suggest that phorate is mutagenic, genotoxic, or carcinogenic (Bingham et al. 2001; California Department of Pesticide Regulation 1996; Lin et al. 1987; Pandita 1986). However, several epidemiologic studies have found associations between exposure to organophosphate pesticides and non-Hodgkin lymphoma (NHL) (Cantor et al. 1992; Zahm et al. 1993) as well as leukemia (Brown et al. 1990; Clavel et al. 1996), and the International Agency for Research on Cancer (IARC) considers insecticide application to be an exposure that is probably carcinogenic in humans (Group 2A) (IARC 1991). Recent findings from the Agricultural Health Study (AHS) linking lung cancer with exposure to diazinon and chlorpyrifos (Alavanja et al. 2004) and prostate cancer with exposure to chlorpyrifos, coumaphos, fonofos, and phorate among applicators with a family history of prostate cancer (Alavanja et al. 2003) prompted us to examine risk for all cancers among phorate users in the same cohort over a longer follow-up period. The aforementioned insecticides belong to the organothiophosphate subgroup, are similar to phorate in chemical structure, and must be converted in the body to their bioactive, neurotoxic oxon forms, which irreversibly inhibit acetylcholine esterase by phosphorylating a serine hydroxyl group in the active site of the enzyme (Pope 1999; Sultatos 1994). Little is known of the carcinogenicity of the oxon species. To our knowledge, this is the largest epidemiologic examination of an occupational group exposed to phorate.

Methods

Cohort enrollment and follow-up. The AHS has been described elsewhere (Alavanja et al. 1996). It is a prospective cohort including 52,395 private applicators (farmers) from Iowa and North Carolina and 4,916 commercial applicators (employees of pest control companies or persons who apply pesticides as employees of businesses whose primary function is not pesticide application) from Iowa licensed to apply restricted-use pesticides (82.4% of eligible applicators). Incident tumors diagnosed between enrollment (31 December 1993 to 31 December 1997) and 31 December 2002 were identified using population-based tumor registries of both states. Subjects were censored in the year they moved out of the state, as determined by an extensive search of address records, or the year they died, as determined using the National Death Index and state death registry records. Less than 2% of the cohort was lost to follow-up. The average follow-up time of 7.5 years represents an increase of 3.2 years over the previously published prostate cancer paper. All participants provided informed consent and the protocol was approved by all appropriate institutional review boards.

Exposure assessment. Applicators were given two self-administered questionnaires upon enrollment. The enrollment questionnaire collected information on days of use per year, years of use, and decade of first use for 22 pesticides, as well as information on ever/never use of 28 additional pesticides (including phorate); application methods; use of personal protective equipment (PPE); smoking; alcohol consumption; farm activities; cancer history in first-degree relatives; and basic demographic data. A supplemental take-home questionnaire collected information on days of use per year, years of use, and decade of first use for the 28 pesticides for which only ever/never use information was collected in the enrollment questionnaire. The take-home questionnaire, which was completed by 25,291 (44%) of the participants, also collected additional information on work practices, physical activity, medical conditions, and occupational exposures from nonfarming jobs. Both questionnaires are

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available at http://www.aghealth.org/questionnaires.html. Applicators who did not return a questionnaire were generally similar to those who did with respect to many characteristics including use of crop insecticides; however, small differences were observed with respect to education, age, family size, and vegetable consumption (Tarone et al. 1997). Because of changing pesticide exposure patterns over time, pesticide exposure information, pesticide handling practices, and other information was updated by computer-aided telephone interview from 1999 to 2003. However, because of the proximity of this interview period to the end of cancer incidence follow-up, it is unlikely that this exposure information was etiologically relevant, and we have not used the interview information here.

We estimated phorate exposure using phorate lifetime exposure-days, calculated by multiplying the frequency of phorate use in an average year and the number of years of use, using the midpoints of the questionnaire categories, and intensity-weighted exposure-days, calculated by multiplying lifetime exposure-days by an intensity score calculated using the following algorithm: intensity score = (mixing status + application status + equipment repair) × PPE (Dosemeci et al. 2002). This algorithm takes into account the effect of exposure-modifying factors by assigning different weights to various activities based on the inverse of their potential contribution to total exposure. For example, because dermal absorption is often the most important exposure route for pesticide applicators (Maroni et al. 2000), the use of chemically resistant gloves was weighted to confer a greater reduction in intensity score than any other single item of PPE; the use of disposable outer clothing reduced the intensity score to a lesser degree.

Statistical analysis. In contrast to the previously published tumor-specific analysis of prostate cancer, which examined prostate cancer risk with respect to the ever/never use of pesticides among all male pesticide applicators without a prior prostate cancer diagnosis, this analysis was limited to the subset of applicators who completed the take-home questionnaire. Prevalent cancer cases (n = 620) and applicators who did not provide information on phorate exposure or other variables (n = 3,655) were excluded from this analysis, leaving 5,903 exposed and 15,113 nonexposed applicators. Participants with missing information tended to be older and to live in North Carolina. They were also likely to have missing information for more than one of the variables listed above.

Cancer sites were selected for analysis if there were 15 or more incident diagnoses among phorate-exposed subjects. Specifically, these sites were *a*) all cancers combined; *b*) cancers of the colon, lung, and prostate; and *c*) the grouping of lymphohematopoietic cancers, which contains both Hodgkin lymphoma and NHL, leukemia, and multiple myeloma. All statistical analyses were conducted in AHS data release 0412.01 using Stata version 8 (StataCorp, College Station, TX; StataCorp 2003). Poisson regression was used to calculate incidence rate ratios (RR) and 95% confidence intervals (CI). Both lifetime exposure-days and intensity-weighted exposure-days for exposed applicators were categorized into tertiles based on the distribution of the exposure metric among cancer cases. To improve resolution at high exposure levels, we further categorized lifetime exposure-days by splitting the top tertile at the median when the split left at least four exposed cases in each new category. With intensity-weighted exposure-days used as the exposure measurement, the highest tertile was not split because of a small number of cases. Finally, because measures of lifetime cumulative exposure do not distinguish between infrequent exposure over a long period of time and more frequent exposure over a short period of time, which could make a difference with respect to cancer, we examined cancer incidence in relation to average days of use per year categorized as none, low, and high (low and high categorized at the median) and stratified by low and high years of use (categorized at median).

Both the lifetime and intensity-weighted exposure-days analyses used the nonexposed and lowest exposed categories as the reference group. Cancer-specific analyses were adjusted for age as a continuous variable; applicator type (private or commercial); state of residence (Iowa or North Carolina); education (≤ high school graduate, > high school graduate); pack-years of smoking categorized at the median (never, ≤ 12, > 12); history of the specific tumor in first-degree relatives (yes or no); and use of the five most correlated pesticides [aldicarb, ethylene dibromide, aldrin, 2,4,5-trichlorophenoxy propionic acid (2,4,5-TP), and butylate]. Pearson correlation coefficients ranged from 0.39 (aldicarb) to 0.36 (butylate). We categorized use of each correlated pesticide as never, low, and high usage, employing the median lifetime exposure-days of use to distinguish between low and high usage. Although the results of these more fully adjusted analyses and the analyses adjusted for age and smoking

Table 1. Characteristics of applicators by phorate exposure in the AHS (1993–1997) [no. (%)].

Characteristic	Nonexposed n = 15,113	Lowest exposed n = 2,407	Other exposed n = 3,496
Age (years)			
< 40	1,981 (13.1)	167 (6.9)	218 (6.2)
40–49	4,161 (27.5)	581 (24.1)	797 (22.8)
50–59	3,630 (24.0)	666 (27.7)	992 (28.4)
≥ 60	5,341 (35.3)	993 (41.3)	1,489 (42.6)
Sex			
Male	14,589 (96.5)	2,387 (99.2)	3,475 (99.4)
Female	524 (3.5)	20 (0.8)	21 (0.6)
State of residence			
Iowa	9,783 (64.7)	2,261 (93.9)	2,945 (84.2)
North Carolina	5,330 (35.3)	146 (6.1)	551 (15.8)
Applicator type			
Commercial	1,779 (11.8)	101 (4.2)	219 (6.3)
Private	13,334 (88.2)	2,306 (95.8)	3,277 (93.7)
Smoking history			
Never	8,262 (54.7)	1,429 (59.4)	1,977 (56.6)
Light (< 12 pack-years)	3,437 (22.7)	542 (22.5)	793 (22.7)
High (≥ 12 pack-years)	3,414 (22.6)	436 (18.1)	726 (20.8)
Education			
≤ High school	8,183 (54.2)	1,273 (52.9)	2,050 (58.6)
> High school	6,930 (45.9)	1,134 (47.1)	1,446 (41.4)
Family history of cancer[a]			
No	8,257 (58.7)	1,225 (53.0)	1,763 (53.0)
Yes	5,809 (41.3)	1,084 (47.0)	1,561 (47.0)
Alcohol[a,b]			
No	5,149 (34.8)	593 (24.9)	944 (27.4)
Yes	9,641 (65.2)	1,789 (75.1)	2,506 (72.6)
Corn farming			
No	4,897 (32.4)	149 (6.2)	366 (10.5)
Yes	10,216 (67.6)	2,258 (93.8)	3,130 (89.5)
Use of correlated pesticides			
Aldicarb	932 (6.2)	111 (4.6)	444 (12.7)
Ethylene dibromide	687 (4.6)	42 (1.8)	102 (2.9)
Aldrin	1,466 (9.7)	674 (28.0)	1,028 (29.4)
2,4,5-TP	590 (3.9)	122 (5.1)	257 (7.4)
Butylate	3,038 (20.1)	940 (39.1)	1,437 (41.1)
No. of pesticides used[c]	10.7 ± 5.9	16.0 ± 5.9	17.0 ± 6.5

[a]Column numbers do not add up to total n because of missing information. [b]Based on reported alcohol consumption in the past 12 months. [c]Mean ± SD.

only were consistent, we present the more fully adjusted results to address the fact that farmers are exposed to many agents. To assess trends in dose–response patterns, we performed linear trend tests by assigning each exposure category the median value in that category and treating the variable as a continuous variable.

To further examine the risk of prostate cancer, we obtained a parsimonious model by removing variables that did not alter point estimates by > 5%, leaving variables for age and state of residence in the model. We used an interaction term obtained by taking the product of family history of prostate cancer and category of lifetime exposure-days to evaluate effect modification between phorate exposure and family history of prostate cancer.

Results

Table 1 displays selected characteristics of applicators by their level of exposure, with "lowest exposed" referring to those in the lowest exposed tertile of lifetime exposure-days, and "other exposed" referring to those in the remaining tertiles. Overall, those in the nonexposed category tended to be less likely to report producing corn, slightly less likely to report family history of any cancer, and younger than those in either of the exposed categories. Additionally, compared with the exposed applicators, the nonexposed were also exposed to significantly fewer of the pesticides that were assessed in the questionnaires. Finally, residents of North Carolina were more likely than residents of Iowa to be nonexposed. These differences between the nonexposed group and either of the two exposed groups suggest that the lowest exposed group may be the more appropriate reference group.

Table 2 displays adjusted associations between selected cancer sites and phorate lifetime exposure-days. Phorate use did not appear to be associated with the incidence of all cancers combined. For prostate and colon cancer, the results differed depending on the reference group. The risk estimates for both cancers increased monotonically with increasing exposure category relative to the lowest exposed, but the point estimates and linear trend tests were not significant. However, the point estimates were not elevated compared with the nonexposed. Phorate use was not related to any other examined cancer.

Because phorate use was uncommon in North Carolina, we repeated our analysis of all cancers combined, restricting the data to applicators from Iowa. The results were similar to those presented above (not shown). They were not meaningfully different when we adjusted for lifetime exposure-days to all pesticides rather than the five most correlated pesticides (not shown). Finally, the results were also consistent when we examined days of use per year by years of use (not shown). Namely, we observed slightly elevated but insignificant point estimates for prostate and colon cancer, and no discernible pattern with any other examined cancer.

Because of the large number of applicators excluded due to missing information on covariates, we repeated the analysis in Table 2 on all applicators without missing phorate exposure information by assigning the missing covariates an unspecified category (not shown). The results of this analysis of 17,051 nonexposed and 6,488 exposed applicators did not substantially differ from the results presented above.

Using the intensity-weighted exposure-days metric, though the number of cases was slightly reduced because individuals were missing data on intensity metric covariates, except for prostate cancer the results were not meaningfully different from those presented above (not shown). Phorate use was not associated with all cancers combined. Colon cancer risk estimates were not elevated using the nonexposed reference group, but compared with the lowest exposed, they increased monotonically with exposure category (RR in highest exposed = 1.81; 95% CI, 0.58–5.60). RRs for prostate cancer were uninfluenced by exposure category regardless of reference group. Phorate use was not associated with any other examined cancers.

Although too few exposed melanoma cases (n = 14) prevented inclusion of results in tables, there were some interesting yet statistically insignificant observations. In particular, melanoma risk estimates tended to increase with lifetime and intensity-weighted exposure-days category regardless of reference group. Relative to the unexposed, risk estimates were also elevated among low and high days of use per year, but only in applicators with many years of use.

When prostate cancer risk was stratified by family history of prostate cancer, risk estimates did not increase across categories of lifetime exposure-days in the stratum of applicators reporting no family history (Table 3). However, in applicators with a family history, the risk estimates tended to increase. The RR in the highest compared to the lowest exposed was 1.91 (95% CI, 0.86–4.24). The interaction RR indicated that the risk associated with an increase in phorate exposure category was 1.53 (95% CI, 0.99–2.37) times higher in those with a family history of prostate cancer compared with those without. To account for latency of exposure, we repeated the analysis among applicators whose first use of phorate was prior to 1980 (not shown). Too few

Table 2. RRs (95% CIs) for selected cancers by phorate lifetime exposure-days among AHS (1993–1997) applicators, using nonexposed and lowest exposed applicators as the reference group.

Lifetime exposure-days[a]	Cases (n)	Nonexposed reference	Lowest exposed reference
All cancer			
0	689	1.00	
> 0–8.75	111	0.84 (0.68–1.04)	1.00
> 8.75–38.75	84	0.94 (0.74–1.19)	1.14 (0.85–1.53)
> 38.75–108.5	62	0.89 (0.68–1.17)	1.08 (0.78–1.49)
> 108.5	38	0.94 (0.67–1.33)	1.19 (0.79–1.78)
p-Trend		0.71	0.53
Lymphohematopoietic cancer			
0	72	1.00	
> 0–8.75	9	0.59 (0.20–1.12)	1.00
> 8.75–38.75	9	0.88 (0.40–2.03)	1.24 (0.48–3.22)
> 38.75	8	0.64 (0.52–2.17)	0.84 (0.30–2.33)
p-Trend		0.30	0.57
Colon cancer			
0	53	1.00	
> 0–8.75	6	0.47 (0.20–1.13)	1.00
> 8.75–38.75	7	0.90 (0.40–2.03)	2.22 (0.72–6.83)
> 38.75	10	1.07 (0.49–2.52)	2.48 (0.84–7.36)
p-Trend		0.74	0.18
Lung cancer			
0	69	1.00	
> 0–8.75	6	0.81 (0.34–1.96)	1.00
> 8.75–38.75	5	1.00 (0.39–2.61)	1.14 (0.34–3.84)
> 38.75–108.5	4	0.82 (0.29–2.37)	0.85 (0.22–3.23)
> 108.5	4	0.95 (0.31–2.94)	0.63 (0.14–2.86)
p-Trend		0.91	0.47
Prostate cancer			
0	286	1.00	
> 0–8.75	53	0.89 (0.65–1.21)	1.00
> 8.75–38.75	38	0.91 (0.64–1.29)	1.08 (0.71–1.66)
> 38.75–108.5	28	0.92 (0.62–1.38)	1.16 (0.73–1.86)
> 108.5	16	0.93 (0.55–1.57)	1.31 (0.72–2.37)
p-Trend		0.78	0.40

p-Trend, p-value for trend test. Incidence RRs adjusted for age, state of residence, applicator type, education, family history of site-specific cancer, smoking, and use of aldicarb, ethylene dibromide, aldrin, 2,4,5-TP, and butylate.
[a]Lifetime exposure-days = years of use × days of use per year.

exposed applicators with a family history of prostate cancer prevented us from conducting the same analysis among those whose first exposure was after 1980. The results were consistent with those presented above.

For comparison with the previously published prostate cancer analysis (Alavanja et al. 2003), we repeated the analysis using methodology comparable to that used in the previously published prostate cancer study. That is, we used logistic regression to calculate odds ratios (OR) among all enrolled male applicators without prior history of prostate cancer, categorizing phorate exposure as ever/never. We used the cross product of ever/never phorated exposure and family history of prostate cancer to assess effect modification (not shown). We found that the age-adjusted prostate cancer risk was significantly elevated in those with a family history of prostate cancer (OR = 1.53; 95% CI, 1.09–2.14; 249 exposed cases), but not in those without (OR = 1.11; 95% CI, 0.95–1.31; 73 exposed cases). The interaction OR was 1.40 (95% CI, 0.96–2.04), adjusted for age and family history of prostate cancer. The corresponding interaction OR from the previous paper was 1.64 (95% CI, 1.02–2.63).

Discussion

Phorate use was not related to the occurrence of all cancers combined in this study. Although previous studies have observed suggestive increases in the risk of NHL and leukemia associated with the use of organophosphate pesticides (Brown et al. 1990; Cantor et al. 1992; Clavel et al. 1996; Zahm et al. 1993), the risk of lymphohematopoietic cancers overall was not associated with phorate use in this cohort. Too few exposed cases of NHL, leukemia, Hodgkin lymphoma, and multiple myeloma prevented evaluations of these cancers individually. As the lymphohematopoietic grouping may not be etiologically homogeneous, it would be prudent for follow-up studies to examine each cancer separately as more cancer cases develop in the cohort.

Prostate cancer risk was not significantly associated with phorate use. However, among applicators reporting a family history of prostate cancer, the risk associated with phorate exposure was elevated, whereas there was no corresponding increase among those without a family history. An elevated interaction term of similar magnitude was observed in an examination of prostate cancer in an article by Alavanja et al. (2003). The study conducted here is a chemical-specific analysis carried out on 135 phorate-exposed prostate cancer cases using information on lifetime exposure-days and intensity-weighted lifetime-exposure days to examine dose–response relationships. In contrast, the previously published prostate cancer study was a tumor-specific analysis and in quantifying phorate use as ever/never use could not examine dose–response trends. In addition, this study was carried out over a longer average follow-up period of 7.5 years, compared with 4.3 years. Despite the analytic differences, the results are generally consistent with the previous paper.

Family history of prostate cancer is strongly and consistently linked to prostate cancer in the scientific literature (Bostwick et al. 2004). For example, monozygotic twins have higher prostate cancer concordance than dizygotic twins (Gronberg et al. 1994). Risk is elevated several-fold in individuals with an affected father or brother (Glover et al. 1998; Spitz et al. 1991; Steinberg et al. 1990; Whittemore et al. 1995) and may increase if a greater number of first-degree relatives are affected (Steinberg et al. 1990). Finally, cancers in individuals with affected family members occur at younger ages compared with individuals without affected family members (Bratt et al. 1999; Gronberg et al. 1999). Farming occupation has also been modestly but significantly associated with prostate cancer (Blair et al. 1985; Checkoway et al. 1987; Sharma-Wagner et al. 2000; van der Gulden and Vogelzang 1996; Van Maele-Fabry and Willems 2004). In particular, the results of several studies suggest that this association may be caused by exposure to pesticides (Potti et al. 2003). Significant associations were found specifically among those individuals who were ever employed in the mixing and application of pesticides (Fleming et al. 1999; Settimi et al. 2001), and cancer risk increased with both the number of days of pesticides applied per year (van der Gulden et al. 1995) and the number of acres sprayed with herbicides in 1 year (Morrison et al. 1993).

Although a number of exposures shared in common between study subjects and their first-degree relatives could lead to a statistical interaction between phorate use and family history of prostate cancer, the presence of family history of prostate cancer may serve as a surrogate for an inherited genetic trait, such as a polymorphism in a metabolism enzyme. The active form of most organophosphates, including phorate and chlorpyrifos, is the corresponding oxon (Pope 1999; Sultatos 1994), and both of these insecticides are metabolized using many of the same enzymes (Tang et al. 2001; Usmani et al. 2004). Polymorphic variants of several cytochrome P450 isoforms vary considerably in their ratio of bioactivation to detoxification of chlorpyrifos (Dai et al. 2001; Tang et al. 2001). Thus, it is possible that the observation of an interaction of family history and phorate exposure reflects the presence of an inherited polymorphism that alters the balance between bioactivation and detoxification in the body.

There are some limitations of this study. At this time, investigation of certain cancers is hindered by small numbers of exposed cases, making it difficult to analyze some cancers. However, as the cohort ages, more cancer cases will accrue and allow for more statistically powerful investigations. Additionally, some exposure misclassification is likely, although there is no reason to believe that it occurred differentially between cancer cases and cancer-free subjects, because exposure information was gathered prior to disease onset.

Another general limitation of studies of pesticide applicators is that few applicators are exposed to one agent. To attempt to control for potential confounding from other pesticides, the risk estimates were adjusted for use of the five pesticides most correlated with phorate. However, the use of other pesticides did not likely confound the observed relationships because the correlation coefficients, which ranged between 0.36 for butylate and 0.39 for aldicarb, were not very high. Moreover, risk estimates were similar when they were adjusted for cumulative lifetime exposure-days to all pesticides. An examination of pesticide usage and specific farming activities found that these activities likely resulted in minimal confounding (Coble et al. 2002).

Table 3. Incidence RRs[a] (95% CIs) for prostate cancer by phorate lifetime exposure-days after stratification by family history of prostate cancer among male AHS (1993–1997) applicators.

Lifetime exposure-days[b]	Cases (*n*)	Nonexposed reference	Lowest exposed reference
No family history			
0	270	1.00	
> 0–8.75	49	1.01 (0.74–1.39)	1.00
> 8.75–24.5	35	1.05 (0.73–1.51)	1.03 (0.67–1.60)
> 24.5	35	0.91 (0.64–1.30)	0.92 (0.59–1.43)
p-Trend		0.66	0.69
Family history			
0	56	1.00	
> 0–8.75	10	0.69 (0.35–1.39)	1.00
> 8.75–24.5	11	1.27 (0.65–2.49)	1.90 (0.80–4.50)
> 24.5	17	1.48 (0.85–2.58)	1.91 (0.86–4.24)
p-Trend		0.11	0.16
Interaction[c]		1.18 (0.96–1.44)	1.53 (0.99–2.37)

p-Trend, *p*-value for trend test.
[a]RRs adjusted for age and state of residence. [b]Lifetime exposure-days = years of use × days of use per year. [c]RR and 95% CI for the cross product of family history of prostate cancer and lifetime exposure-days category, adjusted for age, state of residence, and family history of prostate cancer.

The exposure measures in this study are an improvement. Whereas previous studies of pesticide exposures were limited to qualitative exposure measures, this study attempts to quantify cumulative lifetime exposure by incorporating measures of frequency, duration, and intensity of exposure to specific pesticides. The measure of intensity used information such as application methods and PPE use to calculate a more precise measurement of actual exposure. Furthermore, the rate of recruitment and follow-up of participants was very high, as 82% of eligible participants enrolled, and < 2% were lost to follow-up. Although not all take-home questionnaires were returned, the measured differences between respondents and nonrespondents were not likely to be influential here (Tarone et al. 1997). Thus, the applicators returning the take-home questionnaire were likely representative of the overall cohort in terms of cancer risk.

In summary, no clear association between phorate and any cancer was observed in this study. However, the study findings are not inconsistent with the hypothesis that there is an interaction between phorate exposure and family history of prostate cancer in the incidence of this cancer, suggesting that there may be an inherited genetic variability that alters susceptibility to prostate cancer. Future analyses of the AHS to further investigate the relationship between phorate exposure and the risk of prostate cancer are warranted.

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Pesticide Spray Proves Disastrous In Salkiana Village, Jalandhar

By Kheti Virasat Mission

04 August, 2006
Countercurrents.org

Report of a Fact Finding Visit by Kheti Virasat Mission

21 July 2006 began as any other day for the residents of Salkiana village in Jalandhar district. That was until around 8.30 am - until they started feeling severe suffocation and breathlessness. The worst affected were the school children of the Government-run Elementary School. It was just after Morning Prayers that the students started complaining of a strange smell and breathlessness. The teachers were not aware of what happened either. Suddenly one student fell unconscious near the hand pump [of the drinking water tube well] and then student after student started to faint. Within ten minutes, 16 students fainted after inhaling something that was toxic.

It was not just the school children who were affected. The villagers outside the school were also experiencing and complaining about breathlessness by then. Some women in the adjoining houses are reported to have fainted too.

There was total panic in the village for a while. It was only then the villagers began to realize what happened – IT WAS A DEADLY PESTICIDE SPRAYED IN A NEARBY SUGARCANE FIELD THAT HAD AFFECTED THE VILLAGERS. The villagers then understood that they were experiencing acute poisoning symptoms.

Meanwhile the farm workers, who had sprayed this pesticide, came into the village and disclosed that they had sprayed PHORATE. In this case, it was Sudarshan Chemicals' SUTOX 100 that they had sprayed. The workers had sprayed 15 kilos of Phorate 10G in 3.75 acres by then.

In the school, the situation had become quite serious by then and the teachers started experiencing breathlessness too. Students started complaining of difficulty in breathing, severe headache, body ache, irritation in eyes, uneasiness, dizziness and some of them started vomiting.

The teachers acted very swiftly and informed the higher authorities and the local health officer. Within half an hour, a team of doctors reached the

school and first-aid was administered. The affected students and teachers were shifted to Civil Hospital, Phillour [the nearest town]. However, some parents took their children to private hospitals also. Some children with severe breathlessness were administered oxygen.

Fact Finding Visit:

Following this incident, a fact finding visit was made by Kheti Virasat Mission on 26th and 28 th July to this village. The teachers, the students, the other affected villagers, the sarpanch and other farmers were interviewed as part of the fact finding visit. In addition, the FFT [fact finding team] spoke with the doctors in the Civil Hospital and met with the SDM, Phillour.

This report tries to give a picture of the situation based on all the information gathered from these interviews and discussions.

According to the doctors at civil hospital, patients were admitted with following signs and symptoms [somewhat varying between patients]:

1. Excessive Lacrimation

2. Excessive Salivation

3. Dizziness

4. Nausea & Vomiting

5. Breathlessness

6. Body aches and cramps

These patients were given first-aid there and then in the village and later referred to Civil Hospital, Phillaur. Patients were given-

1. I/V fluids

2. Rangers Lactato

3. DNS

4. And 5% Dextrose

-Inj.Atropine I/M & I/V slow in cloop

-Inj. Aciloc sos

-Inj.Ettacarlin

-Inj. PAM

-Inj.Neomolsos

-O* 2* Inhalation

The list of patients admitted to the Civil Hospital in Phillaur, following this poisoning incident:

1. Parwinder 13 yrs M

2. Jasbir Kaur 40 yrs F

3. Jeeto 50 yrs F

4. Sonia 13 yrs F

5. Dalwinder 15 yrs F

6. Gurdeep Singh 13 yrs M

7. Reena Kumari 13 yrs F

8. Manjit 9 yrs M

9. Kamla Devi 25 yrs F

10. Naresh Kumari 13 yrs F

11. Manjit Kaur 30 yrs F

12. Navneet Kaur 13 yrs F

13. Raman Deep 12 yrs M

14. Amarjeet Kaur 22 yrs F

15. Dalbiro 40 yrs F

16. Suman 18 yrs F

17. Sukhdev 15 yrs M

18. Bhagwan Dass 52 yrs M

Total: 18 persons. One adult male, seven adult females, 5 male children and 5 female children.

All these patients were in the hospital for three days. Two others patients Suman (18 year old young girl) and Sunita (14 year old girl) were admitted in a private nursing home at Phillaur. Sunita, a newly-married girl inhaled the toxic fumes when she had gone near the fields the next day. Her condition deteriorated soon after and she was taken to the Civil Hospital. She was then referred to a hospital in Ludhiana as her condition was found to be critical. Her family members got her admitted in to Arora Hospital. She was here for four days. These are the 21 cases of hospitalization from the inhalation poisoning from the phorate spraying.

At the time of the fact finding visit, some of the students and teachers were still unwell, even after six days. They had irritation in eyes upto six days, itching of the skin and general uneasiness. The teacher in the government school Mr Bhagwan Dass was complaining of disturbance in his digestive system. He is suffering from constipation and urinary problem. Same were the complaints from Hardev Singh M 38 and Ms Asha Sharma F 34, both teachers at the government school. Bakshish Chand, 37, who is also the ex-sarpanch of the village, had similar complaints.

All children and adults, exposed to PHORATE, were experiencing loss of appetite even on sixth day after exposure. Most of the children poisoned were from SC community with low incomes

After this incident, there is widespread resentment and concern amongst the villagers regarding pesticides. They are quite worried about safety from pesticides. The elders at village feel that there should at least be restrictions followed about spraying away from the village that too from schools etc.

Mr Ram Kishan, Harjeet Ram and Ram Swarup (all members of the Panchayat) and other villagers were of the strong view that some concrete steps should be taken to prevent such mishaps in future.

The Doctors who worked tirelessly at Civil Hospital, Phillaur were admitting their limitations in dealing with a case like this, given that none of them is a trained environmental epidemiologist.

About Phorate:

Phorate is a Class IA pesticide – which means that by World Health Organisation's classification, it is "Extremely Hazardous". Phorate 10% G falls under Class IB. The Food & Agriculture Organisation recommends that products that fall under Class IA and Class I B [Extremely Hazardous and Highly Hazardous] should not be used in developing countries given a variety of safety concerns related to these products.

Phorate poisoning in India

Phorate is an organo-phosphorus pesticide implicated in several poisoning cases earlier. For instance, in June 2001, phorate was implicated in poisoning of workers in a tea estate and in a cardamom plantation in Kerala [1]. A 16 year old boy, Kannan, died applying phorate on June 26th in a cardamom plantation. On the tea estate in Idukki district, on the same day, 41 people in all got affected. They all experienced acute poisoning symptoms of blurred vision, vomiting and dizziness.

Mancini et al report in the International Journal of Occupational and Environmental Health, 2005 that acute pesticide poisoning from Organophosphorus pesticides like phorate was quite common, especially amongst low income marginal farmers in cotton growing belts of South India.

There are reports that indicate that upon ingestion of organo phosphate pesticides like phorate and monocrotophos, there is also the danger of Organo Phosphate Induced Delayed Neuropathy which appears 2-4 weeks after poisoning and leads to motor paralysis [affecting the distal muscles of limbs, minimal sensory involvement and calf pain which precedes its onset].

Acute poisoning due to phorate intoxication was reported from Tamluk in West Bengal in the Journal of Indian Pediatrics in 2002. Here, the affected were mainly toddlers.

Significant amongst all the published studies is a report by Kashyap [1986] which reported that " Exposure of 40 formulators to a highly toxic OP insecticide (phorate) showed that over 60% of the workers suffered from toxic effects in spite of using a complete set of protective clothing" [2].

In Wayanad district of Kerala, in July 2002, children exposed to phorate fumes sprayed on banana plantations had to be admitted to hospitals. These children had experienced vomiting, dizziness and headaches.

According to the first information report available with Thanal, on July 10, 2002, children arriving at the Kottathara upper primary school complained of an unbearable stench, obviously from the banana fields where workers were busy applying a mixture of fertilizer and pesticide (Phorate 10%) to the soil before planting the banana rhizomes. As the day progressed, and aided by the breeze, the smell became worse and the children started complaining of severe headache and dizziness. Meanwhile, efforts by the school authorities to stop the workers from continuing using more pesticide were met with a firm refusal. The situation soon began to get out of hand as children began fainting; gram panchayat officials were contacted for jeeps to help carry the students to hospital. On July 17, the children were back in hospital with similar complaints. Doctors confirmed that the symptoms were of acute toxic exposure.

As per a study published in Economic & Political Weekly, December 2004, based on field investigations in high pesticide consumption districts in four states of India, phorate was implicated in creating adverse health effects amongst respondents.

Ch. Srinivas Rao et al reported in the Journal of Tropical Medicine & International Health [Vol. 10, No. 6, June 2005] about acute pesticide poisoning in South India [3]. In this paper, the authors report that Warangal district in Andhra Pradesh alone records more than 1000 pesticide poisoning cases each year and hundreds of deaths. They report that in the district government hospital, between 1997 and 2002, 8040 patients were admitted to the hospital with pesticide poisoning. In 2002, there were 21 cases of poisonings due to phorate, including 4 deaths.

About Class I pesticides in India

It is estimated that 2 to 5 million people every year suffer acute poisonings all over the world and that around 40,000 people die. These are very conservative estimates and these poisonings occur mostly in the developing world, caused mainly due to OP pesticides. Many of these are Class IA and Class IB pesticides. In India, despite the fact that FAO has recommended the non-usage of Class IA and IB pesticides, a number of these products continue to be used.

It is only from July 1 st 2006, after many long years of activist struggles with the company that Bayer, a market leader in pesticides in India , had stopped marketing many of its deadliest pesticides including its Class I

products. In earlier studies done by groups like Centre for Sustainable Agriculture, Hyderabad , many such products were implicated in acute poisoning hospitalizations and deaths [4].

It is also interesting to note that even though the Central Insecticides Board is currently reviewing several pesticides that have been banned in other countries for their continued use in India, Phorate, Hinosan, Oxydemeton-Methyl, Methyl Parathion etc., are not amongst them!

Given the wide extent of acute poisoning and the related socio-economic problems, we strongly demand the following:

To the government:

- Immediately ban aggressive marketing of pesticides and all type of agro-chemicals including all forms of advertisements and publicity of pesticides along with all incentives given to pesticide dealers' network.

- Punjab Government should take –up a proactive campaign on ill effects of pesticides.

- acknowledge the threat and that the problem of serious health effects with pesticides exists

- assess the extent of the problem with various adverse health effects of pesticides

- raise awareness about the dangers through well-financed education campaigns

- ensure the dissemination of information on ill effects of pesticides to all users

- fix liability and get compensation to be paid for medical care and economic rehabilitation for all victims – get the industry to pay up; if not, the government to pay

To the Union of India:

- ban all class I a, I b and II pesticides

- modify pesticide risk assessment procedures – bring in the precautionary principle

- promote better and safer agricultural practices including NPM approach and organic farming

- curb aggressive marketing by pesticide industry

To the health sector:

- train and equip health sector staff and infrastructure to identify and deal with such cases

- set up systems for regular and proper monitoring

- Government should fill posts of District Epidemiologist in all districts on priority basis.

- Citizens committee on epidemiological surveillance shall formed under District Epidemiologists to ensure community participation in mitigation and crisis management process

To the Industry:

- pay compensation to all the persons affected

- pro-actively withdraw all Class I and Class II products from the market

- stop aggressive marketing

PUNJAB AGRICULTURE UNIVERSITY [PAU]

1. Ample funding should be provided to Punjab Agriculture University to ensure adequate education, research and extension on organic farming

2. Policy formulation as well as agriculture recommendations should be such that they are evolved from the original experiences of organic farmers. PAU should draw from such experiences and not just research in agriculture research station campuses

3. Crops that are suitable to the local natural eco-systems should be promoted and research should be taken up on this basis

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INHIBITION AND ACTIVATION OF THE HUMAN LIVER MICROSOMAL AND HUMAN CYTOCHROME P450 3A4 METABOLISM OF TESTOSTERONE BY DEPLOYMENT-RELATED CHEMICALS

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ABSTRACT:

Cytochrome P450 (P450) enzymes are major catalysts involved in the metabolism of xenobiotics and endogenous substrates such as testosterone (TST). Major TST metabolites formed by human liver microsomes include 6β-hydroxytestosterone (6β-OHTST), 2β-hydroxytestosterone (2β-OHTST), and 15β-hydroxytestosterone (15β-OHTST). A screen of 16 cDNA-expressed human P450 isoforms demonstrated that 94% of all TST metabolites are produced by members of the CYP3A subfamily with 6β-OHTST accounting for 86% of all TST metabolites. Similar K_m values were observed for production of 6β-, 2β-, and 15β-OHTST with human liver microsomes (HLM) and CYP3A4. However, V_{max} and CL_{int} were significantly higher for 6β-OHTST than 2β-OHTST (~18-fold) and 15β-OHTST (~40-fold). Preincubation of HLM with a variety of ligands, including chemicals used in military deployments, resulted in varying levels of inhibition or activation of TST metabolism. The greatest inhibition of TST metabolism in HLM was following preincubation with organophosphorus compounds, including chlorpyrifos, phorate, and fonofos, with up to 80% inhibition noticed for several metabolites including 6β-OHTST. Preincubation of CYP3A4 with chlorpyrifos, but not chlorpyrifos-oxon, resulted in 98% inhibition of TST metabolism. Phorate and fonofos also inhibited the production of most primary metabolites of CYP3A4. Kinetic analysis indicated that chlorpyrifos was one of the most potent inhibitors of major TST metabolites followed by fonofos and phorate. Chlorpyrifos, fonofos, and phorate inhibited major TST metabolites noncompetitively and irreversibly. Conversely, preincubation of CYP3A4 with pyridostigmine bromide increased metabolite levels of 6β-OHTST and 2β-OHTST. Preincubation of human aromatase (CYP19) with the test chemicals had no effect on the production of the endogenous estrogen, 17β-estradiol.

The cytochrome P450 (P450[1]) monooxygenase system is comprised of a superfamily of heme-containing enzymes, expressed in many mammalian tissues with the highest levels found in liver, and capable of catalyzing the metabolism of a wide range of both endogenous and exogenous substrates (Nelson et al., 1996). Human CYP3A4 is one of the most important and most abundant drug-metabolizing P450 isoforms in human liver microsomes and accounts for approximately 40% of the total P450 in human liver microsomes (Lehmann et al., 1998). CYP3A4 not only metabolizes xenobiotics but is also responsible for the metabolism of endogenous compounds, such as steroid hormones. Human CYP3A4 plays an important role in the metabolism of testosterone (TST), androstenedione (AD), and progesterone (Waxman et al., 1988). Direct and indirect approaches have been employed to show that isoforms belonging to the CYP3A subfamily are the major contributors to 6β-hydroxylation of testosterone as well as the production of several minor metabolites (Waxman et al., 1988, 1991; Yamazaki and Shimada, 1997).

In the human male, TST is the major circulating androgen. TST is essential for the development and maintenance of specific reproductive tissues as well as for other characteristic male properties such as control of spermatogenesis, retention of nitrogen, promotion of muscle strength, hair growth, bone density, and many aspects of sexually dimorphic behavior (Nieschlag and Behre, 1998; Wilson et al., 1998). Maintaining hormonal balance relies upon a number of variables including rate of hormone synthesis, interactions among hormones, and rates of secretion, transport, and metabolism. P450s are a major controlling element in the maintenance of proper steroid hormone levels in mammalian systems. Exposure to foreign compounds can exert changes in endocrine function both directly (hormone agonists or antagonists) or indirectly (altering circulating levels of hormones by influencing rates of hormone synthesis or metabolism) that can severely affect steroid hormone action (Wilson and LeBlanc, 1998). Steroids such as TST are hydroxylated by P450 in a regioselective and stereoselective manner (Waxman et al., 1988). It follows that perturbation of the P450 system by xenobiotics may in turn affect the subsequent metabolism and disposition of TST. Perturbations in TST metabolism may affect levels of circulating TST with possible reproductive and other consequences, including further modulation of the expression of some P450 proteins.

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[1] Abbreviations used are: P450, cytochrome P450; TST, testosterone; AD, androstenedione; DEET, *N,N*-diethyl-*m*-toluamide; KTST, ketotestosterone; OHAD, hydroxyandrostenedione; HPLC, high-performance liquid chromatography; HLM, human liver microsomes; OHTST, hydroxytestosterone; K_i, inhibition constant; b_5, cytochrome b_5.

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Following the Gulf War some veterans reported illnesses which may have been the result of chemical exposures. Some studies of these veterans have concluded that significant correlations between perceived illnesses and chemical use exist (Haley and Kurt, 1997). The reported chemical exposures included the insect repellent *N,N*-diethyl-*m*-toluamide (DEET), insecticides such as permethrin and chlorpyrifos to protect against insect borne diseases and the neuroprotective agent pyridostigmine bromide to protect against possible nerve gas attack. It has been reported that chlorpyrifos and DEET are metabolized by human P450s (Tang et al., 2001; Usmani et al., 2002) and that interactions of Gulf War related chemicals can inhibit or induce the P450s involved in their metabolism (Usmani et al., 2002). Other studies have reported that interaction of Gulf War related chemicals could produce greater than additive toxicity in rats and mice (Chaney et al., 1997; McCain et al., 1997), increased neurotoxicity in hens associated with increased inhibition of brain acetylcholinesterase and Neurotoxicity Target Esterase (Abou-Donia et al., 1996a,b), and neurobehavioral deficit associated with significant inhibition of brainstem acetylcholinesterase activities in rats (Abou-Donia et al., 2001). However, no studies have been carried out to examine the induction or inhibition potential of these or related compounds on human P450-mediated metabolism of steroid hormones, such as TST. An understanding of how Gulf War related chemicals affects the metabolism of TST could aid in the evaluation of the possible role that these chemicals may play in deployment-related illnesses.

The main objectives of present study were to identify human liver P450 isoforms responsible for TST metabolism and the products of their activity using an improved HPLC method, to study the effects of various deployment-related chemicals on the metabolism of TST using HLM and CYP3A4, and to study the effects of the test compounds on human aromatase (CYP19).

Materials and Methods

Chemicals. DEET, chlorpyrifos, chlorpyrifos-oxon, phorate, fonofos, deltamethrin, fipronil, imidacloprid, and permethrin (isomeric mix 78% *trans*-20% *cis*) were purchased from Chem Service (West Chester, PA). Pyridostigmine bromide was purchased from Roche Diagnostics (Indianapolis, IN). 6α-, 15β-, 15α-, 7α-, 6β-, 16α-, 16β-, 2α-, 2β-, 11β-OHTST, 11-ketotestosterone (11-KTST), 11β-hydroxyandrostenedione (11β-OHAD), AD, and 4-hydroxyandrostenedione (4-OHAD) were purchased from Steraloids (Newport, RI). HPLC grade water, methanol, acetonitrile, and tetrahydrofuran were purchased from Fisher Scientific (Pittsburgh, PA). TST, 17β-estradiol, and all other chemicals were purchased, if not specified, from Sigma-Aldrich (St. Louis, MO).

Human Liver Microsomes and Human P450 Isoforms. Pooled human liver microsomes (HLM) (pooled from 21 donors) and human P450 isoforms expressed in baculovirus infected insect cells (Sf9) (BTI-TN-5B1–4), CYP1A1, 1A2, 2B6, 3A4, 3A5, 3A7, 4A11, 2B6, 2C8, 2A6, 2C9*1 (Arg_{114}), 2C9*2 (Cys_{144}), 2C9*3 (Leu_{359}), 2C18, 2C19, 2D6*1 (Val_{374}), 2E1, and human aromatase (CYP19) were purchased from BD Gentest Corporation.

In Vitro TST Metabolism. Metabolic activity assays for human P450 isoforms were performed by incubation of TST (final concentrations, 250 μM) with an NADPH-regenerating system (0.25 mM NADP, 2.5 mM glucose 6-phosphate, and 2 U/ml glucose-6-phosphate dehydrogenase) in specific buffers recommended by the supplier (BD Gentest Corporation). For CYP1A1, 1A2, 2E1, 2C8, 2D6*1 (Val_{374}), 3A4, 3A5, 3A7, 2B6, 2C18, 2C19, and an insect cell control, a 100 mM potassium phosphate buffer with 3.3 mm $MgCl_2$ (pH 7.4) was used. For 2C9*1 (Arg_{114}), 2C9*2 (Cys_{144}), 2C9*3 (Leu_{359}), 4A11, and 2A6, a 100 mM Tris-HCl buffer with 3.3 mM $MgCl_2$ (pH 7.5) was used. After preincubation at 37°C for 5 min, the reactions were initiated by the addition of ice-cold P450 isoforms (final P450 contents 50 pmol/ml) for 30 min at 37°C. The controls were performed under identical conditions with the insect cell control.

Enzyme kinetic assays for HLM and CYP3A4 were performed by incubation of serial concentrations of TST (final concentrations, 9.375–500 μM) with HLM (final protein concentration, 1 mg/ml) or CYP3A4 (final concentration, 50 pmol/ml) in 100 mM potassium phosphate buffer (pH 7.4 at 37°C) containing *3.3* mM $MgCl_2$. After preincubation at 37°C for 5 min, the reactions were initiated by the addition of ice-cold HLM or CYP3A4 for 10 min.

The effects of test chemicals on TST metabolism were examined in HLM and CYP3A4 after preincubation with test compounds. The HLM (final protein concentration, 1 mg/ml) or CYP3A4 (final concentration, 50 pmol/ml) were incubated with individual test compounds (final concentration, 100 μM), NADPH-generating system, and 100 mM potassium phosphate buffer with 3.3 mM $MgCl_2$, pH 7.4, for 5 min at 37°C before adding TST (final concentration, 250 μM).

Range finding assays were conducted for chlorpyrifos, fonofos, and phorate inhibition of TST major metabolites. Varying concentrations of chlorpyrifos, fonofos, and phorate (0.5–100 μM) were incubated with CYP3A4 (final concentration, 50 pmol/ml), NADPH-generating system, and 100 mM potassium phosphate buffer with 3.3 mM $MgCl_2$, pH 7.4, for 5 min at 37°C before adding TST (final concentration, 100 μM). Reactions were terminated and analyzed as described above. With selected concentration levels based on the range finding assay, the mode of chlorpyrifos, fonofos, and phorate inhibition on TST major metabolites was investigated. For Michaelis-Menten plots, chlorpyrifos (2 μM), fonofos (5 μM), and phorate (30 μM) were incubated with CYP3A4 (final concentration, 50 pmol/ml), NADPH-generating system, and 100 mM potassium phosphate buffer with 3.3 mM $MgCl_2$, pH 7.4, for 5 min at 37°C before adding TST (final concentration, 9.375–500 μM).

To demonstrate whether chlorpyrifos inhibition is reversible or irreversible, incubations with and without chlorpyrifos (2 μM) were conducted with varying concentrations of CYP3A4 (0.78–6.25 pmol), NADPH-generating system, and 100 mM potassium phosphate buffer with 3.3 mM $MgCl_2$, pH 7.4, for 5 min at 37°C before adding TST (final concentration, 100 μM).

To determine (inhibition constant) K_i values, chlorpyrifos (1–8 μM), fonofos (1–25 μM), and phorate (10–100 μM) were incubated for 5 min at 37°C with CYP3A4 (final concentration, 50 pmol/ml), NADPH-generating system, and 100 mM potassium phosphate buffer with 3.3 mM $MgCl_2$, pH 7.4, prior to adding TST (final concentrations, 50, 100, or 200 μM). K_i values were calculated from Dixon plots.

Since cytochrome b_5 (b_5) is not coexpressed with CYP3A5 as supplied by BD Biosciences (San Jose, CA), a comparison of CYP3A5 metabolism of TST was made using 10 pmol 3A5 with and without addition of 20 pmol b_5.

Human aromatase (CYP19) catalyzes the conversion of TST to estradiol. To study the effects of the test chemicals on this conversion, test compounds (final concentration, 200 μM) or a well known competitive inhibitor, 4-OHAD (final concentration, 200 μM) were incubated with CYP19 (final concentration, 50 pmol/ml), NADPH-generating system, and 100 mM potassium phosphate buffer with 3.3 mM $MgCl_2$, pH 7.4, for 5 min at 37°C before adding TST (final concentration, 100 μM). The reaction was terminated after an additional 10 min, and supernatant was analyzed for 17β-estradiol concentration by HPLC.

All assays were conducted in triplicate. All reactions were terminated by the addition of an equal volume of methanol and vortexing. After 10-min centrif-

TABLE 1

HPLC retention times for testosterone and hydroxylated testosterone metabolites

Common Name	Chemical Name	Retention Time
		min
6α-Hydroxytestosterone	4-Androsten-6α,17β-diol-3-one	14.38
15β-Hydroxytestosterone	4-Androsten-15β,17β-diol-3-one	15.11
15α-Hydroxytestosterone	4-Androsten-15α,17β-diol-3-one	15.53
7α-Hydroxytestosterone	4-Androsten-7α,17β-diol-3-one	15.81
6β-Hydroxytestosterone	4-Androsten-6β,17β-diol-3-one	16.25
16α-Hydroxytestosterone	4-Androsten-16α,17β-diol-3-one	17.43
11-Ketotestosterone	4-Androsten-17β-ol-3,11-dione	18.24
16β-Hydroxytestosterone	4-Androsten-16β,17β-diol-3-one	19.34
11β-Hydroxyandrostenedione	4-Androsten-11β-ol-3,17-dione	19.68
2α-Hydroxytestosterone	4-Androsten-2α,17β-diol-3-one	20.68
2β-Hydroxytestosterone	4-Androsten-2β,17β-diol-3-one	21.55
11β-Hydroxytestosterone	4-Androsten-11β,17β-diol-3-one	21.86
Androstenedione	4-Androsten-3,17-dione	24.92
4-Hydroxyandrostenedione	4-Androsten-4-ol-3,17-dione	27.20
Testosterone	4-Androsten-17β-ol-3-one	28.90

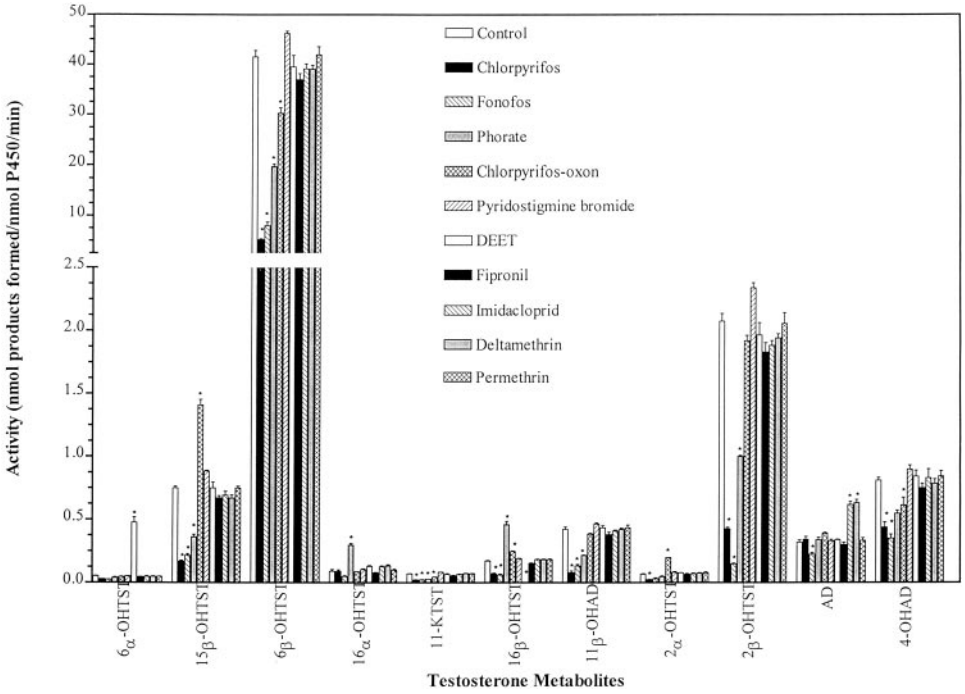

FIG. 1. *Effects of deployment-related chemicals on the rate of testosterone metabolism by pooled human liver microsomes.*

Specific activities were expressed as nanomole products formed per nanomole P450 per minute. ⋆, statistically significantly different when compared with respective control ($P < 0.01$).

ugation at 15,000 rpm in a microcentrifuge, the supernatants were analyzed for TST metabolite concentrations by HPLC. The protein concentrations and incubation times used in the assays were found to be in the linear range in preliminary experiments. No metabolites were detected when incubations were carried out in the absence of an NADPH-generating system.

Analysis of Metabolites by HPLC. Metabolites were analyzed using a Shimadzu HPLC system (Kyoto, Japan). The Shimadzu HPLC system (Kyoto, Japan) used in this study consisted of one pump (LC-10AT VP), a four-position solvent selection proportioning valve (FCV-10AL VP), a degasser (DUG-14A), a Shimadzu autoinjector (SIL-10AD VP), and a Shimadzu UV/VIS detector (SPD-10AV VP). All system components were controlled through the Shimadzu powerline firmware. Data were collected via a Shimadzu system controller (SCL-10A VP) and analyzed using CLASS-VP 4.3 software. A reverse phase HPLC method was modified based on the HPLC method of Purdon and Lehman-McKeeman (1997), for the separation of TST and its potential metabolites. The mobile phase for pump A was 5% tetrahydrofuran, 95% water, for pump B 100% methanol. A gradient system was employed in the following manner: 0 to 1 min (30% B), 1 to 10 min (30–60% B), 10 to 22 min (60–65% B), 22 to 28 min (65–80% B), 28 to 30 min (80–90% B), 30 to 32 min (90% B), 32 to 34 min (90–30% B), and 34 to 36 min (30% B). The flow rate was 0.5 ml/min. Metabolites were separated by a Prodigy column [Prodigy 3 μ, 150 × 4.6 mm, ODS (3), 100A; Phenomenex, Rancho Palos Verdes, CA] and detected at 247 nm. A summary of the retention times of TST and 14 TST metabolites are presented in Table 1. The limits of detection for most of TST metabolites were approximately 0.04 μM except for 6β-OHTST (0.15 μM) and 4-OHAD (0.30 μM). Standards of TST metabolites were made in methanol and 50-μl standard or sample injected on HPLC. Concentrations of metabolites were obtained by extrapolation of peak height from a standard curve. Percentages of individual metabolites are expressed on the basis of the total metabolites produced by the isoform or preparation in question.

For 17β-estradiol, the mobile phase was 60% H_2O and 40% acetonitrile. TST and 17β-estradiol were eluted isocratically at a flow rate of 1.0 ml/min for 15 min, separated by a Prodigy column [Prodigy 3 μ, 150 × 4.6 mm, ODS (3), 100A, Phenomenex, Rancho Palos Verdes, CA] and detected at 200 nm. The retention time of 17β-estradiol and TST was 10.4 and 11.3 min, respectively.

TABLE 2

Testosterone hydroxylation by human cytochrome P450 isoforms expressed in baculovirus-infected insect cells (nanomoles per nanomole isoforms per minute)

Isoforms	6α-OHTST	15β-OHTST	6β-OHTST	16α-OHTST	11-KT	16β-OHTST	11β-OHAD	2α-OHTST	2β-OHTST	AD	4-OHAD
1A1	NDA	NDA	3.01 ± 0.14	NDA	NDA	NDA	NDA	NDA	NDA	NDA	NDA
1A2	NDA	NDA	0.64 ± 0.02	NDA	NDA	NDA	NDA	NDA	NDA	NDA	NDA
2A6	0.05 ± 0.00	0.14 ± 0.01	NDA	NDA	NDA	NDA	NDA	NDA	NDA	0.53 ± 0.05	NDA
3A4	0.22 ± 0.02	3.18 ± 0.11	157.7 ± 6.00	NDA	1.04 ± 0.04	0.44 ± 0.01	1.70 ± 0.02	0.19 ± 0.01	7.05 ± 0.23	0.27 ± 0.01	2.23 ± 0.24
3A5	0.11 ± 0.01	NDA	12.4 ± 1.57	NDA	NDA	0.08 ± 0.01	0.14 ± 0.02	NDA	0.74 ± 0.10	NDA	NDA
3A7	0.09 ± 0.01	0.15 ± 0.02	3.89 ± 0.34	NDA	0.15 ± 0.02	0.27 ± 0.02	0.79 ± 0.05	3.05 ± 0.24	0.61 ± 0.06	0.13 ± 0.00	NDA
4A11	NDA	NDA	NDA	NDA	NDA	NDA	NDA	NDA	NDA	0.62 ± 0.01	NDA
2B6	0.05 ± 0.01	NDA	0.23 ± 0.01	0.17 ± 0.00	0.13 ± 0.03	0.61 ± 0.12	NDA	NDA	0.03 ± 0.00	NDA	NDA
2C8	NDA	NDA	NDA	0.38 ± 0.03	0.14 ± 0.03	NDA	NDA	NDA	NDA	NDA	NDA
2C9*1	NDA	NDA	NDA	NDA	NDA	0.18 ± 0.01	NDA	NDA	NDA	NDA	NDA
2C9*2	NDA	NDA	NDA	NDA	NDA	0.11 ± 0.02	NDA	NDA	NDA	NDA	NDA
2C18	0.05 ± 0.01	NDA	NDA	NDA	NDA	NDA	NDA	NDA	NDA	NDA	NDA
2C19	0.05 ± 0.00	NDA	0.43 ± 0.04	0.15 ± 0.01	NDA	0.32 ± 0.05	NDA	NDA	0.04 ± 0.01	2.53 ± 0.28	NDA
2E1	NDA	NDA	NDA	NDA	0.15 ± 0.03	NDA	NDA	NDA	NDA	NDA	NDA
2D6*1	NDA	NDA	1.49 ± 0.02	NDA	NDA	NDA	NDA	NDA	0.06 ± 0.00	1.40 ± 0.08	NDA

NDA, no detectable activity; no metabolite was formed with 2C9*3.

TABLE 3

Kinetic parameters for the production of major testosterone metabolites by human liver microsomes and CYP3A4

Means in the same column followed by the same letter are not significantly different ($P < 0.01$). Values are the mean ± S.E.M. ($n = 3$).

	Human Liver Microsomes				CYP3A4			
	K_m	V_{max}	CL_{int}	R^2	K_m	V_{max}	CL_{int}	R^2
	μM	*nmol/mg protein/min*	*μl/mg protein/min*		*μM*	*nmolnmol 3A4/min*	*μl/nmole 3A4/min*	
6β-OHTST	120.4 ± 19.4[a]	36.3 ± 2.3[a]	300.0[a]	0.94	107.7 ± 12.5[a]	284.8 ± 12.5[a]	2600.0[a]	0.97
2β-OHTST	119.2 ± 18.0[a]	2.0 ± 0.1[b]	20.0[b]	0.95	122.8 ± 14.1[a]	15.7 ± 0.7[b]	130.0[b]	0.98
15β-OHTST	138.9 ± 28.2[a]	0.8 ± 0.1[c]	6.0[c]	0.91	108.7 ± 16.4[a]	7.1 ± 0.4[c]	70.0[c]	0.96

The limit of detection for 17β-estradiol was approximately 0.10 μM. Concentrations of metabolites were obtained by extrapolation of peak height from a standard curve.

Data Analysis and Statistics. The apparent K_m and V_{max} parameters were calculated using nonlinear regression analysis program (Prism, GraphPad software Inc., San Diego, CA), and the K_i values were estimated by nonlinear regression analysis from the Dixon plot (Segel, 1975) using SigmaPlot Enzyme Kinetics Module (Chicago, IL). Significant differences between data sets were determined by one-way analysis of variance, and multiple comparisons were performed with the Dunnett's method using a JMP 4.0.2, SAS program (SAS, 1989).

Results

Four major metabolites were formed after incubation of TST with pooled HLM: 6β-, 2β-, 15βOHTST, and 4-OHAD as well as seven minor metabolites (Fig. 1). Among 16 different human P450 isoforms screened, only 2C9*3 (Leu_{359}) had no detectable activity toward TST (Table 2). All other P450 isoforms were active in generating one or more than one TST metabolites, although the extent of metabolism and the ratios of metabolites varied widely among isoforms. In this comparison of metabolite production by equal quantities of each isoform, CYP3A4, 3A5, and 3A7 were most active in TST metabolism among all the P450 isoforms tested (93.5% of the metabolites produced by all isoforms). Among members of the CYP3A subfamily, CYP3A4 produced the highest amount of total TST metabolites (88.5%) compared with 3A5 (6.9%) and 3A7 (4.6%). 6β-OHTST, the most prominent TST metabolite, mainly produced by the CYP3A subfamily, accounts for 86% of all TST metabolites. Among the CYP3A subfamily, CYP3A4 produced the highest amount of 6β-OHTST (90.6%) compared with 3A5 (7.1%) and 3A7 (2.2%). Other major TST metabolites formed by CYP3A4 were 15β-, 2β-OHTST, and 4-OHAD, whereas 6α-, 16β-, 11β-, 2α-OHTST, 11-KTST, and AD were minor metabolites. Among the P450 isoforms tested, CYP3A5 and 3A7 were significantly more important in forming the major TST metabolites than most of the others, but their activity was 10- to 20-fold less than that of CYP3A4. Interestingly, CYP3A7 produced 16 times more 2α-OHTST than CYP3A4. CYP1A1 is involved in the oxidation of TST at the 6β-position (3.0 nmol/nmol isoform/min), whereas CYP1A2 oxidized TST poorly at the 6β-position (0.6 nmol/nmol isoform/min). As can be observed in Table 2, the other P450 isoforms tested generally produced small amounts of one or more TST metabolites. CYP2C19 metabolized TST to AD more actively

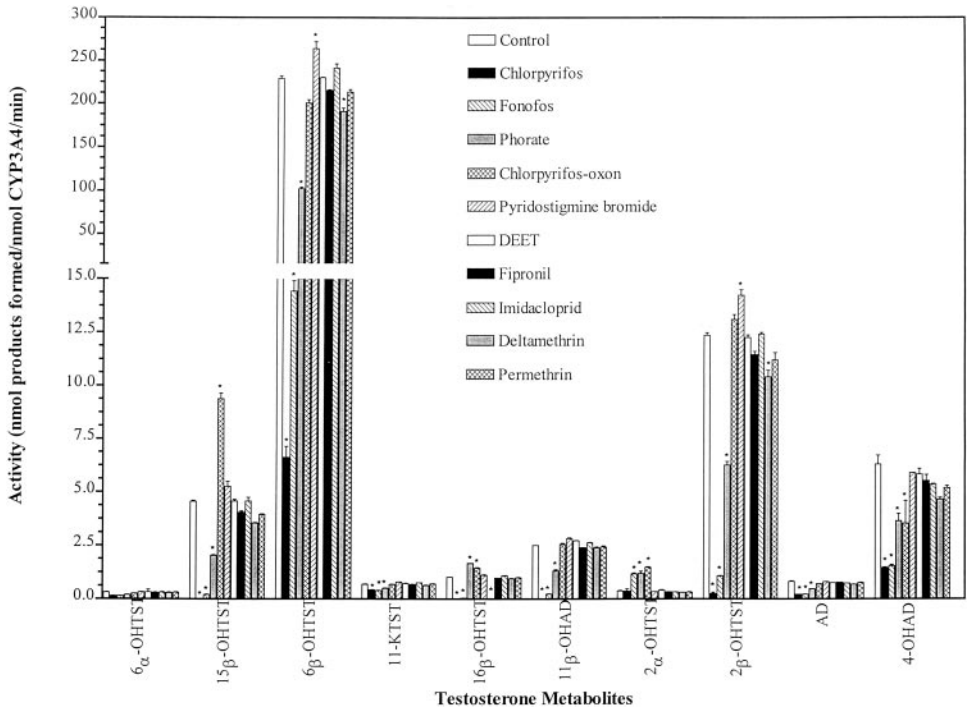

FIG. 2. *Effects of deployment-related chemicals on the rate of testosterone metabolism by CYP3A4.*

Specific activities were expressed as nanomole products formed per nanomole CYP3A4 per minute. ★, statistically significantly different when compared with respective control ($P < 0.01$).

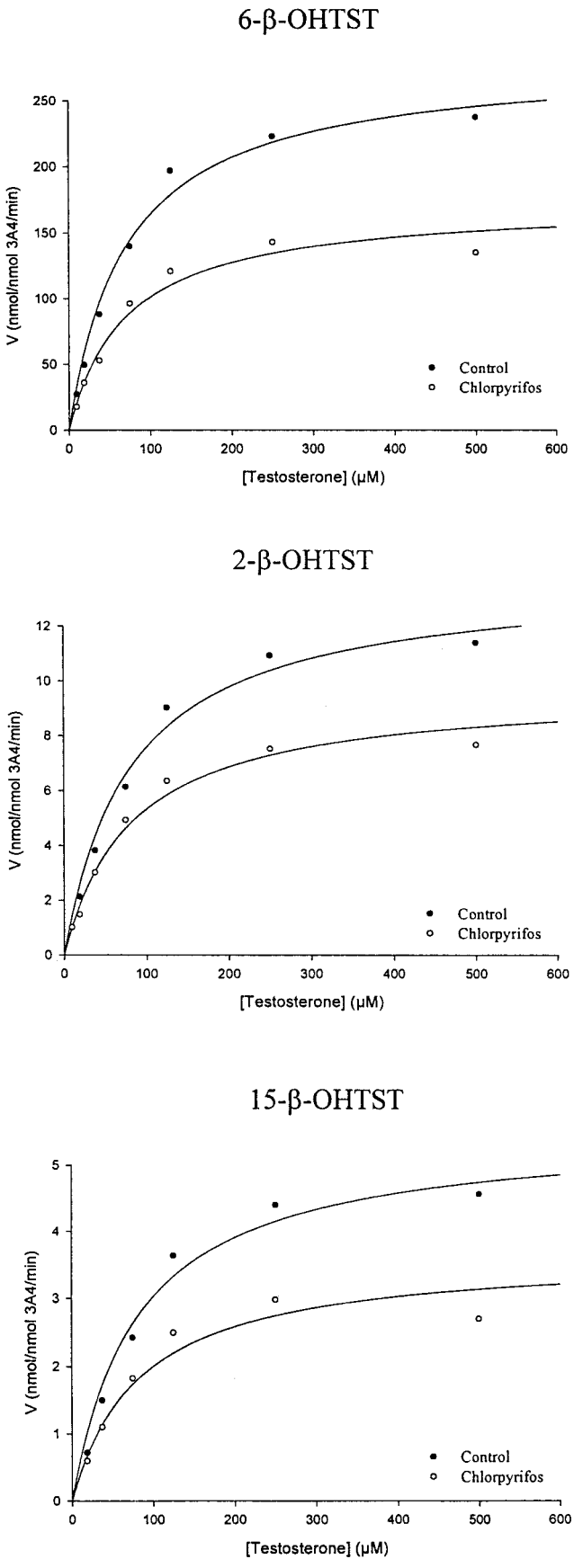

FIG. 3. *Representative Michaelis-Menten plots for the inhibition of CYP3A4-mediated testosterone hydroxylation by chlorpyrifos (2 μM).*

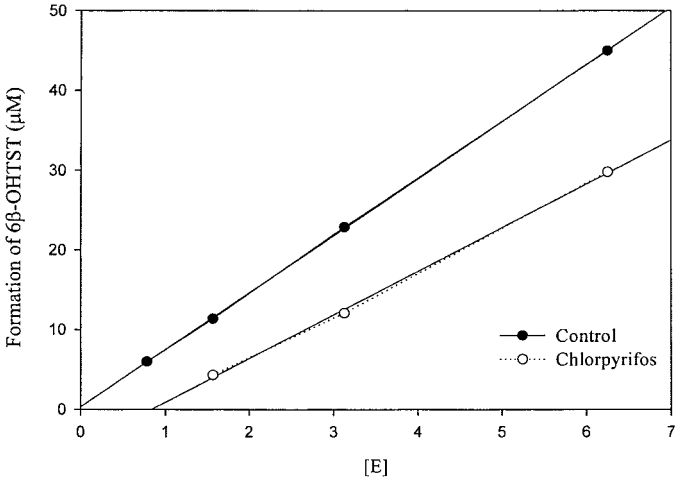

FIG. 4. *Representative plot of V_{max} versus amount of enzyme (CYP3A4) added to distinguish between a reversible and an irreversible noncompetitive inhibitor chlorpyrifos (2 μM).*

than any other isoform tested, whereas it catalyzed the formation of 6α-, 6β-, 16α-, 16β-, and 2β-OHTST poorly.

HLM and CYP3A4 displayed similar K_m values for 6β-, 2β-, and 15β-OHTST (Table 3). V_{max} and intrinsic clearance rate [Cl_{int} (V_{max}/K_m)] for 6β-OHTST was significantly higher than 2β-OHTST (~18-fold) and 15β-OHTST (~40-fold), respectively.

The effects of various deployment-related chemicals on TST metabolism were investigated by preincubating them with pooled HLM (Fig. 1). Preincubation of pooled HLM with chlorpyrifos, phorate, and fonofos resulted in significant inhibition of 6β-, 2β-, 15β-OHTST, 11-KTST, 11β-OHAD, and 4-OHAD. Preincubation of pooled HLM with DEET, chlorpyrifos-oxon, phorate, imidacloprid, and deltamethrin in some cases caused small but significant increases in the production of some TST metabolites by HLM.

Preincubation of CYP3A4 with a variety of chemicals resulted in varying levels of activation and inhibition of TST metabolism (Fig. 2). The greatest inhibition of TST metabolism was observed for the organophosphorus compound chlorpyrifos with up to 98% inhibition of major (6β-, 2β-, 15β-OHTST, and 4-OHAD) and several minor (11-KTST, 16β-OHTST, 11β-OHAD, and AD) TST metabolites. However, chlorpyrifos-oxon, an active metabolite of chlorpyrifos, has no inhibitory effect on the major TST metabolites. Two other organophosphorus compounds, phorate and fonofos, also significantly inhibited formation of several TST metabolites including 6β-, 2β-, 15β-OHTST, 11-KTST, 11β-OHAD, AD, and 4-OHAD. In contrast, preincubation of CYP3A4 with pyridostigmine bromide resulted in the production of small but significantly greater levels of the 6β- and 2β-OHTST metabolites. Some other TST metabolites were also significantly increased by preincubation of CYP3A4 with chlorpyrifos-oxon, phorate, and fonofos.

To investigate the type of inhibition of CYP3A4 by chlorpyrifos, fonofos, and phorate on major TST metabolites, chlorpyrifos (2 μM), fonofos (5 μM), and phorate (30 μM) were preincubated for 5 min before adding the varying concentrations of TST. Michaelis-Menten plots showed that the V_{max} values were significantly reduced without affecting K_m values, indicative of a noncompetitive inhibition of major TST metabolites by chlorpyrifos (Fig. 3). Similar results were obtained with fonofos and phorate (data were not shown). Further investigation of noncompetitive reversible or nonreversible inhibition data revealed that the inhibition is nonreversible (Fig. 4).

The K_i, an indicator of inhibitor affinity to target enzyme, was calculated by Dixon plot (Table 4; Fig. 5). Chlorpyrifos was the most

TABLE 4

Kinetics parameters for the inhibition of CYP3A4-mediated production of major testosterone metabolites by chlorpyrifos, fonofos, and phorate

Inhibitors	6β-OHTST		2β-OHTST		15β-OHTST	
	K_i	R^2	K_i	R^2	K_i	R^2
Chlorpyrifos	2.0 ± 0.2	0.99	3.6 ± 0.3	0.98	3.7 ± 0.4	0.97
Fonofos	5.8 ± 0.6	0.98	10.1 ± 0.7	0.98	6.3 ± 0.6	0.97
Phorate	34.1 ± 2.7	0.98	42.9 ± 4.1	0.98	33.8 ± 3.6	0.97

potent inhibitor of major TST metabolites with K_i values ranges from 2.0, 3.6, and 3.7 μM for 6β-, 2β-, 15β-OHTST, respectively. Fonofos was the second best inhibitor with K_i values ranging from 5.8, 10.1, and 6.3 μM for 6β-, 2β-, 15β-OHTST, respectively. Phorate K_i values ranged from 34.1, 42.9, and 33.8 μM for 6β-, 2β-, 15β-OHTST, respectively.

We investigated the possibility that b_5 may stimulate CYP3A5 catalytic activity by incubating b_5 (20 pmol) and CYP3A5 (10 pmol), which, in the preparations used, does not have b_5 coexpressed, with 250 μM of TST for 10 min. Addition of b_5 resulted in a more than 2-fold increase in TST 6β- and 2β-OHTST activity.

The possibility that conversion of TST to estradiol, which is catalyzed by aromatase (CYP19), could be inhibited by the test compounds was also investigated. Preincubation of human aromatase (CYP19) with various chemicals (chlorpyrifos, chlorpyrifos-oxon, permethrin, pyridostigmine bromide, DEET, phorate, fonofos, fipronil, imidacloprid, and deltamethrin) had no significant effect on the production of estradiol (data not shown). However, incubation with 4-OHAD, a well known competitive aromatase inhibitor, resulted in 90% inhibition of the aromatase enzyme activity.

Discussion

P450-dependent hydroxylation appears to be a major pathway of oxidative metabolism of TST in mammalian liver. Studies carried out using human P450 isoforms provide further insight into the range of TST hydroxylation reactions that can be catalyzed by human P450 enzymes. Our isoform data corroborates earlier findings (Waxman et al., 1988, 1991; Yamazaki and Shimada, 1997) that CYP3A4 is one of the major isoforms responsible for TST metabolism, and 6β-OHTST is the major TST metabolite. Greater than 82% of the TST metabolites are formed by CYP3A4, and 87% of the major 6β-OHTST metabolite is formed by CYP3A4. The mean metabolic intrinsic clearance rates, as estimated by V_{max}/K_m, also indicated that 6β-OHTST is the major metabolite of TST. Interestingly, CYP3A4 also metabolized TST to 4-OHAD, a potent inhibitor of extrahepatic aromatase (CYP19). It has been reported that 4-OHAD was able to inhibit 90% of the aromatase activity at a concentration of 1 μM (Mak et al., 1999). The physiological significance or consequence of this reaction is unclear and will require further investigation. Our results indicate that CYP1A1 and 1A2 were able to metabolize TST to 6β-OHTST, however, activity of CYP1A1 was much higher (4.7-fold) than CYP1A2. Consistent with a previous report (Yamazaki and Shimada, 1997), our data also indicated that CYP2C19 catalyzed oxidation of TST to form AD as a major TST metabolite. However, CYP2C18, which has 81% amino acid sequence identity to CYP2C19, exhibited distinctly poor hydroxylation activity in comparison with CYP2C19. Furthermore, our data indicated that CYP2D6*1, 4A11, and 2A6 metabolized TST to form AD but not as actively as CYP2C19. Guengerich et al. (2002) characterized the affinity of CYP2D6 for testosterone.

Endogenous steroids, such as TST, always exist in vivo, and considerable amounts of these steroids are metabolized by the P450s expressed in the human liver, where foreign compounds are mainly metabolized. If xenobiotics substantially affect TST metabolism, it may alter the rate of TST metabolism, which may ultimately disrupt TST homeostasis. Preincubation of pooled HLM with organophosphorus compounds, such as chlorpyrifos, phorate, and fonofos, resulted in the extensive inhibition of major and some minor TST

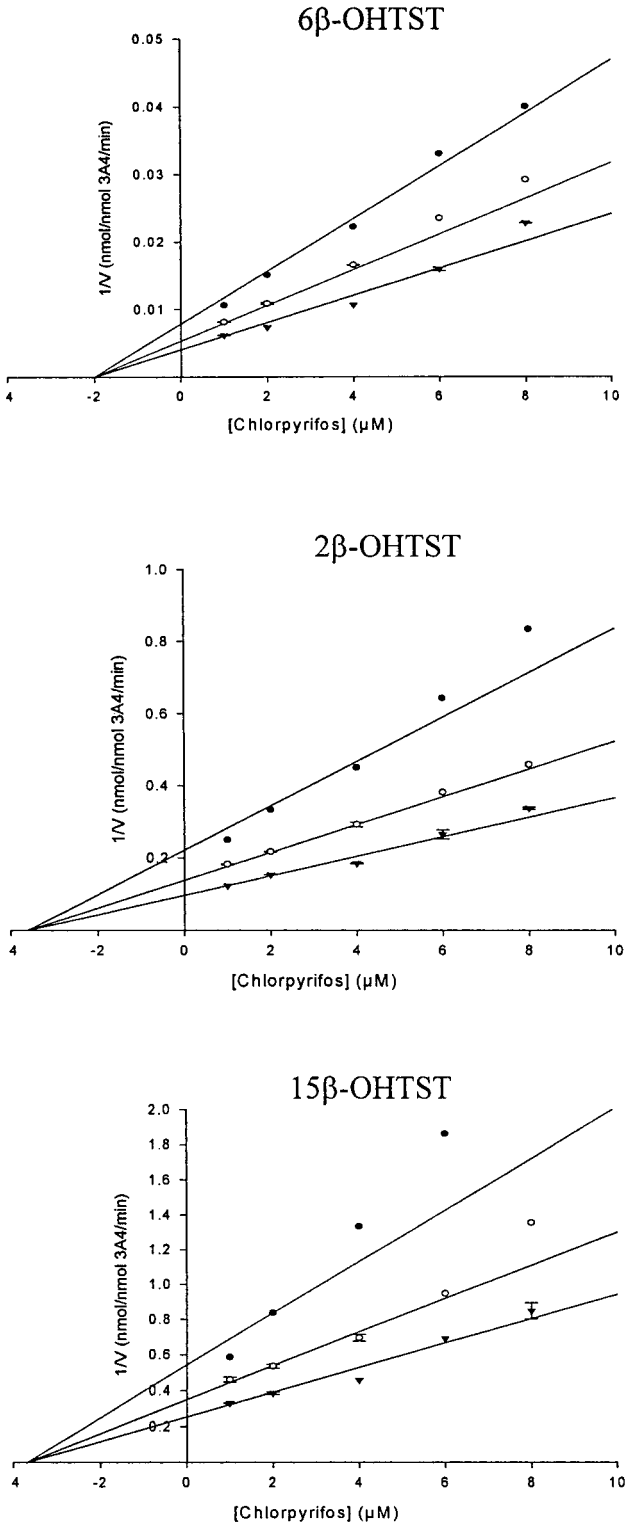

FIG. 5. *Representative Dixon plots for the inhibition of CYP3A4-mediated testosterone hydroxylation by chlorpyrifos.*

Testosterone concentrations were 50 μM (●), 100 μM (○), and 200 μM (▼).

metabolites. Chlorpyrifos, fonofos, and phorate inhibited major TST metabolites noncompetitively and irreversibly, and it is clear that organophosphorus compounds are some of the most potent inhibitors of the CYP3A4-dependent oxidation of TST yet described. Organophosphorus pesticides, such as chlorpyrifos, phorate, and fonofos are activated by a P450-catalyzed desulfuration reaction (Fukuto, 1990). The sulfur atom released from these pesticides in this reaction is highly reactive and is believed to bind immediately to the heme iron of P450 and inhibit its activity (Neal, 1980). On the other hand, enzyme stimulation is a process by which direct addition of one chemical to an enzyme stimulates the rate of reaction of the substrate (Guengerich, 1997). Our data indicated that some compounds, such as pyridostigmine bromide, DEET, chlorpyrifos-oxon, phorate, imidacloprid, and deltamethrin may stimulate the production of some of the TST metabolites.

Several studies, including this, have shown that CYP3A4 is the major P450 involved in the metabolism of TST in human liver microsomes (Waxman et al., 1988, 1991; Yamazaki and Shimada, 1997). Either inhibition or induction can modulate the activity of an enzyme; P450s may exhibit stimulation or inhibition in the presence of certain xenobiotic compounds (Guengerich, 1997; Szklarz and Halpert, 1998). It has been suggested that CYP3A4 is an allosteric enzyme, even though the identity of the allosteric site is not known (Shimada and Guengerich 1989; Lee et al., 1995). In addition, little is known about the active site topology of CYP3A4, although it is generally recognized that the active site of this enzyme has the capacity to accommodate large molecules and even more than one substrate (Shou et al., 1994). Inhibition may, in some interactions, be more serious than enzyme induction since inhibition happens more rapidly, not taking time to develop, as with induction (Guengerich, 1997). Preincubation of CYP3A4 with chlorpyrifos resulted in almost complete inhibition of major TST metabolites. The K_i value indicated that chlorpyrifos is one of the most potent inhibitors yet shown for the production of major TST metabolites. This inhibition was not due to inhibition by the metabolite, chlorpyrifos-oxon, since the latter had no inhibitory effect on the production of the major TST metabolites. Phorate and fonofos also inhibited the production of major and some minor metabolites of TST. The K_i value indicated that fonofos was a much better inhibitor of major TST metabolites than phorate. The possibility exists that inhibition of CYP3A4 may lead to higher levels of TST and may alter hormonal properties. However, in vivo studies are necessary to understand the impact of these changes. Preincubation with pyridostigmine bromide resulted in higher production of 6β- and 2β-OHTST, suggesting stimulation of CYP3A4. Preincubation with chlorpyrifos-oxon, phorate, and fonofos with CYP3A4 also resulted in activation of the production of some TST metabolites. A number of in vivo studies in rodents have shown that organochlorine pesticides increased the overall rate of TST metabolism (Cassidy et al., 1994; Wilson and LeBlanc, 1998; Dai et al., 2001).

Several studies have demonstrated that simultaneous expression of CYP3A4 and P450 reductase in bacterial or baculovirus-based insect cell membranes can produce high catalytic activity for TST 6β-OHTST in the absence of b_5 (Guengerich and Johnson 1997; Shaw et al., 1997), although addition of b_5 to the system can enhance the reaction rates (Yamazaki et al., 1999). In contrast to the CYP3A4 used in these experiments, cytochrome b_5 was not coexpressed in CYP3A5. A comparison of CYP3A5 with and without the addition of exogenous b_5 demonstrated a 2-fold increase in the activity of 6β- and 2β-OHTST in the presence of b_5.

Human aromatase (CYP19), an extrahepatic P450, catalyzes the conversion of TST via three hydroxylation steps to estradiol. Inhibitors of aromatase currently in use have received considerable attention as treatments for postmenopausal breast cancer and other estrogen-dependent diseases (Bordie et al., 1999). Endocrine disruptors are hormone mimics that modify hormonal action in humans. Currently, inhibitors of human aromatase have been identified as potential endocrine disruptors or environmental toxicants (Mak et al., 1999). The chemicals used in this study have no significant effect on the activity of aromatase.

In conclusion, the hydroxylation of TST by P450 isoforms indicates important functions for these enzymes other than detoxification of xenobiotics. The present study provided further insight into the range of TST hydroxylation reactions that can be catalyzed by different human P450 isoforms. The deployment-related chemicals used in this study, including pesticides, caused a marked modification of P450-mediated TST metabolism in vitro. Organophosphorus pesticides were very potent inhibitors of the production of the primary metabolites of CYP3A4 and inhibited major TST metabolites noncompetitively and irreversibly. Addition of b_5 to CYP3A5 increased the catalytic activity of this enzyme. Preincubation of the test chemicals had no effect on the production of estradiol from TST.

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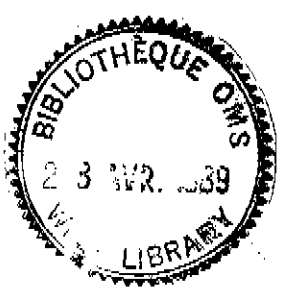
ORIGINAL: ENGLISH

Distr.: Limited

DATA SHEET ON PESTICIDES

No. 75

PHORATE



It must be noted that the issue of a Data Sheet for a particular pesticide does not imply endorsement of the pesticide by WHO or FAO for any particular use, or exclude its use for other purposes not stated. While the information provided is believed to be accurate according to data available at the time when the sheet was compiled, neither WHO nor FAO are responsible for any errors or omissions, or any consequences therefrom.

CLASSIFICATION:

Primary use: Insecticide

Secondary use: Acaricide, nematocide

Chemical group: Organophosphorus compound

Date issued: July 1988

1.0 GENERAL INFORMATION

1.1 COMMON NAME: phorate (E-ISO, F-ISO, BSI ANSI, ESA), timet (U.S.S.R.)

1.1.1 Identity:

IUPAC: O,O-diethyl S-ethylthiomethyl phosphorodithioate

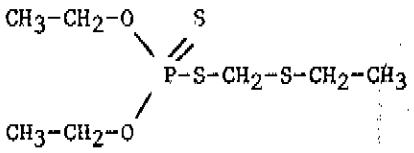
CAS: O,O-diethyl S-[(ethylthio)methyl] phosphorodithioate

CAS Reg. No.: 298-02-2

Molecular formula: $C_7H_{17}O_2PS_3$

Relative molecular mass: 260.4

Structural formula:



1.1.2 Synonyms: AC 3911, Agrimet^R, CL 35024, EI 3911, ENT 24 042, foraat, Geomet^R, Granutox^R, L 11/6, phorat, Rampart^R, Thimenox^R, Thimet^R, timet, Vergfru Foratox^R.

1.2 SYNOPSIS: Phorate is a broad spectrum, non-biocumulative organophosphorus insecticide and acaricide, an indirect inhibitor of cholinesterase with good contact, stomach and fumigant action against target organisms. It is extremely toxic to mammals and other non-target organisms. It is a plant systemic with no residual action. Granular formulations are most commonly used. The sulphone metabolite may persist in soil, and relatively long pre-harvest intervals are recommended.

1.3 SELECTED PROPERTIES

1.3.1 Physical characteristics: Phorate is a clear, pale yellow mobile liquid. It has a boiling point of 118-120 °C (107 Pa), and a freezing point of -42.9 °C; a density (d^{25}) of 1.167. The technical material is over 90% pure.

1.3.2 Solubility: Water, 50 mg/L at 25 °C; miscible with carbon tetrachloride, dioxane, vegetable oils, xylene, alcohols, ethers and esters.

1.3.3 Stability: Phorate is stable for at least two years at room temperature in media between pH 5 and 7. In very acidic (pH <2) or very alkaline (pH >9) media, hydrolysis occurs at rates dependent upon pH and temperature.

1.3.4 Vapour pressure: 85 mPa (25 °C)

1.4 AGRICULTURE, HORTICULTURE AND FORESTRY

1.4.1 Common formulations: Emulsifiable concentrates of various concentrations including 960 g tech./L and 250 g a.i./L are still available, however, granular formulations containing 50, 100, 150, 200 g a.i./kg are almost universally used.

1.4.2 Susceptible pests: Mites, aphids, greenbugs, thrips, leafhoppers, sorghum shootfly, leafminers, corn rootworms, psyllids, cutworms, Hessian fly, foliar nematodes, wireworms, flea beetles, whiteflies, pine tip moth, and others.

1.4.3 Use pattern: Phorate may be used in foliar or soil treatments on alfalfa, barley, beans, brassicas, coffee, corn, cotton, grapes, hops, lettuce, oats, peanuts, potatoes, rice, sorghum, soybeans, sugar cane, sugar beets, tomatoes, watermelon, wheat and on ornamentals and pine nursery stock.

Soil applications may be applied as a band treatment on each side of the seed row or incorporated into the soil as a side dress, irrigation should follow as soon as possible. Care should be taken to avoid contact with seed in the furrow. In foliar treatments apply under dry conditions into plant crowns as insects appear.

1.4.4 Unintended effects: Phorate is toxic to many seeds and to many non-target organisms, including bees and fish. Contamination of standing water and waterways must be avoided. Good agricultural practices and the use of granular formulations diminish these adverse effects.

1.5 PUBLIC HEALTH USE: No recommended use.

1.6 HOUSEHOLD USE: No recommended use.

2.0 TOXICOLOGY AND RISKS

2.1 TOXICOLOGY - MAMMALS

2.1.1 Absorption route: Phorate may be absorbed from the gastrointestinal tract, through the intact skin and by inhalation of spray mists or fine dust.

2.1.2 **Mode of action:** Several metabolites of phorate inhibit the activity of both acetylcholinesterase and pseudocholinesterase.

2.1.3 **Metabolism and excretion products:** Phorate is metabolized in animals to yield phorate sulphoxide and phorate sulphone and the oxygentated analogues (phoratoxon, phoratoxone sulphoxide and phoratoxone sulphone) which are excreted as diethyl phosphoric acid, O-O-diethyl phosphorothioic and O-O-diethylphosphorodithioic acid.

Oral administration of a single dose of 2 mg/kg of labelled phorate to rats resulted in 35% of material being excreted in urine and 3.5% in the faeces, within six days.

Six daily doses of 1 mg/kg/day of phorate to rats resulted in 12% and 6% being excreted in the urine and faeces respectively within seven days. At necropsy brain, liver and kidney tissue contained unidentified and largely unextractable residues.

2.1.4 **Toxicity, single dose** (technical material)

Oral LD_{50}:

Rat (M)	2.3-3.2 mg/kg b.w.
Rat (F)	1.1-1.6 mg/kg b.w.
Mouse (M,F)	3.5-6.5 mg/kg b.w.

Dermal LD_{50}:

Rat (M)	5.7-9.3 mg/kg b.w.
Rat (F)	2.5-3.9 mg/kg b.w.
Rabbit	5.2 mg/kg b.w.

Inhalation LC_{50}:

Rat (M)	60 mg/m^3 (1 hour)
Rat (F)	11 mg/m^3 (1 hour)

I.V. LD_{50}:

Rat (M)	2.2 mg/kg b.w.
Rat (F)	1.2 mg/kg b.w.

I.P. LD_{50}:

Rat	1.98 mg/kg b.w.
Mouse	3.0 mg/kg b.w.

2.1.5 **Toxicity, repeated doses**

Oral: Mongrel dogs given 0.05 mg/kg/day for 15 weeks had significantly depressed plasma and erythrocyte cholinesterase activity. At 2.5 mg/kg two dogs died following a single dose.

2.1.6 Dietary studies

Short term: The plasma, erythrocyte and brain cholinesterase activity of rats fed on a diet containing phorate at levels greater than 0.66 ppm was depressed. In rats fed on a diet containing up to 6 ppm of phorate no effect on growth, food consumption, or histopathology was noticed.

Long term: In a two year rat dietary study at dose levels of 1, 3 or 6 ppm, growth was depressed at the highest dose level, among females only, and only 36% of animals in this group survived terminally. Erythrocyte cholinesterase activity was not affected at any dose level whereas plasma cholinesterase activity was depressed at 6 ppm in males and at 3 and 6 ppm in females.

In an 18 month study in mice fed on a diet containing phorate 1, 3 or 6 ppm, growth was retarded in females at 6 ppm. However, in all dose groups food aversion was observed for both sexes. There were no observed compound related adverse effects other than clinical signs of cholinesterase inhibition.

2.1.7 Supplementary studies of toxicity

Carcinogenicity: Results of rat and mouse chronic toxicity studies have not demonstrated any carcinogenic potential for phorate.

Mutagenicity: Phorate was not mutagenic in a dominant lethal test in mice and in several microbial systems including Salmonella typhimurium (reverse mutation), Saccharomyces cerevisiae (mitotic recombination) and in several Ames tests with E. coli and B. subtilis.

Teratogenicity: In a rat study phorate was not observed to be teratogenic at oral doses up to 0.25 mg/kg/day. At 0.5 mg/kg/day (high dose level), an increased incidence of hypertrophy of the heart was observed. In another rat study, exposure to phorate by inhalation at the dose of 1.94 mg/m^3/day, during the seventh through fourteenth day of gestation, caused increased foetal mortality and decreased foetal weight gain but teratogenic effects were not observed.

Reproduction: In a three generation study in mice, with phorate levels in diet up to 3 ppm, no compound related effects on the fertility, gestation, viability or lactation indices were observed.

Neurotoxicity: Phorate produced no adverse effects on nerve fibres or the myelin sheath in hens fed dietary levels of 40 ppm for four weeks. In a separate study no delayed neurotoxicity was noted in hens given phorate as a single dose of 14.2 mg/kg.

2.1.8 Modification of toxicity: No potentiation of toxicity was observed in male rats treated with equitoxic portions of phorate and each of 10 other pesticides.

2.2 TOXICOLOGY - MAN

2.2.1 Absorption route: Phorate may be absorbed from the gastrointestinal tract, through the intact skin, or by inhalation of dust.

2.2.2 Dangerous doses

Single: 5 mg/kg b.w.

Repeated: Not known

2.2.3 Observations in occupationally exposed workers: There have been no reports of fatalities associated with the use of phorate. A 16 year old youth became ill after working with phorate treated cotton seed. Symptoms included coma, undetectable blood pressure, pinpoint pupils, blood tinged frothy sputum and occasional convulsions. One day after onset, erythrocyte and plasma cholinesterase activities were 21% and 49% of normal respectively. Two illnesses occurred in the same formulating plant where phorate concentrations in the air were measured at 0.07-14.60 mg/m^3. In another incident, a formulator experienced neurological symptoms following exposure to phorate while cleaning a mixing tank. This was accompanied by a 50% reduction in plasma and erythrocyte cholinesterase and increased urinary levels of diethyl phosphate, a metabolite of phorate.

2.2.4 Observations on exposures of the general population: No published information available. However, when recommended agricultural practices are followed no adverse effects are expected.

2.2.5 Observations on volunteers: No published information available.

2.2.6 Reported mishaps: No published information available.

2.3 TOXICOLOGY TO NON MAMMALIAN SPECIES

2.3.1 Birds

Oral LD_{50} (technical material):

Mallard (F)	0.62-2.55 mg/kg b.w.
Pheasant (F)	7.12 mg/kg b.w.
Chukar (F)	12.8 mg/kg b.w.
Red-winged blackbird	1.00 mg/kg b.w.
Starlings	7.5 mg/kg b.w.
Grackles	1.30 mg/kg b.w.

Dermal LD_{50} (technical material):

Mallard (F)	203 mg/kg b.w.

Dietary LD_{50} (5 day):

Bobwhile quail	373 ppm
Japanese quail	200 ppm
Ring-necked pheasant	441 ppm
Mallard	248 ppm

2.3.2 Fish: Highly toxic to fish

Bluegill TLM	5.5 µg/L (48 hour)	technical material
Rainbow trout LC_{50}	13.0 µg/L (96 hour)	technical material
Channel catfish LC_{50}	280.0 µg/L (96 hour)	technical material

2.3.3 Other species: Highly toxic to bees

Oral LD_{50}:

Bullfrog (F)	85.2 mg/kg b.w. (technical material)

3.0 FOR REGULATORY AUTHORITIES - RECOMMENDATIONS ON REGULATION OF COMPOUND

3.1 RECOMMENDED RESTRICTIONS ON AVAILABILITY

(For definition of categories see the Introduction to Data Sheets.)

Liquid formulation of 10% and over, Category 1.

Other liquid formulations, Category 2.

Solid formulations of 40% and over, Category 1.

Other solid formulations, 4-40%, Category 2, less than 4%, Category 3.

3.2 TRANSPORTATION AND STORAGE

All formulations: Phorate should be transported and stored in clearly labelled impermeable containers under lock and key, secure from access by children and other unauthorized persons. No food or drink should be stored in the same compartment.

3.3 HANDLING

All formulations: Full protective clothing (see 4.3) should be used by those handling the compound. Adequate washing facilities should be available at all times during the handling and should be close to the site of handling. Eating, drinking and smoking should be prohibited during handling and prior to washing after handling.

3.4 DISPOSAL AND/OR DECONTAMINATION OF CONTAINERS

All formulations: Container must first be decontaminated and then crushed and buried below topsoil. Care must be taken to avoid subsequent contamination of water sources. Decontamination of containers in order to use them for other purposes should not be permitted.

3.5 SELECTION, TRAINING AND MEDICAL SUPERVISION OF WORKERS

All formulations: Pre-employment medical examination of workers is necessary. Workers suffering from active hepatic or renal disease should be excluded from contact. Pre-employment and periodic blood cholinesterase tests for workers is desirable. Special account should be taken of the worker's mental ability to comprehend and follow instructions. Training of workers in techniques to avoid contact is essential.

3.6 ADDITIONAL REGULATIONS RECOMMENDED IF DISTRIBUTED BY AIRCRAFT

All formulations: Not recommended for aerial application.

3.7 LABELLING

All formulations

"DANGER - POISON"
(skull and cross-bones insignia)

Phorate is an organophosphorus compound which inhibits cholinesterase. It is extremely toxic. Contact with the skin, inhalation of dust or spray, or swallowing may be fatal. Wear protective gloves, clean protective clothing, and a respirator of the organic-vapour type when handling this material. Wash immediately after work. Ensure that containers are stored under lock and key. Empty containers must be disposed of in such a way as to prevent all possibility of accidental contact with them. Keep the material out of reach of children and well away from foodstuffs, animal feed and their containers.

In case of contact, immediately remove contaminated clothing and wash the skin thoroughly with soap and water; for eyes, flush with water for 15 minutes.

If poisoning occurs, call a physician. Atropine sulfate is the principal antidote, repeated doses may be necessary. Pralidoxime chloride (2-PAM or protopam chloride) may be effective as an adjunct to atropine treatment. Artificial respiration also may be needed.

3.8 RESIDUES IN FOOD

Maximum residue levels: Maximum residue levels were estimated by the Joint FAO/WHO Meeting on Pesticide Residues in 1984 for several commodities as temporary.

4.0 PREVENTION OF POISONING IN MAN AND EMERGENCY AID

4.1 PRECAUTIONS IN USE

4.1.1 General: Phorate is an extremely toxic organophosphorus pesticide. It penetrates the intact skin and is also absorbed by inhalation and from the gastrointestinal tract. Repeated exposure may have a cumulative effect on the cholinesterase activity. Most formulations should be handled by trained personnel wearing protective clothing.

4.1.2 Manufacture and formulation - T.L.V.: 0.05 mg/m^3 (TWA); 0.2 mg/m^3 (STEL) for skin absorption. Closed systems and forced ventilation may be required to reduce, as much as possible, the exposure of workers to the chemical.

4.1.3 Mixers and applicators: When opening the container and when mixing, protective impermeable boots, clean overalls, gloves and respirator should be worn. Mixing, if not mechanical, should always be carried out with a paddle of appropriate length. When spraying all crops a face mask should be worn, as well as an impermeable hat, clothing, boots and gloves. The applicator should avoid working in spray mist and avoid contact with the mouth. Particular care is needed when equipment is being washed after use. All protective clothing should be washed immediately after use, including the insides of gloves. Splashes must be washed immediately from the skin, or eyes, with large quantities of water. Before eating, drinking, smoking, hands and other exposed skin should be washed.

4.1.4 Other associated workers: Persons exposed to the compound and associated with its application should wear protective clothing and observe the precautions described above in 4.1.3 under "mixers and applicators".

4.1.5 Other populations likely to be affected: Respecting good agricultural practice, subject to 4.2 below, other persons should not be exposed to hazardous amounts of the compound.

4.2 ENTRY OF PERSONS INTO TREATED AREA

Unprotected persons should be kept out of tall crops for four days and out of other crops for 24 hours.

4.3 DECONTAMINATION OF SPILLAGE AND CONTAINERS

Residues in containers should be emptied in a diluted form into a shallow pit, taking care to avoid contamination of ground waters. The empty container may be decontaminated prior to disposal by rinsing two or three times with water and scrubbing the sides. Impermeable gauntlets should be worn during this work, and a soakage pit should be provided for the rinsings. Decontamination of containers in order to use them for other purposes including storage of food and drink should not be permitted. Spillage of the compound and its formulations should be removed by washing with 5% sodium hydroxide solution and then rinsing with large quantities of water.

4.4 EMERGENCY AID

4.4.1 Early symptoms of poisoning: Early symptoms of poisoning may include tightness of chest, non-reactive pinpoint pupils, excessive sweating, headache, weakness, giddiness, nausea, vomiting, hypersalivation, diarrhoea, abdominal pains, blurred vision, slurred speech and muscle twitching. Advanced symptoms are: convulsions, coma, loss of reflexes and loss of sphincter control.

4.4.2 Treatment before person is seen by a physician, if these symptoms appear following exposure: The person should stop work immediately, remove contaminated clothing and wash the affected skin with soap and water and flush the area with large quantities of water. If swallowed and if the person is conscious, vomiting should be induced. In the event of collapse, artificial respiration should be given, bearing in mind that if mouth-to-mouth respiration is used, vomit may contain hazardous amounts of the pesticide. Call a physician immediately and transport the patient to the nearest medical facility if necessary.

5.0 FOR MEDICAL AND LABORATORY PERSONNEL

5.1 MEDICAL DIAGNOSIS AND TREATMENT IN CASES OF POISONING

5.1.1 General information: Phorate, an organophosphorus pesticide, is extremely toxic to mammals. It is readily absorbed from the gastrointestinal tract, through the intact skin and by inhalation. It is converted *in vivo* to the oxygen analogue which inhibits cholinesterase. It does not accumulate in body tissues.

5.1.2 Symptoms and signs: Initial symptoms of poisoning may include tightness of chest, non-reactive pinpoint pupils, excessive sweating, headache, weakness, giddiness, nausea, hypersalivation, vomiting, diarrhoea, abdominal pains, blurred vision, slurred speech and muscle twitching. More advanced symptoms of poisoning may be convulsions, coma, loss of reflexes and loss of sphincter control.

5.1.3 Laboratory: The most important finding is reduction of activity of blood cholinesterases. Urinary levels of organic phosphorus containing metabolites may also be used as a measure of exposure. Neither method is specific for the compound.

5.1.4 Treatment: If the pesticide has been ingested, unless the patient is vomiting, rapid gastric lavage should be performed using 5% sodium bicarbonate, if available. In case of skin contact, the skin should be washed with soap and water. If the compound has entered the eyes, they should be washed with large quantities of isotonic saline or water.

Persons without signs of respiratory impairment but with manifest peripheral symptoms should be treated with 2-4 mg of atropine sulfate by intravenous or intramuscular injection and 1 000 mg of pralidoxime chloride or 250 mg of toxogonin (adult dose) by slow intravenous infusion. More atropine may be given as needed. Persons with severe intoxication, with respiratory difficulties, convulsions and unconsciousness should immediately be given ventilatory support followed by atropine and a reactivator. In such severe cases 4-6 mg of atropine sulfate should be given initially followed by repeated doses of 2 mg at 5-10 minute intervals. Diazepam may be given to control convulsions which are unresponsive to atropine and pralidoxime. The patient's condition including respiration, blood pressure, pulse frequency, salivation and convulsions should be carefully observed as a guide to further administration of atropine. If the patient is cyanotic artificial respiration should be given at the same time as atropine sulfate. The airways should be kept free and artificial respiration should be applied if required, preferably by mechanical means. If necessary, intubation should be performed.

Contraindications are morphine, aminophylline, phenothiazines, reserpine, furosemide or ethacrynic acid.

Pralidoxime and toxogonin alone are not regarded as effective antidotes in organophosphorus poisoning, but may be effective as an adjunct to atropine.

5.1.5 **Prognosis**: If the acute poisoning episode is survived and if needed adequate artificial respiration has been given the chances of complete recovery are good. However, in very severe cases, particularly if artificial respiration has been inadequate, prolonged anoxia may give rise to permanent brain damage.

5.1.6 **References of previously reported cases**: Phorate has been implicated in a number of cases of pesticide poisoning.

Brokopp, C.D., Wyatt, J. L and Gabica, J., Dialkyl Phosphates in Urine Samples from Pesticide Formulators to Disulfoton and Phorate. (1981), Bull. Environ. Contam. Toxicol., 26, 524-529.

Hayes, W. J. (1982), "Pesticides Studied in Man" p. 360, Williams and Wilkins, Baltimmore, USA

Young, R. J., Jung, F. P. and Ayer H. E., Phorate Intoxication at an Insecticide Formulating Plant. (1979), Amer. Indust. Hyg. Assoc. 40, 1 013-1 016.

5.2 SURVEILLANCE TESTS

Test	Normal level*	Action level*	Symptomatic level*
Plasma cholinesterase	100%	50%	variable
Whole blood or erythrocyte cholinesterase	100%	70%	usually 40%

5.3 LABORATORY METHODS

5.3.1 **Detection and assay of compound**: Product analysis is by I.R. spectroscopy. Residues of phorate and its oxidation products may be determined by GLC.

Boshoff, P.R. and Pretorius, V., (1979), J. Agric. Food Chem. 27, 626-630.

Boyd, J.G. (1972), Anal. Methods Pestic. Plant Growth Regul. Food Addit., 6, 493-510.

Brokopp, C.D., Wyatt, J. L and Gabica, J., (1981), Bull. Environ. Contam. Toxicol., 26, 524-529.

Carson, L.J. (1981), J. Assoc. Off. Anal. Chem., 64, 714-719.

Mount, M.E. and Oehme F. W. (1981), Vet. Hum. Toxicol., 23, 34-42.

Sans, W.W. (1978), Assoc. Off. Anal. Chem., 61, 837-840.

Stan, A.J., Abraham, B., June, J., Kellert, M., Steinland, K. (1977), Fresenius Z. Anal. Chem., 287, 271-285.

* Expressed as a percentage of pre-exposure activity.

The toxicological effects of phorate are associated with inhibition of acetylcholinesterase activity. Inhibition of acetylcholinesterase activity and clinical signs occurred at similar doses in rats, rabbits and dogs, while mice appeared to be somewhat less sensitive. The NOAELs for toxicologically significant inhibition of brain acetylcholinesterase activity were 0.05–0.07 mg/kg bw per day in 13-week and 2-year studies in rats and in 1-year studies in dogs. The NOAELs for clinical signs were generally higher. The Meeting noted that the dose–response curve for acetylcholinesterase inhibition is steep.

In an 18-month study in mice and in a 24-month study in rats, phorate did not increase the incidence of tumours or cause any non-neoplastic effects other than clinical signs secondary to inhibition of acetylcholinesterase activity.

Phorate was tested for genotoxicity in vitro and in vivo in an adequate battery of assays. In view of the lack of genotoxicity in vitro and in vivo and on the basis of the results of studies of carcinogenicity in rodents, the Meeting concluded that phorate is not likely to pose a carcinogenic risk to humans.

In a multigeneration study of reproductive toxicity in mice, the NOAEL was 1.5 ppm (equal to 0.30 mg/kg bw per day) on the basis of slightly reduced lactation indices in four out of the six litters at 3 ppm (equal to 0.60 mg/kg bw per day).

In a two-generation study of reproductive toxicity in rats, phorate showed effects on pup growth and mortality at maternally toxic doses. The NOAEL was 2 ppm (equal to 0.17 mg/kg bw per day) on the basis of decreased brain acetylcholinesterase activity, decreased parental and pup body weights and decreased pup survival at 4 ppm (equal to 0.35 mg/kg bw per day).

In a study of developmental toxicity in rats, the NOAELs for maternal and developmental toxicity with phorate were 0.3 mg/kg bw per day on the basis of mortality, cholinergic signs of toxicity, significantly decreased body weights and food consumption in the dams, decreased fetal body weights and delays in skeletal ossification at 0.4 mg/kg bw per day. No fetal malformations were produced, even at the lethal dose (0.4 mg/kg bw per day), the highest dose tested. The Meeting concluded that phorate is not teratogenic in rats.

Phorate was not embryotoxic, fetotoxic or teratogenic in rabbits at doses of up to and including 1.2 mg/kg bw per day, a dose that produced severe maternal toxicity. The NOAEL for maternal toxicity with phorate was 0.15 mg/kg bw per day on the basis of mortality observed at 0.5 mg/kg bw per day. The NOAEL for developmental toxicity was 1.2 mg/kg bw per day, the highest dose tested.

The Meeting concluded that the existing database on phorate was adequate to characterize the potential hazards to fetuses, infants and children.

In a study of acute neurotoxicity in rats treated by gavage, phorate at a dose of 1 mg/kg bw caused miosis in 2 out of 20 males and 5 out of 20 females, tremors in 2 out of 20 females, fasciculations, slightly impaired locomotion and splayed or dragging hindlimbs in one female and significant inhibition of brain and erythrocyte acetylcholinesterase activity in females (65%), but not in males (14–21%). No histopathological signs were observed. At 0.5 mg/kg bw, miosis was observed in 2 out of 20 males and 2 out of 20 females. Although miosis was observed in a small number of animals (and in 1 out of 20 controls) in the absence of inhibition of erythrocyte and brain acetylcholinesterase activity, it could not be dismissed as a compound-related effect. The NOAEL was 0.25 mg/kg bw on the basis of miosis.

Phorate did not cause acute delayed neurotoxicity in hens. Although measurements of neuropathy target esterase were not carried out, the Meeting noted that the dose used (approximately equal to the LD_{50}) was sufficiently high to indicate that dietary exposure to phorate would not cause delayed polyneuropathy.

The mammalian and plant metabolites of phorate, phorate sulfone and phorate sulfoxide, had similar toxicity to the parent compound. In rats, the oral LD_{50} values for these metabolites were 1.2–3.5 and 2.2–2.6 mg/kg bw, respectively. The NOAELs for inhibition of brain acetylcholinesterase activity were 0.80 ppm (equal to 0.08 and 0.06 mg/kg bw per day) for phorate sulfone and sulfoxide, respectively, in 90-day studies in rats.

Several cases of occupational and non-occupational poisoning in humans have been reported. The subjects showed typical cholinergic symptoms, including gastrointestinal effects, bradycardia and neurological effects (headache, giddiness, fatigue). Skin and eye irritation were also observed.

Toxicological evaluation

An ADI of 0–0.0007 mg/kg bw was established on the basis of a overall NOAEL of 0.07 mg/kg bw per day for inhibition of brain acetylcholinesterase activity in rats and dogs and a safety factor of 100. This ADI includes the phorate metabolites, phorate sulfone and phorate sulfoxide.

An ARfD of 0.003 mg/kg bw was also established based on the NOAEL of 0.25 mg/kg bw for miosis in the study with single doses in rats. Although inhibition of acetylcholinesterase activity is a C_{max}-dependent phenomenon, a safety factor of 100 was used in view of the steep dose–response curve and the slow recovery of brain acetylcholinesterase activity because of irreversibility of its inhibition. This ARfD includes the metabolites of phorate, phorate sulfone and phorate sulfoxide.

A toxicological monograph was prepared.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	18-month study of toxicity and carcinogenicity[a]	Toxicity	3 ppm, equivalent to 0.45 mg/kg bw per day	6 ppm, equivalent to 0.90 mg/kg bw per day
		Carcinogenicity	6 ppm, equal to 0.90 mg/kg bw per day[d]	—
	Multigeneration study of reproductive toxicity[a]	Parental and offspring toxicity	1.5 ppm, equal to 0.30 mg/kg bw per day	3 ppm, equal to 0.60 mg/kg bw per day
Rat	2-year study of toxicity and carcinogenicity[a]	Toxicity	1 ppm, equal to 0.05 mg/kg bw per day	3 ppm, equal to 0.16 mg/kg bw per day
		Carcinogenicity	6 ppm, equal to 0.32 mg/kg bw per day[c, d]	—
	Multigeneration reproductive toxicity[a]	Parental and offspring toxicity	2 ppm, equal to 0.17 mg/kg bw per day	4 ppm, equal to 0.35 mg/kg bw per day
	Developmental toxicity[a]	Embryo- and fetotoxicity and maternal toxicity	0.3 mg/kg bw per day	0.40 mg/kg bw per day
	Single-dose study[c]	Miosis	0.25 mg/kg bw	0.50 mg/kg bw per day
	13-week study of neurotoxicity[a]	Neurotoxicity	0.07 mg/kg bw per day	0.3 mg/kg bw per day
Rabbit	Developmental toxicity[a]	Maternal toxicity	0.15 mg/kg bw per day	0.50 mg/kg bw per day
		Embryo- and fetotoxicity[a]	1.2 mg/kg bw per day[d]	—
Dog	1-year study of toxicity[b]	Toxicity	0.05 mg/kg bw per day	0.25 mg/kg bw per day

[a] Diet
[b] Capsules
[c] Gavage
[d] Highest dose tested

Estimate of acceptable daily intake for humans

0–0.0007 mg/kg bw

Estimate of acute reference dose

0.003 mg/kg bw

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

Further observation in humans

Critical end-points for setting guidance values for exposure to phorate

Absorption, distribution, excretion and metabolism in animals	
Rate and extent of oral absorption	Rapid, approximately 90% within 24 h
Dermal absorption	Extensive based on acute toxicity
Distribution	Rapid and extensive
Potential for accumulation	None
Rate and extent of excretion	89% within 24 h; urinary excretion predominated (77%); faecal excretion (12%)
Metabolism in animals	Major pathway: cleavage of phosphorus–sulfur bond, methylation of the liberated thiol group and oxidation of the resulting divalent moiety to the sulfoxide and sulfone
Toxicologically significant compounds (plants, animals and the environment)	Parent, phorate sulfoxide and phorate sulfone
Acute toxicity	
Rat, LD_{50}, oral	3.7 mg/kg bw in males, 1.4 mg/kg bw in females
Rat, LD_{50}, dermal	9.3 mg/kg bw in males, 3.9 mg/kg bw in females
Rat, LC_{50}, inhalation	0.06 mg/l of air in males (1-h), 0.011 mg/l of air (1-h) in females
Rabbit, skin irritation	Highly toxic by skin contact — could not be tested
Rabbit, eye irritation	Highly toxic by eye contact — could not be tested
Skin sensitization	Highly toxic by skin contact — could not be tested
Short-term studies of toxicity	
Target/critical effect	Brain and erythrocyte acetylcholinesterase activity and miosis (rats)
Lowest relevant oral NOAEL	0.07 mg/kg bw per day
Lowest relevant dermal NOAEL	No data
Lowest relevant inhalation NOAEC	No data
Genotoxicity	Negative results in vivo and in vitro
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Inhibition of erythrocyte and brain cholinesterase activity
Lowest relevant NOAEL	0.07 mg/kg per day (rat)
Carcinogenicity	Not carcinogenic in mice and rats
Reproductive toxicity	
Reproduction target/critical effect	Reduced pup growth at maternally toxic dose
Lowest relevant reproductive NOAEL	2 ppm, equivalent to 0.17 mg/kg bw per day
Developmental target/critical effect	Decreased pup weights and delayed ossification at maternally toxic doses (rats)
Lowest relevant developmental NOAEL	0.3 mg/kg bw per day (rats)
Neurotoxicity/delayed neurotoxicity	
Single dose study of neurotoxicity	
Target/critical effect	Signs consistent with acetylcholinesterase inhibition; no neuropathological effects
Relevant NOAEL	0.25 mg/kg bw
Delayed neuropathy	No delayed neurotoxicity in hens

Medical data	Findings consistent with inhibition of acetylcholinesterase activity; no record of permanent sequelae

Summary	*Value*	*Study*	*Safety factor*
ADI	0–0.0007 mg/kg bw	Rats and dogs, short- and long-term studies, inhibition of brain acetylcholinesterase activity	100
ARfD	0–0.003 mg/kg bw	Rats, single-dose study, miosis	100

DIETARY RISK ASSESSMENT

Long-term intake

The estimated theoretical maximum daily intakes in the five GEMS/Food regional diets, based on recommended MRLs, were in the range of 40–200% of the ADI (Annex 3). Further refinements of dietary intake estimates will be undertaken during the periodic review of phorate residues scheduled for 2005.

Short-term intake

The Meeting established an ARfD for phorate of 0.003 mg/kg bw but was unable to finalize the risk assessment before the residue evaluation, scheduled for 2005, had been completed.

4.21 PIRIMICARB (101)

TOXICOLOGY

Pirimicarb is the ISO approved common name for 2-dimethylamino-5,6-dimethylpyrimidin-4-yl dimethylcarbamate. It is a selective aphicide that is used extensively on a broad range of crops, including vegetable, cereal and orchard crops. The mode of action of pirimicarb is by inhibition of acetylcholinesterase activity.

Pirimicarb was evaluated by the JMPR in 1976, 1978 and 1982; an ADI of 0–0.02 mg.kg bw was established in 1983. Pirimicarb was reviewed by the present Meeting within the periodic review programme of CCPR, using new data not previously reviewed and relevant data from previous evaluations.

Kinetic studies in rats have demonstrated that pirimicarb administered orally to male and female rats is rapidly and extensively absorbed (> 70% of the administered dose) and widely distributed. Radioactivity from [^{14}C]pyrimidinyl-labelled pirimicarb was excreted predominantly in the urine, while radioactivity from [^{14}C]carbamoyl-labelled pirimicarb was excreted predominantly in expired air. Tissue retention of radioactivity was low. There were no pronounced sex differences in the routes or rates of excretion. Pirimicarb was extensively metabolized, giving rise to 24 metabolites, 17 of which were identified. The main metabolic pathway involves the loss of the carbamate moiety to produce a range of substituted hydroxypyrimidines, some of which are glucuronide conjugates.

The acute oral median LD_{50} for pirimicarb was 152 mg/kg bw in male rats and 142 mg/kg bw in female rats, while the acute dermal median LD_{50} of pirimicarb was > 2000 mg/kg in both male and female rats. The 4-h inhalation median LC_{50} of pirimicarb in rats was 0.948 and 0.858 mg/l of air in males and females respectively. Pirimicarb is not irritating to the rabbit eye or skin. It does, however, have skin sensitizing potential under the conditions of the Magnusson & Kligman maximization test.

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CLINICAL EFFECTS AND CHOLINESTERASE ACTIVITY CHANGES IN WORKERS EXPOSED TO PHORATE (THIMET)

Key words: Phorate, Formulators, Cholinesterase inhibition.

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ABSTRACT

Phorate (Thimet), an aliphatic derivative of phosphorus is a highly toxic insecticide. In order to implement the safety measures, the clinical manifestations and cholinesterase (ChE) activity were evaluated before and after 2 weeks of exposure to this insecticide in 40 male formulators.

The 2 week's exposure reveal signs and symptoms of toxicity in 60% of the formulators. Gastrointestinal symptoms and lowering of heart rate (bradycardia) were more prominent as compared to the neurological symptoms. A significant depression in plasma ChE activity was observed at the end of 1st week (55%) and 2nd week (71%) as compared to the respective pre-exposure values. A recovery up to 79% of the pre-exposure activity of this enzyme was noticed 10 days after cessation of the above exposure.

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INTRODUCTION

The use of organophosphorus insecticides is increasing due to their non-persistance which diminish the risk of environmental pollution by such compounds. Also the increase in the incidence of pest resistance to the hitherto more widely used organochlorine insecticides, this group of insecticides are put into use in greater quantities. Some of the organophosphates are highly toxic and are highly effective against pests but this property also requires proper hygienic precautions to be observed including the use of personal protective devices in order to combat the health nazards during their use, formulation and manufacture.

Phorate (O,O-diethyl S-(ethylthio) methyl phosphorodithioate) an alipnatic derivative of the phosphate group of insecticides is highly toxic with an oral LD_{50} in male rats of only 3.7 mg/kg and is used in a granular form in agriculture against sap feeding insects (Martin, 1971). This chemical like other organophospnorus insecticides is a cholinesterase inhibitor. Since the toxicity hazards from these highly toxic insecticides will depend on various factors of which the principal ones are, the characteristics of the exposed population and the intensity of exposure, an attempt was made in the

present study to evaluate the clinical manifestations of ChE enzyme depression in Phorate formulators after 2 weeks of exposure.

MATERIALS AND METHODS

Description of the Formulation Plant: The study was conducted in a plant formulating 10% Phorate granules from technical grade material. The plant was running in 3 shifts and the total production capacity of the plant was about 3 tonnes per day during the three shifts. The plant was running 7 days a week and each worker had to work for 8 hours per day.

Subjects: A group of 40 male workers between the ages of 19-45 years were selected for the study. These workers, in the past, were exposed to a combination of pesticides (organochlorines, organophosphates and carbamates) for periods ranging between 2-19 years. However, these workers had not been exposed to Phorate for at least one week before the start of the present study. The workers were then allowed to work in the Phorate formulation plant for 2 weeks.

Hygiene and Protective Devices Used: All the workers were maintaining the normal personal hygienic procedures like, washing of hands and face before and after taking the meals and at the end of the work shift. All the workers were provided with hand gloves, face mask and washed overalls everyday, before the

work which was discarded at the end of the work. Investigations: In all the workers, detailed personal history, occupational history, past episodes of poisoning and occurrence of signs and symptoms of toxicity were recorded in precoded performe. Each worker was clinically examined before and after 2 week period of Phorate exposure. In each case Electrocardiogram (ECG) was also recorded to detect any cardiac abnormality attributable to Phorate exposure.

Whole blood, plasma and RBC's ChE activity was determined by the method of Voss and Sachsse (1970) before, during (after 1st week) and after the 2nd week period of Phorate exposure. The ChE activity was also determined 10 days after the cessation of the 2 weeks' exposure in order to assess the recovery of ChE activity. The workers were not allowed to work with any cholinesterase inhibitor during the 10 days after the cessation of exposure.

RESULTS

It was observed that 60% of the formulators during 2 weeks of Phorate exposure showed toxicity signs and symptoms like neurological (headache, giddiness, fatigue etc.), gastrointestinal (nausea, vomiting, stomachache etc.), skin and eye (irritation, watering etc.) and bradycardia. Table I shows the frequency of prevalence of these toxicity symptoms. It was observed that the

TABLE I

Prevalence of Various Symptoms in Workers Exposed to Phorate

Symptoms	No. of subjects showing symptoms
Asymptomatic	16 (40.0%)
Symptomatic	24 (60.0%)
A. Neurological	
Headache	18 (45.0%)
Giddiness	15 (37.5%)
Easy fatigability	11 (27.5%)
B. Gastrointestinal	
Nausea	17 (42.5%)
Vomiting	14 (35.0%)
Stomachache	21 (52.0%)
C. Bradycardia	19 (48.0%)
D. Eyes	
Irritation	16 (40.0%)
Watering	06 (15.0%)

symptoms related to the gastrointestinal system were more pronounced as compared to those of other systems.

Table II shows the mean of whole blood, plasma and RBC's ChE activity before, during (after one week of exposure), at the end of 2 weeks of exposure and 10 days after cessation of exposure. A significant depression was observed in whole blood and plasma ChE after 1st and 2nd week of exposure as compared to the pre-exposure values. However, only the depression of plasma ChE activity persisted even after 10 days of cessation of exposure.

TABLE II

Pre and Post Exposure Whole Blood, Plasma and RBC's Cholinesterase Activity (Klett Units) in Workers Exposed to Phorate

Exposure	Cholinesterase activity (Mean + SD)		
	Whole blood	Plasma	RBC's
Pre-exposure	133.56±18.68	38.51±10.04	95.00±13.21
Pre-exposure			
- 1st week	119.70±15.26**	17.68±04.17**	102.03±14.37*
- 2nd week	115.23±20.05**	11.15±01.41**	104.39±19.45
- 10 days after cessation of exposure	135.86±16.67	30.56±10.31**	105.75±13.51*

* = $P < 0.05$; ** = $P < 0.01$.

A depression of 55% and 71% in mean plasma ChE activity after 1st and 2nd week of exposure respectively, was observed as compared to pre-exposure value. This depression was recovered to the extent of 79% to the mean of pre-exposure values after 10 days of cessation of exposure. However, the post exposure RBC's ChE activity showed a continuous increase as compared to the pre-exposure values.

The percentage changes of mean plasma ChE activity in relation to the occurrence of various toxicity signs before and after 2 weeks of exposure to this insecticide are shown in Table III. No definite pattern of association of various signs and the levels of depression in plasma ChE activity could be observed.

TABLE III

Plasma ChE activity (Klett Units) in Relation to Various Signs After 2 Weeks of Exposure

Symptoms	Pre-exposure	Post-exposure	Percentage inhibition
Neurological	39.00	11.17**	71.36
Gastrointestinal	41.24	11.29**	72.62
Eyes	40.41	11.37**	71.86
Decrease in heart rate	38.64	11.14**	71.17
No symptoms	35.33	10.80**	69.74

** = $P < 0.01$.

There was no evidence that the occurrence of toxicity manifestations relating to any of the different organ systems were correlated with the severity of depression of cholinesterase activity even when they were separately considered.

DISCUSSION

The results of a study of toxic manifestations amongst exposed workers in a Phorate formulation plant with reasonably satisfactory hygienic conditions and in which the workers had been regularly using adequate protective clothings are presented. Exposure to Phorate for 2 weeks revealed that in 60% of workers there are clinical manifestations which were predominantly gastrointestinal, neurological and of the skin and eyes. The ECG records did not show any evidence of

cardiac damage in the form of conduction defects. The only abnormality observed was bradycardia in 48% of workers. The toxicity signs encountered have also been reported by many investigators in subjects exposed to organophosphate insecticides (Baker et al., 1978; Shihab., 1976; Wadia et al., 1977). These toxicity manifestations might be due to depression of ChE activity as it has been observed that the toxic symptoms appear when ChE activity is reduced to 50% or more of the baseline value in acute malathion poisoning (Namba et al., 1971) and in workers occupationally exposed to organophosphorus insecticides (Kashyap and Gupta., 1971).

The observation of depression in plasma ChE activity with significant rise in RBC's ChE activity confirm that following acute exposure to organophosphorus insecticides there is innibition of plasma ChE. The depression in RBC's ChE is a delayed phenomenon which takes comparatively more time. The significant depression of whole blood and plasma ChE activity after the 1st and 2nd week of Phorate exposure does indicate the absorption of the compound by the workers inspite of using apron, hand gloves and face masks. It has also been demonstrated from case histories and environmental concentration that routine supply of personal protective equipment is not necessarily adequate to

prevent poisoning due to this highly toxic insecticide (Young et al., 1976). The post exposure clinical examination also indicates the absorption of the chemical by the workers. The 79% recovery of plasma ChE of the pre-exposure values within 10 days after the cessation of exposure indicate that majority of these toxicity signs were self-limiting and gradually disappeared following the withdrawal of the exposure.

A significant rise in RBC's ChE activity after Phorate exposure might be due to long term low levels of exposure to an ChE inhibitor causing an increase in RBC's ChE activity by enzyme induction which has also been reported earlier (Burges and Robert 1980). This could be the possible mechanism of this phenomenon in these subjects also, since they had been practically exposed to organophosphate insecticides periodically before the present exposure.

The lowering of heart rate (bradycardia) in 48% of the subjects with no ECG abnormality also point to the detrimental effect of Phorate exposure. The sinus bradicardia due to exposure to other organophosphates has also been reported in a number of cases of acute poisoning and in occupationally exposed subjects (Baker et al., 1978; Chhabra et al., 1970). The bradycardia could be due to the effect of ChE inhibitors acting either directly or through neurogenic mechanisms.

The results of this study indicate that 2 weeks of exposure to Phorate in formulators do involve health risks inspite of the use of standard hygienic and protective measures. A reduction of 71% in plasma ChE activity after 2 weeks as observed, need safety measures and it is advisable to perform a periodical monitoring regularly to safeguard against any severe poisoning from this highly toxic chemical.

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HI-6 in Man: Efficacy of the Oxime in Poisoning by Organophosphorus Insecticides

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The efficacy of the oxime HI-6 was studied as a treatment for organophosphorus poisoning. HI-6 was given four times daily as a single intramuscular injection of 500 mg accompanied by atropine and diazepam therapy. Oxime treatment was started on admission and continued for a minimum of 48 h and a maximum of 7 d. HI-6 rapidly reactivated human blood acetylcholinesterase inhibited by diethoxy organophosphorus compounds, while the dimethoxy-inhibited enzyme was mainly resistant to the treatment by HI-6. Although both HI-6 and pralidoxime chloride reactivated the red blood cell cholinesterase in quinalphos-poisoned subjects, the return of enzyme activities was more rapid following the use of HI-6. The general improvement of poisoned patients, which was sometimes more rapid than the rise of acetylcholinesterase activity, pointed to direct pharmacological effects of HI-6. No undesirable side-effects were noted in patients when HI-6 plasma concentrations were maintained at levels far above the 'therapeutic' concentration for up to 7 d.

Introduction

Among a large series of oximes that have been synthesized and studied with the aim of improving the specific treatment of organophosphorus (OP) poisoning, HI-6 (1–///4-(aminocarbonyl)-pyridinio/methoxy/methyl/ -2- /(hydroxyimino) methyl/-pyridinium dichloride) (asoxime chloride) appears to be the most promising compound. Its development, as with other cholinesterase reactivators, was initiated by Military Establishments with the aim of improving the treatment of chemical warfare agent poisoning, with particular emphasis on soman. The advantages of HI-6 over the oximes so far available for routine clinical use (i.e. pralidoxime, obidoxime and trimedoxime) are based on the ability of HI-6 to reactivate soman – inhibited cholinesterase *in vitro* and *in vivo*,[1–7] low toxicity,[3,7,8] and the lack of undesirable side-effects in humans receiving either single[9] or the multiple intramuscular (i.m.) injections of the oxime.[10,11]

Despite the evident efficacy of HI-6 against nerve agents,[7,12,17] antidotal properties of this oxime were less studied in experimental and human poisoning by OP insecticides.[10,11,18,19] Because of the paucity of such information, the present study was designed to evaluate the effectiveness of HI-6 in human intoxication by the commercial preparations of OP insecticides. Thus, the paper summarizes some of the clinical observations following the therapeutic use of an oxime.

Subjects and methods

Sixty patients of both sexes (21 males and 39 females) were included in the study (Table 1). All of them were admitted to the Clinic of Toxicology and Clinical Pharmacology of the Military Medical Academy of Yugoslavia with a definite history of OP poisoning. Fifty-two persons attempted suicide by taking the OP insecticide orally; the remainder were occupational inhalation or percutaneous exposures. Among the OP insecticides studied, malathion, quinalphos, dimethoate and phorate were the

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Table 1 Patient data and organophosphorus insecticides involved.

Insecticide	*Number of patients*	*Sex*		*Source*		*Organophosphorus intoxication*						*Outcome*	
						Route			*Severity*				
		M	*F*	*Suicide*	*Accident*	*Oral*	*Resp.*	*Dermal*	*Severe*	*Moderate*	*Mild*	*Survived*	*Died*
Malathion	24	5	19	20	4	21	3	–	13	5	6	20	4
Quinalphos	17	5	12	16	1	16	1	–	9	7	1	16	1
Dimethoate	4	2	2	4	–	3	–	1	2	2	–	4	–
Phorate	3	3	–	2	1	2	1	–	3	–	–	3	–
Others*	12	6	6	10	2	10	2	–	7	3	2	12	–
Total:	60	21	39	52	8	52	7	1	34	17	9	55	5
%		(35)	(65)	(87)	(13)	(87)	(11)	(2)	(57)	(28)	(15)	(92)	(8)

* Including: dichlorvos, fenitrothion, fenthion, phosphamidon, phosalone, pyridafenthion and parathion.

most frequently involved. More than 50% of subjects revealed signs of severe cholinergic effects accompanied by red blood cell cholinesterase (RBC-ChE) activity below 10% of the lower limit of normal laboratory values (range 6900–9100 IU l^{-1}; titrimetric method of Glick).[20] Five out of 60 patients died.

If a history of OP intoxication has been accompanied by the typical clinical picture and the persistent decrease in the activity of acetylcholinesterase, the specific treatment was started. Treatment protocol included the use of cholinolytics (atropine, 1 mg s.c. or i.v.), anticonvulsants (diazepam, 10 mg i.m. or 5 mg p.o.), and the oxime HI-6 (asoxime chloride, Bosnalijek, Drug company, Sarajevo). A single i.m. dose of HI-6, (500 mg, dissolved in 3 ml of distilled water), was given four times daily. Additional group of nine severely quinalphos-poisoned subjects received a 1000 mg dose of pralidoxime chloride four times daily, also at 6-h intervals. Depending of the severity of OP poisoning the oxime therapy was continued for between 48 h and 7 d.

Liver enzymes and kidney functions, the activities of serum (S-ChE; normal range 2600–6900 IU l^{-1}, method of Ellman *et al.*[21] and RBC cholinesterases, and the content of OP insecticides in blood and urine were measured daily. The concentrations of the oxime HI-6 in plasma within the one dosing interval were also determined by the spectrophotometric method of Maksimović and Vojvodić.[22]

The antidotal efficacy of HI-6 was evaluated by the clinical improvement of the patients and its reactivating potency. The activities of RBC-ChE and S-ChE were expressed as a percent of their respective lower normal laboratory range limits. The corresponding half-times of reactivation (calculated from the regression line) in comparison to the rates of spontaneous enzyme recovery served for an evaluation of the reactivating effectiveness of HI-6.

Results

The time between exposure to OP insecticides and admission varied from 60 min to 24 h. On admission all the patients required either gut lavage or skin decontamination. Patients clinically classified as having moderate to severe poisoning revealed the following symptomatology of cholinesterase inhibition: pin-point pupils (85%), hypersecretion of exocrine glands (75%), bradycardia (15%), muscle fasciculations (58%) and disturbances of consciousness (stupor or coma; 33%). Urgent blood analyses showed mainly hyperglycaemia (64%), low serum potassium (42%) and an acidosis in 60% of cases.

Initial treatment was started with atropine, diazepam and the oxime HI-6 (in some cases the alternative oxime was pralidoxime chloride). Peripheral cholinergic blockade was maintained by further intermittent boluses of atropine or its continuous intravenous infusion. Total doses of atropine depended on the severity of poisoning; the averages amount to 50 mg (mild), 130 mg (moderate) and 300 mg (severe), respectively. Diazepam 30 mg to 230 mg) was given either as an anticonvulsant or as a drug which minimized the central effects of atropine. Depending on the duration of HI-6 treatment, patients received from 4 g to 14 g of the oxime. Symptomatic therapy (alkaline infusion, infusion with potassium, antibiotics, etc.) and mechanical ventilatory support (one-third of treated subjects) were also included. Haemodialysis (one case), haemoperfusion (two cases) or plasma exchange (two cases) were used in a few cases.

The results related to the reactivating effectiveness of HI-6 in poisoning by various OP insecticides are presented in Table 2.

The recovery of RBC-ChE activity was rapid in patients suffering from severe phorate, quinalphos, dichlorvos and pyridafenthion poisoning. Malathion-inhibited enzyme was reactivated much more slowly by HI-6, while the oxime was ineffective against inhibition by dimethoate and phosphamidon. The half-times of reactivation of S-ChE were very close and varied from 10 to 20 d.

Table 2 The reactivating effectiveness of HI-6 in organophosphorus poisoning.

Insecticide	*Half-time reactivation (days ± s.d.)*	
Control*	27	7–15
Phorate (3)	1.4 ± 0.8	18.8 ± 1.3
Quinalphos (17)	1.5 ± 1.4	17.3 ± 7.1
Dichlorvos (3)	3.5 ± 1.8	10.2 ± 4.3
Pyridafenthion (2)	0.5	9.8 ± 0.7
Malathion (24)	10.1 ± 8.4	16.6 ± 11.7
Dimethoate (4)	29.6 ± 9.4	13.6 ± 3.2
Phosphamidon (1)	27.5	13.8

The results are the means ± s.d. for the number of subjects denoted in parenthesis.
* From references 23–6.

Time courses of RBC-ChE and S-ChE in patients suffering from the severe quinalphos poisoning treated by HI-6 or pralidoxime chloride are shown in Figure 1.

Both HI-6 and pralidoxime reactivated human blood acetylcholinesterase inhibited by quinalphos. However, the return of RBC-ChE to the control level was more rapid following treatment by HI-6. By contrast, the oximes were ineffective against the inhibition of S-ChE. S-ChE activities recovered slowly at the rate of several percent per day.

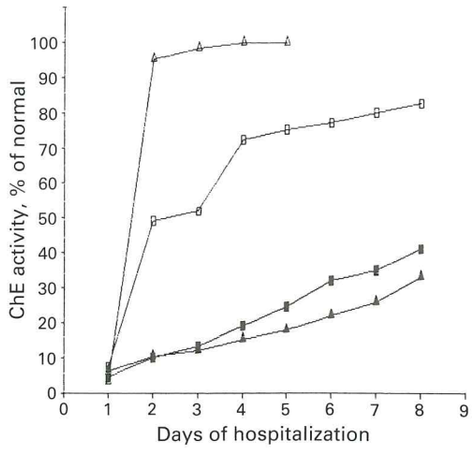

Figure 1 The recovery of RBC and serum cholinesterase activities in quinalphos-poisoned subjects treated with HI-6 and pralidoxime chloride. Points are the averages of enzyme activities in nine patients expressed as a percent of the lower limits of normal laboratory ranges (△ – RBC-ChE HI-6; ▲ – S-ChE HI-6; □ – RBC-ChE pralidoxime; ■ – S-ChE pralidoxime).

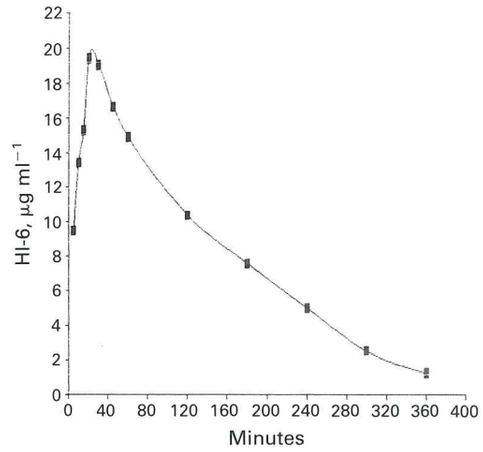

Figure 2 Time–plasma concentration curve of the oxime HI-6 in patients with acute organophosphorus poisoning. Each point represents the mean concentration of HI-6 determined in 15 subjects.

The time–plasma concentration curve of HI-6 in patients is shown in Figure 2.

Peak plasma concentrations of HI-6 (21.8 μg ml^{-1}) were reached at 31 min. Oxime levels were maintained above the level of 4 μg ml^{-1}, often and rather arbitrarily accepted as a therapeutic concentration for oximes according to Sundwall,[27] over a 260-min period.

Twenty percent of patients showed a moderate elevation of BUN and serum creatinine and/or a decrease in the clearance of creatinine. Transient elevation of serum enzymes, including alanine and aspartate aminotranspherases (ALAT, ASAT), lactate dehydrogenase (LDH), hydroxybutyrate dehydrogenase (HBDH), gamma-glutamyl transferase (gamma-GT), and 5′ nucleotidase (5′ ND), were also noted in a group of subjects suffering from severe poisoning. The disturbances lasted from 2 to 5 d, required no specific medical treatment and all the subjects were released from hospital with a normal renal and hepatic function.

Discussion

Previous reports on the efficacy of HI-6 in experimental OP poisoning[18,19] and the low toxicity of HI-6 in healthy men,[9,28] have led to further clinical development. HI-6 has been under careful and critical evaluation in the Clinic

of Toxicology and Clinical Pharmacology of the Military Medical Academy of Yugoslavia since 1985.

The monitoring of RBC-ChE in our patients showed that HI-6 effectively antagonized acetylcholinesterase inhibition by phorate, quinalphos, dichlorvos and pyridafenthion. The reactivation was slow in malathion-poisoned subjects and negligible after the inhibition of acetylcholinesterase by dimethoate and phosphamidon. The most reasonable explanation for these differences in the activity of HI-6 lies in the chemical structure of the OP insecticides being tested. Phorate, quinalphos and pyridafenthion form the diethoxy-type inhibited cholinesterase which, in contrast to dimethoxy-inhibited enzyme, 'ages', slowly.[29,30] When 'aging' has occurred, reactivation of the cholinesterase – inhibitor complex, either spontaneously or by oximes, can no longer take place.[31] Aside from 'aging', the toxicity of malathion impurities,[32] steric factors,[3] and unknown mechanisms[33] have also to be considered. In these respects HI-6 does not differ from pralidoxime and/or obidoxime.[29,34–37] However, those circumstances in which the reactivation of RBC-ChE was significant (quinalphos; Figure 1), HI-6, unlike the more commonly used pralidoxime chloride, seemed to be very active and might even have shown some therapeutic benefit.

Both HI-6 and pralidoxime failed to reactivate S-ChE. The rate of recovery of S-ChE (Table 2) points at *de novo* synthesis of the enzyme rather than to an oxime-induced reactivation. Serum enzyme, therefore, was not a convenient parameter for the evaluation of the efficacy of HI-6 in this study.

General improvement in the patients was sometimes faster than the rise of blood cholinesterases. According to the data on the pharmacodynamics of HI-6 in animals,[3,38–43] it seems reasonable to assume that the oxime also exerts some direct pharmacological effects in man. A recently reported case of suicidal poisoning by dimethoate treated by HI-6 supports such an assumption.[11] Reactivation effects of HI-6 on neuromuscular junction or brain acetylcholinesterase (being less likely), which are evident in experimental studies (for review see Rousseaux and Dua)[43] are not likely to be significant in the present study.

Plasma concentrations of HI-6 in treated patients (Figure 2) were at any given time higher than Kušić and co-workers[9] reported for the same dose of the oxime in volunteers. In spite of this, the lack of undesirable effects even under the conditions of maintaining HI-6 at levels far above 'the minimal effective concentration' for oximes (accepted as 4 μg ml^{-1} for monopyridinium compounds[27] and being much lower for the more effective bispyridinium reactivators)[19,44–46] for up to 7 d, confirms its exceptional tolerance by man.

Nearly one-third of the poisoned subjects had transient symptoms of renal or hepatic damage. OP insecticides, having high volumes of distribution, can affect the most of parenchimatous organs causing their dysfunction.[47–55] This seemed to be the case in our study. A direct renal, and/or hepatic toxicity of HI-6, which in experimental animals is two to three times less toxic than pralidoxime or obidoxime,[7,56] is, therefore, not likely to be the explanation. Four out of five patients who died ingested large quantities of malathion and were admitted to hospital more than 12 h after the suicide attempt. Gross pathomorphological damages in the brain, confirmed on autopsy, were assumed to be the main cause of death.

The addition of diazepam in the present study, which afforded protection against convulsions,[57] agitation[58,59] and brain pathology,[60] was based on the observations that the drug greatly improved morbidity and mortality in OP-poisoned animals, independent of its anticonvulsive action.[3] After its regular use in more than 200 OP-poisoned subjects, we have come to the conclusion that diazepam, besides its own favourable GABA-ergic action, also minimized the central side-effect manifestations of hyperatropinization in man.

In conclusion, the therapeutic use of HI-6 against poisoning by various OP insecticides showed it to be as effective as, or superior to the other oximes clinically available. Exceptional tolerance, the reactivation of acetylcholinesterase and its direct pharmacological effects make HI-6 the main contributory drug to the standard atropine – diazepam treatment of OP poisoning.

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Delayed Calcium Channel Blockade with Diltiazem Reduces Paracetamol Hepatotoxicity in Mice

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1 Diltiazem (30 mg kg^{-1} body weight, intraperitoneally) given to mice 9 h after paracetamol (450 mg kg^{-1}, orally) reduced liver damage, as judged by plasma aspartate aminotransferase activity (median 186, range 6–602 IU l^{-1}, $n = 18$ *vs* 466, range 23–3872 IU l^{-1} in 18 saline-treated controls; $P < 0.05$) with comparable reductions in mortality (14% *vs* 33%, respectively; NS).

2 Regenerative activity, as judged by mitotic figures in tissue removed at 30 h after paracetamol, was significantly higher in mice treated at 9 h with diltiazem (median 0.83 per high power field *vs* 0.1 in saline-treated controls; $P < 0.05$).

3 Diltiazem administered earlier or later than 9 h showed reduced efficacy and in some cases potentiated toxicity, as did nifedipine (40 mg kg^{-1} in divided doses up to 9 h).

Introduction

Paracetamol hepatotoxicity continues to be the major cause of drug-induced hepatic failure in Britain with current annual fatalities exceeding 200[1]and late presentation remaining the principal adverse prognostic indicator.[2] Although intravenous N-acetylcysteine is an effective treatment for preventing liver damage in patients presenting within 15 h of overdose,[3] there is diminished efficacy within the subsequent 20 h[4,5] when no alternative drug therapy is available.

Depletion of reduced glutathione and the arylation of hepatic proteins with paracetamol metabolites were the two critical events leading to paracetamol hepatotoxicity identified by Mitchell colleagues in 1973.[6,7] More recent findings suggest that neither hepatic glutathione depletion[8] nor total covalent binding[9] are absolute indicators for damage, and the disruption of intracellular calcium homeostasis is increasingly considered as the mechanism central to the development of hepatic damage[10,11] with the accumulation of Ca^{++} a terminal event in the progression to cell death.[12]

Agents as diverse as extracellular ATP,[13] cyclosporine[14] and paracetamol[15] can impair calcium homeostasis in isolated hepatocytes. Recently, calcium channel blockade was shown to reduce the cytotoxic sequelae of this impairment in hepatocytes incubated with chenodeoxycholic acid.[16,17] Landon and colleagues[18] have also demonstrated that nifedipine or chlorpromazine prevented liver damage in rats treated with carbon tetrachloride, thioacetamide or paracetamol, but the calcium channel blockers were administered prior to, as well as after, the hepatotoxins. In mice, diltiazem reduced liver damage when given 20 min after paracetamol, but was less effective at 3 h.[19] The aim of the present study was to investigate *in vivo* the hepatoprotective effects of calcium channel blockade late after paracetamol administration when the conventional antidote, N-acetylcysteine, is of diminished efficacy.

Materials and methods

Adult male CD1 mice (Charles River, Margate, Kent) weighing 18–26 g were starved for 10 h prior to administration by gavage of 450 mg kg^{-1} body weight paracetamol (acetaminophen) (Sigma; Poole, Dorset) as a warm aqueous

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Delayed-onset encephalopathy and coma in acute organophosphate poisoning in humans

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Abstract

The objective of the study was to describe the clinical characteristics and course of delayed-onset organophosphate (OP) poisoning. In our clinical experience, we have noticed patients with onset of deep coma 4–7 days after hospital admission, clinical features that have not been previously described. We set up a prospective observational study over 1 year to formally characterize this observation. Thirty-five patients admitted to the intensive care unit (ICU) with severe OP poisoning and treated with atropine and supportive therapy were followed up. Oximes were not administered. Three patients developed delayed-onset coma after presenting with normal or near normal Glasgow coma score (GCS). They developed altered conscious state rapidly progressing to deep coma, 5.0 ± 1.0 (mean ± S.D.) days after OP ingestion. The GCS persisted at 2T for 4.3 ± 2.1 days despite the cessation of sedative drugs at the onset of coma. During this period, the patients had miosed non-reacting pupils and no clinically detectable cortical or brainstem activity. Computed tomography of the brain and cerebrospinal fluid analysis were normal. Electroencephalogram showed bihemispheric slow wave disturbances. Two patients required atropine during this period to maintain heart rate and reduce secretions. In all three patients, no metabolic, infective or non-infective cause of altered conscious state was identified. With supportive therapy the GCS improved to 10T in 8.0 ± 2.0 days. All patients survived to hospital discharge. Three other patients who developed a reduction in GCS (3T–7T) by 4.7 ± 1.2 days but not progressing to coma and recovering (GCS 10T) in 3.3 ± 0.6 days may have manifested delayed-onset encephalopathy. Delayed-onset coma appears to have a distinct clinical profile and course with complete resolution of symptoms with supportive therapy. Although persistent cholinesterase inhibition is likely to have contributed to the manifestations, the mechanism of coma and encephalopathy need to be explored in further trials. The good outcomes in these patients suggest that therapy should not be limited in OP-poisoned patients developing profound coma or encephalopathy during hospitalization.

Keywords: Poisoning; Organophosphate; Insecticide; Intermediate syndrome; Central nervous system effects

1. Introduction

Deliberate ingestion of organophosphate (OP) compounds is common in the agricultural farming communities in Asia (Jeyaratnam, 1990; Tsai et al., 2007). Although the clinical features of OP poisoning are protean with multi-organ involvement, neurological manifestations are frequent and range from anxiety and restlessness to weakness, convulsions and coma (Peter and Cherian, 2000). Neuromuscular weakness following OP exposure can develop acutely within a few hours (Type I paralysis), after a few days (Type II paralysis) or late (Type III paralysis) as in OP-induced delayed polyneuropathy (Peter and Cherian, 2000).

Type II paralysis, termed ''intermediate syndrome'', is a clinical entity of proximal muscle weakness and respiratory insufficiency that occurs in conscious patients 24–96 h after the acute cholinergic crisis and prior to the expected onset of delayed neuropathy (Senanayake and Karalliedde, 1987). Intermediate syndrome is generally self-limiting and resolves in days to weeks with supportive therapy. Alteration in conscious state or coma, a centrally mediated phenomenon (Peter and Cherian, 2000), may occur acutely following OP ingestion and indicates severe intoxication (Peter et al., 2006) and poorer prognosis (Lin et al., 2007). Although this acute decline in conscious state often reverses within the first 24–48 h, when the cholinergic crisis settles, it may be prolonged in some patients (Lin et al., 2007).

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We have observed over several years, some patients who maintain a normal conscious state in the first 4–6 days following OP ingestion, subsequently develop either a decline in their conscious state that resolves in a few days, or manifest progression to profound coma with absent brainstem reflexes. Although a delayed-onset reversible extrapyramidal syndrome (Brahmi et al., 2004) as well as OP poisoning presenting as brainstem stroke has been previously reported (Hollis, 1999), to our knowledge, delayed-onset encephalopathy and coma have not been described. This paper describes a prospective observational study to ascertain the clinical characteristics of this phenomenon as well as describe the clinical course and outcomes following the development of this syndrome.

2. Methods

2.1. Study design

This was a 1 year (May 2006–April 2007) prospective observational study of adult patients admitted with OP poisoning to an 11-bedded Level 3 medical intensive care unit (MICU) of a 2000-bedded university affiliated teaching hospital.

2.2. Diagnosis and general management of OP-poisoned patients

The diagnosis of significant OP poisoning was made on the basis of typical toxidrome of cholinergic and nicotinic manifestations and pseudocholinesterase level of <1000 IU/l (reference 3000–6000 IU/l). Every effort was made to reliably identify the ingested compound based on the container brought by the patient's relatives. Red-cell cholinesterase and identification of the OP by chromatography was not done.

Patients were admitted and monitored for hypotension, arrhythmias and oxygen de-saturation. Continuous blood pressure monitoring via an arterial line was undertaken if patients manifested hemodynamic instability despite adequate atropinisation. A single lumen peripherally inserted central catheter was inserted in all patients; triple lumen lines considered with hemodynamic instability or the need for multiple infusions. The neurological state was monitored by the Glasgow coma score (GCS) four-hourly and more frequently if reduced. Cardio-respiratory and ventilatory parameters, GCS, dose of atropine infusion, sedatives, inotropes, list of medications, hourly intake and output were recorded on the MICU flow-charts. In addition to regular assessment of muscle power, in all spontaneously breathing patients and patients weaned from respiratory supports and close to extubation, a forced vital capacity (FVC) was performed thrice daily. In ventilated patients, given fluctuations in FVC and muscle weakness, patients were considered for extubation only if they were hemodynamically stable, were able to protect their airway, had no significant muscle weakness or gross metabolic derangement, and a FVC of at least 15 ml/kg on three consecutive occasions.

A logbook was maintained in the MICU of all OP admissions. Patients developing a decline in the conscious state during hospitalization were enrolled and data abstracted into data abstraction forms until recovery.

2.3. Specific treatment

All OP-poisoned patients received atropine infusion, adjusted to achieve *adequate* atropination, i.e. heart rate around 100 beats/min, clear lung fields and pupils mid-position. Oximes were not administered, as per the unit protocol (Sudarsanam et al., 2006), based on absence of benefit in our population (Cherian et al., 1997; Johnson et al., 1996) and paucity of evidence of improved outcomes (Peter et al., 2006, 2007a; Rahimi et al., 2006). The lack of oxime benefit has been attributed to late presentation, mega-dose intoxications, propensity of the OP compounds to induce ageing, possible toxicity of oximes (Peter et al., 2006, 2007b) as well as methodological problems with the oxime trials (Eddleston et al., 2002).

The need for ventilatory support was determined by the treating physician and initiated for respiratory distress, persistent hypoxemia not improving with non-invasive respiratory support, severe hemodynamic instability, inability to protect airway, or GCS of <8. Other supportive measures were provided including antibiotics for superadded infections and vasoactive agents for hypotension not responding to fluid resuscitation.

2.4. Definition of delayed-onset OP encephalopathy and OP coma

Patients were suspected to have delayed-onset OP encephalopathy or coma if, after an initial period of normal consciousness of >72 h following OP ingestion, they developed altered conscious state. Patients with altered consciousness from time of admission were excluded. Sedative drugs were discontinued when the GCS declined. If a low GCS persisted despite cessation of sedatives, detailed physical examination and investigations were done to evaluate the cause.

Coma was defined as a GCS of 3 in an unintubated patient or 2T in an intubated patient given that the verbal component cannot be assessed when intubated (Buechler et al., 1998). Full metabolic work-up, computed tomography of the brain, cerebrospinal fluid analysis, electroencephalogram (EEG), septic screen (including appropriate cultures), chest radiograph and procalcitonin levels were performed to evaluate for metabolic, sepsis, structural lesions or intra-cranial infectious and non-infectious causes of coma. Pseudocholinesterase levels were repeated in patients developing delayed coma. If no cause of altered conscious state/coma was identified, given our earlier experience, supportive therapy including atropine (if required) was continued in these patients until resolution of encephalopathy and coma. The clinical course of altered conscious state was recorded with patients followed up until hospital discharge or death.

3. Results

Of the 124 patients presenting to hospital with suspected OP poisoning over 1 year, 31 patients were admitted to the MICU within 24 h of poisoning and 4 were transferred late (>24 h). Excluded patients (Fig. 1) comprised of patients managed in the wards ($n = 66$) and those with non-OP poisoning ($n = 21$). One excluded patient with low pseudocholinesterase levels, not manifesting the cholinergic toxidrome or requiring atropine, may have belonged to a community in this part of the world that has no detectable butyrylcholinesterase activity (Manoharan et al., 2006).

The study cohort ($n = 35$) presented with a well-defined cholinergic phase with miosis, salivation, lacrimation, sweating and bradycardia. Some patients treated outside with atropine and referred, had a normal heart rate or tachycardia at presentation. The majority of patients were young males (Table 1). Although the lag-time to presentation to our hospital was 13.9 ± 24.2 h (mean ± S.D.), only two patients presented early (<2.5 h). Atropine requirements (31 patients) were high (97.3 ± 125.5 mg) in the first 24 h following poisoning. Thirty of thirty-five patients (85.7%) were mechanically ventilated during stay, representing a cohort of very sick patients.

Three patients developed coma 5.0 ± 1.0 days following OP ingestion, whilst three other patients had a reduction in their GCS (>2T) by 4.7 ± 1.2 days, but without further progression to coma (Table 2). The three patients who developed delayed-onset coma did not exhibit significant alterations in conscious state during the initial cholinergic phase and required 58.7 ± 23.6 mg of atropine in the first 24 h of hospital stay. Intermediate syndrome developed in all six patients and they all required ventilatory support (Table 3).

At the onset of coma, patients first manifested tremors and shaking movements of the limbs associated with increased muscle tone. During this period, increased sweating, reduced respiratory effort and reduced response to verbal and subsequently painful stimuli were noted. Atropine was required in two patients to maintain heart rate and minimise secretions. In the three patients, despite cessation of sedation, increased mechanical ventilatory support, and a search for metabolic causes, the conscious state progressed rapidly over the next 12–24 h to deep coma. There were no tonic–clonic movements of the limbs or other focal repetitive movements to suggest seizures, nor did the patients develop hypoxemia or hypotension. Clinical examination during this phase revealed, in addition to depressed conscious state, absent oculo-cephalic, pupillary-light, corneal and deep tendon reflexes, miosed pupils and no spontaneous respiratory excursions. Extensive work-up (detailed above) did not reveal alternate causes for coma. The EEG in all three patients showed bihemispheric slowing without any epileptiform discharges suggesting severe cortical dysfunction or diffuse encephalopathy. Pseudocholinesterase levels remained low during coma. Deep coma (GCS 2T) lasted for 4.3 ± 2.1 days with GCS normalising by day 15 with just supportive therapy (Fig. 2).

Three other patients developed delayed-onset encephalopathy (GCS 3T–7T) without progression to coma (Fig. 2). The GCS improved marginally with cessation of sedative drugs but normalised only after 3.3 ± 0.6 days. Although these patients were discharged alive from MICU, one patient died following a cardio-respiratory arrest due to an unrecognized tube block in the ward.

The duration of hospital stay was longer in patients developing delayed-onset coma or encephalopathy, but not statistically significant, probably because of small numbers.

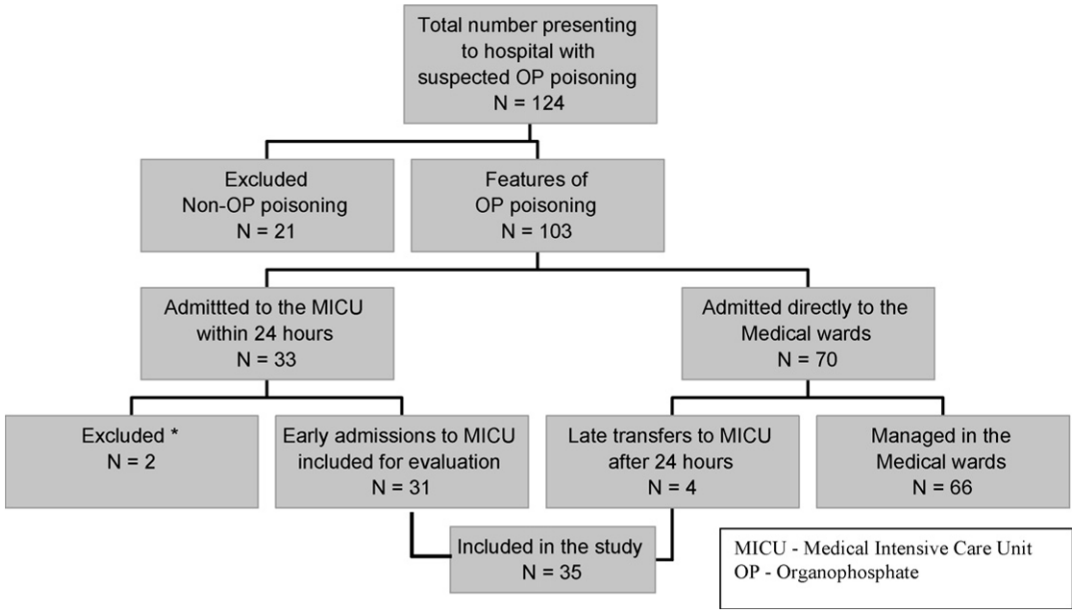

Fig. 1. Flow-chart of patient recruitment, inclusion and exclusion. Number of patients evaluated at each stage of the recruitment process and number of patients excluded from the trial with reasons for exclusion. *Excluded patients: one patient had low pseudocholinesterase levels but did not manifest the toxidrome. In the other patient, the relatives brought the container that was identified as an organocarbamate.

Table 1
Baseline characteristics

Characteristic	Patients not developing delayed onset encephalopathy ($N = 29$)	Patients developing delayed onset encephalopathy ($N = 6$)
Age, mean (S.D.) years	28.31 (10.7)	29.83 (7.6)
Male:female ratio	16:13	5:1
Type of organophosphate compound		
Monochrotophos	6	0
Quinalphos	5	1
Phorate	3	1
Chlorpyriphos	3	0
Methylparathion	0	1
Others	4	0
Unknown	8	3
Number (%) had gastric lavage at local hospital	22 (75.9)	3 (50)
Mean (S.D.) time to presentation in hours	15 (26.4)	8.5 (4.2)*
Number (%) with bradycardia/tachycardia on admission	14 (48.3)	2 (33.3)
Admission mean (S.D.) pseudocholinesterase levels[a]	348.7 (143.7)	418.0 (280.6)*
Atropine dose[b], mean (S.D.) in the first 24 h (mg)	102.8 (125.5)[c] Range 15–630 mg	74.2 (32.1)* Range 24–125 mg

[a] Pseudocholinesterase levels, reference range 3000–6000 IU/l.
[b] Atropine requirement reported only in patients admitted to hospital within 24 h of ingestion of the organophosphate compound.
[c] Mean atropine dose calculated only for the 26 patients who presented within 24 h of poisoning. Three patients who presented late (>72 h) were excluded from this analysis.
* P values > 0.4.

Prolonged stay reflected delayed-onset encephalopathy or coma coupled with longer ventilation time and increased rate of nosocomial infections (Table 3). The in-hospital mortality of the entire cohort treated in the MICU was 5.7% (2/35).

4. Discussion

Neuromuscular weakness without decline in conscious state (Samuel et al., 1995) as well as severe poisoning presenting

Table 2
Characteristics of the three patients who developed delayed-onset organophosphate coma

Variable	Patient 1	Patient 2	Patient 3
Age/sex	30 male	28 male	28 female
Compound	Quinalphos [a]	Unknown	Phorate[a]
Approximate quantity ingested	200 ml	Unknown	50 ml
Time from ingestion to presentation	7.5 h	14 h	9.5 h
Initial pseudocholinesterase level[b]	381	286	286
Atropine requirement in the first 24 h	61 mg	81 mg	34 mg
Time from OP ingestion to ventilation	4 days	1 day[c]	2 days[c]
Time to onset of reduced GCS	5 days	6 days	4 days
Sedation stopped on	Day 5	Day 4	Day 4
Duration of lowest GCS of 2T	2 days	5 days	6 days
Time to normal GCS	8 days	6 days	10 days
Atropine requirement during low GCS	Nil	12 mg/day	12–24 mg
Maximum temperature during-delayed coma	100.2 °F	102 °F	99.4 °F
Pseudocholinesterase levels[b]	317	190	254
CSF findings	Not done	Normal[d]	Normal[d]
Computed tomography	Calcified granuloma	Nil significant	Nil significant
EEG	Bihemispheric slow waves	Bihemispheric slow waves	Bihemispheric slow waves
Duration of mechanical ventilation	9 days	21 days[e]	36 days[e]
Duration of hospitalization	22 days	29 days	39 days
Hospital outcome	Alive	Alive	Alive

[a] Quinalphos and phorate are diethyl organophosphate compounds.
[b] Pseudocholinesterase levels: reference range—3000–6000 IU/ml.
[c] Ventilation required at this time because of the development of muscle weakness.
[d] Normal cerebro-spinal fluid (CSF) studies suggested by normal cell count as well as normal protein and sugar levels in the CSF and negative cultures, EEG—electroencephalogram.
[e] Prolonged ventilation because of the development of nosocomial infections.

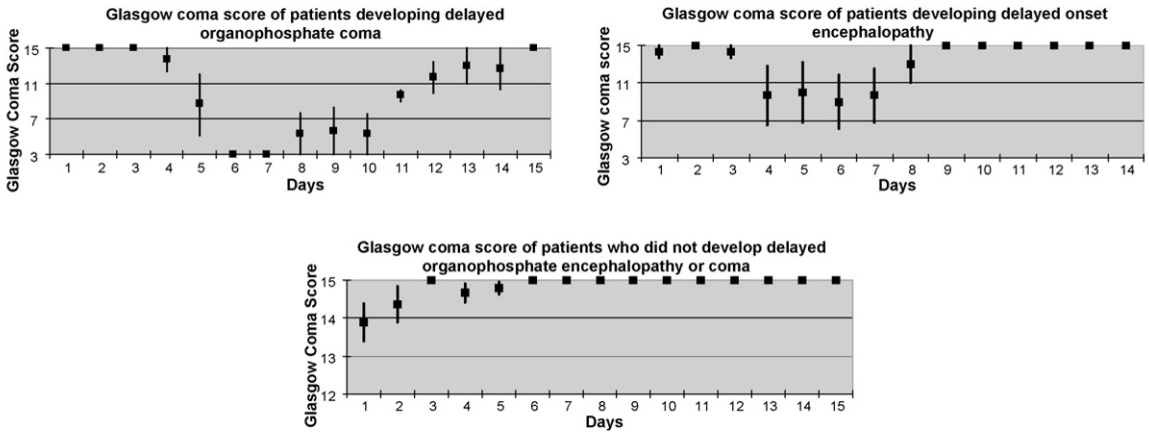

Fig. 2. Glasgow coma score during intensive care stay. Glasgow coma score (GCS) during the period of stay in the intensive care unit (ICU) depicted as mean ± standard error of mean (S.E.M.) separately for patients who developed delayed-onset coma ($n = 3$ patients), delayed-onset encephalopathy ($n = 3$ patients) and those who did not develop encephalopathy or coma ($n = 29$ patients). The GCS of the patient who was transferred to the ICU following a cardiac arrest in the ward was excluded. In patients who were intubated the verbal component was scored as "T". However to be able to provide a standardised GCS for the purposes of the figure during intubated and un-intubated periods a numerical score was assigned for "T" commensurate with motor and eye opening scores. Thus a GCS score of 3 was assigned if the "intubated" GCS score was 2T and 15 for an "intubated" score of 10T. Intermediate values were assigned intermediate scores.

with coma (Brahmi et al., 2006; Tsai et al., 2007) and persisting for several days (Lin et al., 2007) occur commonly in OP poisoning. Delayed-onset OP encephalopathy/coma has hitherto not been reported in the literature and may be part of the spectrum of delayed manifestations (Fig. 3) that include delayed-onset cholinergic symptoms (Davies et al., 1975), intermediate syndrome and respiratory failure (Eddleston et al., 2006) and extrapyramidal manifestations (Arima et al., 2003; Brahmi et al., 2004; Shahar et al., 2005). A recent publication of late-onset intermediate syndrome occurring 114 h post-ingestion of methamidophos, a highly lipophilic OP (Yardan et al., 2007), also strengthens the case for the recognition of delayed-onset symptoms as a distinct clinical entity.

In acute intoxication, *early* coma is multi-factorial and related to neurologic (central), cardiac (hypotension) and respiratory (hypoxemia, hypercarbia) effects. Although the effects of OP vary depending on the species and involvement of a particular OP, its administration evokes excitatory EEG (Carpentier et al., 2000; McDonough et al., 1998) changes that result in focal seizures in the respiratory centre in guinea pigs (Chang et al., 1990) and cat (Rickett et al., 1986). These manifestations are inhibited by diazepam and centrally acting anti-cholinergics such as atropine but not the peripherally acting anti-cholinergics glycopyrrolate or ipratroprium (Bird et al., 2003; Carpentier et al., 2000). Improvement in respiratory function and conscious level have also been

Table 3
Outcomes

Outcome	Patients not developing delayed onset encephalopathy ($N = 29$)	Patients developing delayed onset encephalopathy ($N = 6$)	P value
Number (%) needing ventilation	24 (82.8)	6 (100)	0.56†
Duration of ventilation, mean (S.D.) days	8.8 (6.6)	14.5 (4.3)	0.22
Number (%) developing intermediate syndrome	19 (65.5)	6 (100)	0.15†
Number (%) underwent tracheostomy	15 (51.7)	5 (83.3)	0.21†
Hospital length of stay, mean (S.D.) days	15.1 (11.1)	27.5 (8.7)	0.14
ICU outcome			
Alive	28	6	1.0†
Died	1	0	
Hospital outcome			
Alive	28	5	0.32†
Died	1	1	
Infections			
No infections	11	0	
Pneumonia	13	3	0.15†
Other infections	5	3	

Continuous variables analysed by *t*-test, categorical variables analysed by Fisher exact test (†).

Table 4
Qunialphos and phorate poisoning: comparison of characteristics of patients who developed delayed coma vs. those who did not

Parameter	Quinalphos		Phorate	
	Patients developing delayed onset coma ($n = 1$)	Patients not developing delayed coma ($n = 5$)	Patients developing delayed coma ($n = 1$)	Patients not developing delayed coma ($n = 3$)
Estimated dose (ml)	200	5[a]	50	140[a]
Lag-time to presentation (h)	7.5	3.4 ± 1.6	9.5	6 ± 0[b]
Gastric lavage done (yes/total)	1/1	3/5	1/1	3/3
Admission pseudocholinesterase[c]	381	463 ± 279	286	343 ± 312
Atropine dose first 24 h (mg)	61	65.8 ± 18.3	34	77.5 ± 31.8[b]

[a] Data available only for one patient.
[b] Calculated only for the two patients who presented to our hospital within 24 h of ingestion of organophosphate.
[c] Pseudocholinesterase levels: reference range—3000–6000 IU/ml.

reported in humans administered diazepam rapidly prior to intubation (Eddleston et al., 2006).

The persistently low pseudocholinesterase levels and higher atropine requirements at the onset of coma support a similar mechanism in delayed coma. The occurrence of intermediate syndrome in all patients manifesting delayed-onset encephalopathy/coma is again consistent with generalized overstimulation of the cholinergic synapses although neuromuscular transmission defect (De Bleecker et al., 1994), oxidative stress (Venkatesh et al., 2006) and down-regulation of protein-coupled receptors (Eddleston et al., 2006) have also been implicated in intermediate syndrome. It is interesting to note however, that overall atropine requirements were lower during the cholinergic phase, albeit not significantly, in those who developed encephalopathy (Table 1). Again this may not be surprising given earlier observations that some fenthion-poisoned patients in one series, required little if any atropine before developing delayed respiratory failure (Eddleston et al., 2006). Nevertheless, the observance of bihemispheric slow wave disturbances in the EEG of all three patients and the absence of any seizure activity suggest central nervous system depression rather than stimulation due to persistent cholinesterase inhibition. Other mechanisms such as mitochondrial dysfunction related to persistent inhibition by OP need to be explored given recent evidence of impaired mitochondrial

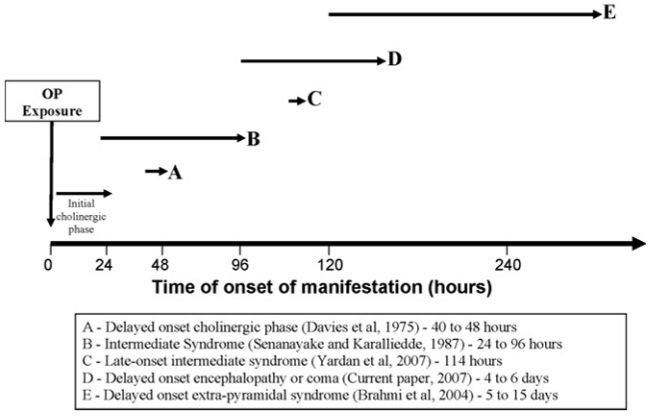

Fig. 3. Delayed manifestations in acute organophosphorus poisoning. A schematic depiction of the time of onset of delayed symptoms. These are derived from studies reported in the literature and summarised in the text box.

bioenergetics and apoptotic neuronal degeneration after chronic low-level exposure to dichlorvos (Kaur et al., 2007).

The toxicokinetic properties of the OP compounds implicated in delayed-onset coma may explain the delay in symptom onset. Both quinalphos and phorate are highly toxic with oral LD50 (in rats) of 19.95 mg/kg for quinalphos (Raizada et al., 1993) and 1 mg/kg for phorate (National Library of Medicine, ChemID plus). Both compounds are also highly lipid soluble with log P of 4.45 for quinalphos and 3.71 for phorate (Benfenati et al., 2003) and hence likely to have large volumes of distribution. These lipophilic OPs may thus share toxicokinetic properties with dichlofenthion (Davies et al., 1975), chlorpyriphos and fenthion (Eddleston et al., 2005) and methamidophos (Yardan et al., 2007) and delayed symptom onset may reflect rapid distribution to the fat tissues and subsequent re-distribution (Davies et al., 1975; Tush and Anstead, 1997). When patients with phorate and quinalphos poisoning who developed coma were compared with those who did not develop it, we were however unable to identify any specific factor (e.g. estimated poison ingested, lag-time to presentation, pseudocholinesterase level, atropine requirements, etc.) that predisposed to the development of delayed coma (Table 4).

The pharmacodynamic properties of diethyl OP compounds (phorate and quinalphos) may provide insight on another possible reason for delayed coma. Since the half-life of ageing of dimethyl and diethyl phosphorylated AChE (as determined in isolated human red-cells in vitro) is not similar (3.7 versus 33 h), a differential response to oxime therapy has been suggested (Eddleston et al., 2005), although not demonstrated in clinical trials. Lack of oxime administration may have contributed to the observance of this syndrome in our population, given that delayed coma has not been reported from centres using oxime therapy and this aspect warrants further study.

Although this report is a small case-series, the incidence of this phenomenon may be overestimated by the exclusion of OP-poisoned patients managed outside MICU. The referral of sicker patients surviving initial intoxication in the peripheral centres may have also favoured referral and survival bias. The best marker of classical cholinergic syndrome would have been red-cell acetylcholinesterase activity, which unfortunately was

not done for these patients. This is a limitation when trying to distinguish the basis of this coma. Notwithstanding these limitations, this first description of delayed-onset coma is of importance and interest to clinicians and toxicologists.

5. Conclusion

The spontaneous resolution of delayed-onset coma and encephalopathy in our cohort of patients with only supportive therapy suggests that this syndrome is self-limiting and has a good prognosis. Delayed-onset OP encephalopathy should be suspected in patients who develop altered conscious state several days (>96 h) after OP poisoning and the observation of miosed (not dilated) non-reacting pupils. It is hoped that further work would throw light on the mechanism of delayed symptom onset. However the impact of prolonged ventilation on outcomes (Cox et al., 2007) need to be borne in mind whilst managing these patients.

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Phorate intoxication at an insecticide formulating plant

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The use of phorate requires precautions similar to those taken with other organophosphate pesticides, as demonstrated by the case histories and environmental concentrations found in a formulating plant using phorate. Routine supply of personal protective equipment is not necessarily adequate to prevent poisoning.

introduction

The earliest organic phosphorus pesticide was apparently tetraethyl pyrophosphate, developed by Clermont in 1854.[1] The biological activity of organophosphorus esters was discovered by chance in 1932 by Lange and Kreuger.[2] However, the basic research and developmental work on these compounds is attributed to Schrader of Germany,[1,2] whose studies were published in 1947.[3] The first recorded fatality from a phosphate ester pesticide occurred in California in 1949.[4]

In 1954, the American Cyanamid Company introduced o,o-diethyl S-(ethylthio)methyl ester under the trade name "Thimet". The ISO has approved the common name "phorate" for this chemical. Phorate is a systemic insecticide used for protection against sap-feeding insects.[5] The major end uses include application to sugar beets, potatoes, lettuce, peanuts, corn, rice, barley, cotton, wheat, tomatoes, beans, ornamentals, and cotton seeds.[6]

phorate poisoning case history #1

On February 13, 1971, a 34-year old black male employed by a formulation plant was admitted to a hospital around noon. He was unconscious and his employer reported that he was suffering from organic phosphate insecticide poisoning. The following symptoms were noted during his treatment period: confusion, dizziness, nausea, vomiting, and constricted pupils.

Treatment was initiated on the basis of the employer's report and symptomatology of the illness since the hospital was not equipped to perform a blood cholinesterase determination. From admission until 4:30 p.m., February 15, 1971, the patient received 1 gm I.V. of 2-PAM (2-pyridine-aldoxime methiodide, reactivates the phosphorylated enzyme) and 36.2 mg of atropine (blocks parasympathetic effects of the accumulated acetylocholine). He was released from the hospital on February 16, 1971 and appeared to be in good health.

For approximately 30 days, the patient had worked in a formulating plant in the "dust house," where technical grade "Thimet" was mixed with ground clay and repackaged. He had been supplied with and reported that he had always worn a respirator, goggles, rubber gloves, and coveralls. Clean coveralls had been supplied by the company every two days. Although shower facilities were available, no one showered after work because the shower rooms were cold, due to inoperative heaters.

The company used a screening blood test to monitor organic phosphate exposure. The test required a "finger prick" and was based on the time required for a color change to develop. It was administered on Tuesday and Friday of each week and was required of all workers exposed to organic phosphate chemicals. The patient said that he had been tested on Tuesday, February 9, and on Friday morning, February 12 and that

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the results of both tests had been reported to him as "okay".

The employee had reported for work on Saturday, February 13, 1971 at 8:00 a.m. He recalled feeling a little tired, but otherwise normal. While on break from 10:30-11:00 a.m., he began to feel sick to his stomach and to feel occasionally dizzy. Upon returning to work, he became increasingly dizzy and confused, and lost consciousness.

phorate poisoning case history #2

On May 21, 1971 an 18-year old black male employed at the same formulating plant was brought to a hospital emergency room in severe respiratory distress. He was exhibiting the following symptoms: cardiac arrhythmia (severe tachycardia), excessive salivation, respiratory distress, muscle fasciculation, and pinpoint pupils. He was treated with atropine and 1 gm I.V. 2-PAM, followed with an additional 1 gm 2 hours later. He received a total of 5 mg of atropine I.M. over a 48-hour period. On May 24, 1971 the patient was in good condition, except for feeling very tired.

The patient had worked for approximately five months in the "dust house" area on the "Thimet" and "Mocap" packing line. He did not recall having been warned of any particular danger at the onset of his employment. He had been given a blood test once during the week that he became sick. During that week, the dust conditions had been worse than he had ever noticed in the past. He stated that he had had to change the cartridges in his respirator at least once a day on several different days. He also reported that he had been furnished with clean coveralls each day and that he showered at the end of each day.

During the week he had noticed that he was feeling more fatigued than normal at the end of each day, but had exhibited no other symptoms. On May 21, 1971, he reported to work at 8:00 a.m. and changed filters in his respirator around noon. About 6:00 p.m. he began feeling nauseated and was sweating profusely. He vomited, became very dizzy, lost his muscle coordination and almost collapsed. At this point, he was removed from the work area, was given two small white tablets and taken to the hospital.

environmental concentrations

After the first reported poisoning, the Tennessee Department of Public Health began an investigation to determine the atmospheric concentrations of phorate in the workroom environment. Air samples were collected utilizing midget impinger flasks containing 15 mL of iso-octane with a flow rate of 2.3 liters per minute. The impinger was hand held in the immediate vicinity of the worker's breathing zone. The duration of each sample was 20 minutes.

Analysis was made with a gas chromatograph equipped with a dual-flame-photometric detector. One detector was equipped with 526 mμ interference filter to monitor phosphorus compounds while the other detector had a 394 mμ filter for sulfur compounds. Standards were prepared from technical grade material obtained from the company and from purer material acquired from the U.S. Environmental Protection Agency. In the worker's breathing zone, phorate concentrations ranged from 0.07 to 14.60 mg/m^3. Complete sample results are presented in Table I.

After the second poisoning, the Tennessee Department of Public Health adopted a tentative 8-hour time-weighted average limit of 0.05 mg/m^3 based upon comparison of the LD_{50}'s of parathion and phorate and the existing TLV of 0.1 mg/m^3 for parathion.[7] (See Table II.) Subsequently the Committee on Threshold Limit Values (TLV's) for Airborne Contaminants of the American Conference of Governmental Industrial Hygienists proposed a TLV of 0.05 mg/m^3 in their "Notice of Intended Changes" in 1972.[8] This value was based on unpublished data of a "no effect" daily dose of phorate for dogs and rats[9] and was adopted in 1974.[10]

discussion

In the formulating plant described in this paper, personal protective equipment was used to supplement inadequate ventilation controls in the mixing and bagging of phorate. Because the

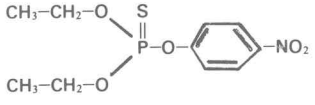
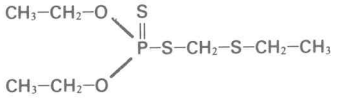
TABLE I
Breathing Zone Concentrations of Phorate

Operation/Location	Remarks	Concentration (mg/m^3)
Bagging Operator	Exhaust system on	0.30
	" "	0.95
	" "	1.16
	Exhaust system off	7.84
Bag Sealer	Exhaust system on	0.57
	" "	0.46
	" "	0.61
	Exhaust system off	1.75
Control room	Mixer in operation	0.87
	No mixing	0.11
Formulation warehouse	Full operation	2.05
	No equipment operating	0.07
Main warehouse	Full operation	0.16
Mixer-screen area	Full operation	4.43
	" "	3.99
	" "	14.60

air samples taken by the Tennessee Department of Public Health were not on the same days that the two reported intoxications occurred, and the workers were partially protected by respirators and clothing, the relative safety of the TLV cannot be judged from these data. It should be noted, however, that the half-mask dust respirators used for protection against inhalation of the phorate-containing dust are normally assigned a protection factor of 10, i.e., airborne concentrations should not exceed 10 times the concentrations which would produce an effect in the absence of respiratory protection.[11] In this instance, all samples taken during the formulating operation exceeded 10 times what eventually was adopted as the TLV. One sample was almost 300 times this value. From the worker's description of conditions, dust levels must have been even higher for the second poisoning incident. It is of utmost importance that a respirator program include the elements of proper selection of respirators, individual fitting, worker training in respirator use, and maintenance of the units. However, even with a good program there will probably be some exposures close to 10 percent of the ambient concentration.

The biological monitoring program described may have been of some value in detecting subclinical cases of phorate intoxication. It was demonstrably ineffective in preventing acute intoxication. Likewise, in the absence of process revision, enclosure and ventilation to reduce potential worker exposures, provision of clothing, respirators and a shower room was ineffective. The case histories also demonstrate

TABLE II
Comparisons of Parathion and Phorate

	Parathion	Phorate
Chemical Name	o,o-diethylo-p-nitrophenyl phosphorothioate	o,o-diethyl S-(ethylthio)-methyl phosphorodithioate
Structure		
LD_{50} (mg/kg)	6-15	1.6-3.7
TLV (mg/m^3)	0.10	0.05

the need for periodic monitoring of workplace air concentrations and regular testing of ventilation control systems.

On the market today there are approximately 1,500 active ingredients formulated into more than 40,000 pesticidal products.[12,13] Federal regulations govern occupational exposure to only 100 of these compounds.[14] A generic work practices standard is sorely needed.

acknowledgement

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Risk Assessment of Pesticides for Soils of the Central Amazon, Brazil: Comparing Outcomes with Temperate and Tropical Data

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Original Research

ABSTRACT

The risk of 11 pesticides to the soil environment was assessed in a 3-tiered approach at 4 sites located in Central Amazon, near Manaus, the capital of the Amazonas State in Brazil. Toxicity–exposure ratios (TERs), as routinely used for the registration of pesticides in the European Union, were calculated. First, the predicted environmental concentration (PEC) values in soil on the basis of real application rates and soil properties but temperate DT50 (degradation time of 50%) values were compared with temperate effect values (earthworm LC50s; median lethal concentrations), both gained from literature. Second, the risk assessment was refined by the use of DT50 values from tropical soils (measured for 7 compounds and estimated for 4) but still with temperate effect values because only a few results from tests performed under tropical conditions are available. Third, the outcome of this exercise was evaluated in a plausibility check with the use of the few results of effect tests, which were performed under tropical conditions. However, the lack of such data allowed this check only for 6 of 11 pesticides. The results are discussed in light of pesticide use in the Amazon in general, as well as compared with the registration status of these pesticides in other countries. Finally, suggestions are given for which kinds of studies are needed to improve the environmental risk assessment of pesticides in tropical regions.

Keywords: Case studies Pesticides Soil Environmental risk assessment Tropics

INTRODUCTION

The tropical areas, covering only 25% of the terrestrial surface of the planet, concentrate the highest biodiversity and generate nearly 60% of the primary productivity of the world (Deshmukh 1986). Approximately 70% of the species of flora and fauna can be found in the tropical areas. Moreover, these areas are also considered to be more susceptible to anthropogenic influences than temperate areas (Allen 1985). Nowadays, tropical ecosystems are threatened by deforestation and postdeforestation land uses, mainly by the intensification of agriculture, that promote not only habitat and biodiversity losses, but also contamination of ecosystems with the pesticides used to improve agricultural productivity (Dasgupta et al. 2001; Ecobichon 2001).

Brazil, the country with the world's largest remaining tropical forest, is not exempt, and the liberalization of agricultural trade has transformed the country into the 3rd largest consumer of pesticides in the world and Latin America's largest consumer (Waichman et al. 2007). Although most of the agricultural expansion has taken place in the south and southeast areas of Brazil, the Amazon region constitutes the main agricultural frontier of the country, mainly for soybean production, one of the main pesticide consumers in the country. The contamination of soil and water associated with soybean cultivation has been reported in several areas of the country (Laabs, Amelung, Pinto, Wantzen, et al. 2002). However, the widespread use of pesticides in the Amazon combined with a lack of training and education programs for safe use increased human and environmental risk (Waichman et al. 2002, 2007).

Besides soybean production for export, agriculture for food production is increasing to compensate for population growth in the Amazon. There, the main areas of food production are in the floodplains, which are very productive because of the seasonal flooding regime that annually fertilizes the soil. This area is favorable for annual crops like fruits and vegetables. However, the use of nontraditional crops in horticultural production in the Amazon demands a heavy use of pesticides because of their susceptibility to pests and competition with native vegetation. Although a rigorous Brazilian Pesticide Law (Law 7.802, July 11, 1989) has been introduced in Brazil, it is poorly implemented because the government does not have a sufficient number of trained personnel to enforce and monitor the law. Even when pesticides require a prescription by law for sale and highly hazardous or toxic pesticides require trained technicians registered with the Ministry of Agriculture for application, they are freely sold and used. In this way, pesticides are central tools for controlling the production process. By minimizing economic risks and maximizing yields, pesticides play an essential role in food production in small-scale farming in the Brazilian Amazon because they are perceived as a component of economic safety against the uncertainties of agricultural production in the region.

Amazonian farmers do not receive technical assistance from official extension services, and they are not adequately prepared and trained for the use of pesticides. Besides their intensive use, several factors contribute to the incorrect use of pesticides in the Amazon, including a poor knowledge of pesticide hazards and careless handling during preparation and application and with disposal of empty packages (Waich-

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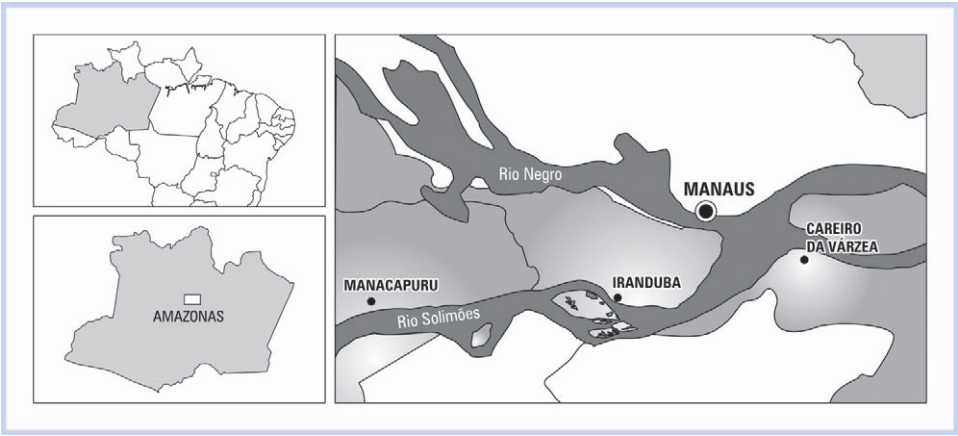

Figure 1. Location of the study area (dark gray = river; light gray = study areas).

man et al. 2002, 2007). Under such circumstances, environmental exposure is probably high. Therefore, research in Amazonian areas is needed to determine the risks and the effects of pesticide contamination on ecosystems. However, most of the data currently used in the risk assessment of pesticides in tropical countries like Brazil are generated in North America or Europe. Consequently, an extrapolation of temperate data to tropical conditions without a scientific basis can lead to erroneous results.

To better understand the environmental risk associated with the use of pesticides in Amazonian agriculture and the further management of this risk, the aim of this work was to conduct an environmental risk assessment (ERA) of pesticides in the soil compartment of the Brazilian Amazon in a 3-tiered approach.

MATERIALS AND METHODS

The study was undertaken in floodplain areas near Manaus (Amazonas State), the main urban center of the Brazilian Amazon. It focuses on the situation at 4 sites: Manaus, Manacapuru, Iranduba, and Careiro da Várzea (Figure 1), where the production of fruits and vegetables are concentrated. All sites are located in the Solimões–Amazon River floodplain, with a seasonal variation of water level. The rinsing phase starts in December, with the highest water level in May or June, and the receding period occurs in July, with the lowest water level in October or November. The average annual temperature is 26.7 °C, with the warmest period between August and November and the coldest months between December and March. The rainfall annual average is 2,296.4 mm and is unevenly distributed throughout the year. The rainy season lasts from December to June and the dry season from July to November.

Data were gathered from February to June 2005 in 26 floodplain villages (7 in Iranduba, 9 in Careiro da Várzea, 9 in Manacapuru, and 1 in Manaus). A total of 220 farmers were interviewed. Standardized questionnaires were used to obtain information on variables such as pesticides used and general knowledge and attitudes on pesticide handling, safety, and risk perception (Waichman et al. 2007). At the 4 sites, 18 crops had been treated with pesticides in the past years: Banana (*Musa paradisiaca*), bunching onion (*Allium fistulosum*), kale (*Brassica olearacea* var. *acephala*), cabbage (*Brassica olearacea* var. *capitata*), long coriander (*Eryngium foetidum*), cucumber (*Cucumis sativa*), eggplant (*Solanum melagena*), gherkin (*Cucumis anguria*), lettuce (*Lactuca sativa*), melon *Cucumis melo*), okra (*Hibiscus esculentus*), papaya (*Carica papaya*), paprika (*Capsicum annuum*), passion fruit (*Passiflora edulis* f. *flavicarpa*), pepper (*Capsicum chinense*), sweet potato (*Ipomea batatas*), tomato (*Lycopersicum esculentum*), and watermelon (*Citrullus lanatus*). Several crops are highly valuable at the Manaus market (e.g., eggplant, gherkin), whereas others are basic food sources of the local population (e.g., banana).

The soil properties of the study area were measured with established methods in Brazil (EMBRAPA 1997). These soils are considered typical for Central Amazonian floodplains (Junk and Piedade 2005). They are classified as gley soils (Entisols), which are slightly acid with pH (KCl) values ranging between 3.7 (Manacapuru) and 5.3 (Iranduba). Organic carbon content differs between 0.14% and 2.63% in the uppermost 10 cm of soil and between 0.01% and 1.12% in the 2nd layer of topsoil (10–20 cm). Accordingly, the content of organic matter varies. The data most relevant to the predicted environmental concentration (PEC) calculation in soil are bulk density values. In particular, the average soil density was determined as 1,010 kg/m^3 for the first 10 cm of the top soil. It is expected that the mean density of the first 5 cm (the topsoil layer, which is usually considered when calculating PECs), is even slightly lower, but because the soil density of this layer was not determined separately, it was decided the value for the uppermost 10 cm would be used.

First, an ERA for the soil compartment was performed for the 11 pesticides used regularly in the study area (Table 1). In addition, the range of doses, number of applications per cycle and interval between applications (in days) are given in Table 2. In comparison, the same parameters recommended for Brazilian crop sites are also presented. On average, farmers use higher doses, more applications per cycle, and shorter intervals between applications than recommended, but the ranges are very broad. Data on the effects of the selected pesticides on soil organisms were compiled from the literature (Table 3). Toxicity data from nonstandard tests (e.g., those confirming a high toxicity of copper oxychloride to earthworms; Helling et al. 2000), are not listed because such results are difficult to compare. The acute toxicity (LC50 [median lethal concentration] values) to earthworms as nontarget organisms was identified as the main measurement endpoint because earthworms are widely accepted as the most important soil invertebrate, not only in temperate

Table 1. Percentage of farmers using a specific pesticide (total numbers can be >100% because farmers use more than 1 pesticide; Waichman et al. 2002) and the toxicological classifications according to World Health Organization (WHO) guidelines

Active ingredient	Use %				
	WHO classification [a]	Careiro da Várzea	Iranduba	Manacapuru	Manaus
Insecticide					
Abamectin	II	1.7	1.3	0.0	37.5
Deltamethrin	II	20.0	26.9	19.5	2.5
Indoxacarb	II	3.3	0.0	0.0	0
Lambda-cyhalothrin	II	33.3	1.3	0.0	17.5
Malathion	III	18.3	0.0	7.3	2.5
Methamidophos	Ib	5.0	6.4	9.8	32.5
Methyl-parathion	Ia	5.0	38.5	26.8	17.5
Fungicide					
Copper oxychloride	III	31.7	29.5	9.8	80.0
Mancozeb	U	6.7	5.1	14.6	52.5
Herbicide					
Glyphosate	U	6.7	9.0	26.8	0.0
Linuron	III	0.0	0.0	0.0	2.5

[a] Ia = extremely hazardous; Ib = highly toxic; II = moderately toxic; III = slightly toxic; U = unlikely to present acute hazard in normal use.

regions but also in many tropical soils (Lavelle et al. 1997). In addition, it was the only (temperate) data set covering all pesticides because it must be reported during pesticide registration in the European Union (EU 1991, 1997; EC 2002). When only acute data (LC50 values) were found, chronic data (no observed effect concentration; NOEC) were estimated by dividing the LC50 values by a factor of 10 (Van den Brink et al. 2003). On the basis of experiences with copper oxychloride in soil, even a factor of more than 10 has been proposed (Maboeta et al. 2004).

Risk was estimated with the toxicity exposure ratio (TER) by dividing the LC50 or NOEC values by the respective PECs. Toxicity exposure ratios were compared with trigger values proposed by the European Union: 10 for the acute test data and 5 for the chronic toxicity data. In total, 120 use scenarios were generated, consisting of real combinations of sites, crops, and pesticide applied (including application rates, frequencies, etc.). The aim was to determine the initial PECs in the soil for each scenario on the basis of the use pattern of the 11 pesticides in the different crops. The PEC was calculated with the FOCUS model (FOCUS Soil Modeling WorkGroup 1997) because no specific model is available for tropical soils. The PEC after a single application was estimated from the application rate, the relevant soil depth, and the soil bulk density. The PECs for multiple applications were calculated with the same information as described above, plus the initial PEC for 1 application, the dissipation rate constant of pesticide, the number of applications, and the days between applications. For this 1st step, we used median disappearance time (DT50) determined under temperate conditions (Table 4). For cases in which several values were found, either the mean value was taken or, most often, a realistic worst case was used.

In the 2nd step, we performed a refined ERA by calculating PECs with DT50 values determined under tropical conditions (i.e., field soils from Brazil, high temperature, etc.; some performed in the laboratory, some directly in the field). Only for cases in which no data were found (indoxacarb, linuron, malathion, and mancozeb) were the respective DT50 values were estimated by dividing the temperate DT50 values by a factor of 2. This factor was used because the ratio between temperate and tropical DT50 values for the 6 other pesticides listed in Table 4 was on average about 2. In addition, the same factor was proposed in a review on the fate of pesticide in tropical soils (Racke et al. 1997). In the 3rd step, we performed a plausibility check of the results of the refined ERA. For this check, the effect data available for tropical soil organisms were used to discuss whether the outcome of the refined ERA is useful to fulfill the goal of the ERA of a pesticide: to protect the respective environmental compartment (here, the soil and its fauna) from the environmental risk that can be caused by the use of a certain pesticide. That is, we assessed whether the outcome of this ERA is over- or underprotective for the Amazonian soil ecosystem.

RESULTS

First step: Risk assessment

The number of use scenarios for which a potential risk could be identified differs strongly between the 11 pesticides (Table 5). A risk is indicated in 10% of all scenarios with the use of TER acute values and in 26.7% of all scenarios with TER chronic values. No risk for earthworms was found for abamectin, deltamethrin, indoxacarb, lambda-cyhalothrin, and linuron. Only moderate risks were identified for mancozeb. A consistent risk was indicated for the remaining

Table 2. Range of doses (g ai/ha), number of applications per cycle, and interval between applications (in days) of the pesticides used in the study region compared with the numbers recommended by the producing companies for the use of these pesticides in Brazil. On demand = the application depends on the occurrence of pest organisms or on the pressure of an infection (i.e., the necessity of application is decided by the individual farmer)

	Determined in the study region (questionnaire)			Recommended dose for Brazilian crops		
Active ingredient	Applied dose (g ai/ha)	Nr of applications ($cycle^{-1}$)	Application interval (d)	Applied dose (g ai/ha)	Nr of applications ($cycle^{-1}$)	Application interval (d)
Abamectin	2.8–21.1	5–27	7–10	7.2–14.4	2–4	7–14
Copper oxychloride	11.76–1,201.4	3–66	5–15	1,470–1,764	3–6	10–15
Deltamethrin	0.2–15.5	2–60	2–45	3.7–6.2	On demand	7–21
Glyphosate	9.6–1,135.5	2–61	1–110	480–2,880	On demand (maximum, every 2 d postemergence)	
Indoxacarb	78.7–111.3	9–22	6–12	18–27	$\leq$5	7
Lambda-cyhalothrin	2.0–111.1	6–35	3–12	7.5–15	On demand	5
Linuron	20.1–42.9	1–6	30–45	720–990	1 only, 21 d after emergence	
Malathion	5.0–1,000.0	5–45	2–8	500–750	3	7–15
Mancozeb	6.0–32,000.0	3–52	7–45	1,600–2,400	5	7–15
Metamidophos	6.6–1,666.7	5–43	5–21	300–600	On demand	On demand
Parathion-methyl	9.0–4,000.0	2–54	4–15	180–480	On demand	On demand

Table 3. Effect values used for the TER calculations (including data sources and main test conditions), separated for temperate conditions (upper part) and tropical conditions (lower part). Tests were performed with artificial soil except those with copper oxychloride[a]

Pesticide	LC50 (mg/kg)	Source	Species	NOEC (mg/kg)	Source	Species
Temperate conditions						
Abamectin	17.1	Sun et al. 2005	*Eisenia fetida*	9.8	Kolar et al. 2007	*Eisenia andrei*
Copper oxychloride	98.0[b]	Haque and Ebing 1983	*Lumbricus terrestris*	—	—	—
Deltamethrine	>1,290	SEEM 2002	*E. fetida*	—	—	—
Glyphosate	>480	SEEM 2002	*E. fetida*	—	—	—
Indoxicarb	>1,250	EU 2005	*E. fetida*	>15.6	EU 2005	*E. fetida*
Lambda-cyhalothrin	99.8	Garcia 2004	*E. fetida*	3.6	Garcia 2004	*E. fetida*
Linuron	>1,000	SEEM 2002	*E. fetida*	—	—	—
Malathion	42	Kuperman et al. 1999	*E. fetida*	—	—	—
Mancozeb	>299	SEEM 2002	*E. fetida*	20	SEEM 2002	*E. fetida*
Methamidophos	17	Haque and Ebing 1983	*E. fetida*	—	—	—
Parathion-methyl	60	Kula 1995	*Aporrectodea caliginosa*	0.72	Van Gestel et al. 1995	*E. fetida*
Tropical conditions						
Abamectin	28.0	Wislocki et al. 1989	*E. fetida*	—	—	—
Copper oxychloride	1,900[b]	Garcia, unpublished data	*E. fetida*	12[a]	Garcia, unpublished data	*E. fetida*
Glyphosate	>1,000	Garcia et al. 2007	*E. fetida*	360	Garcia et al. 2007	*E. fetida*
Lambda-cyhalothrin	23.8	Garcia 2004	*E. fetida*	6.2	Garcia 2004	*E. fetida*
Mancozeb	867.8	Garcia, unpublished data	*E. fetida*	—	—	—
Parathion-methyl	99.7	Garcia, unpublished data	*E. fetida*	—	—	—

[a] LC50 = median lethal concentration; NOEC = no observed effect concentration.
[b] Field soil.

Table 4. Median degradation time (DT50) in soil of the pesticides used in the study region measured under temperate and tropical conditions (data provided by FAO [2003] not considered because of unclear test conditions)

Active ingredient	Use type	DT50 temperate	Source	DT50 tropical	Source
Abamectin[a]	Insecticide	56[b]	Halley et al. 1993	28	Van den Bosch et al. 2005
Copper oxichloride	Fungicide	Not applicable	—	Not applicable	—
Deltamethrin	Insecticide	35	Laabs, Amelung, Fent, et al. 2002	12.4[c]	Laabs, Amelung, Pinto, Zech 2002; Laabs, Amelung, Fent, et al. 2002
Glyphosate	Herbicide	32[d]	Giesy et al. 2000	27.7[e]	de Andrea et al. 2003
Indoxacarb	Insecticide	117	EU 2005	58.5[f]	—
Lambda-cyhalothrin	Insecticide	35.5[c]	Laabs, Amelung, Fent, et al. 2002; Van den Bosch et al. 2005	8.8[5]	Laabs, Amelung, Pinto, Zech 2002; Reichenberger et al. 2002
Linuron	Herbicide	62.9[c]	Beyer and Matthies 2001; Paraiba et al. 2003	31.5[4]	—
Malathion	Insecticide	10	Van den Bosch et al. 2005	5.0[d]	—
Mancozeb	Fungicide	4.9	Van den Bosch et al. 2005	2.5[d]	—
Metamidophos	Insecticide	2.0	Beyer and Matthies 2001	2.6	Van den Bosch et al. 2005
Parathion-methyl	Insecticide	49	Van den Bosch et al. 2005	22.5[g]	Sattar 1990

[a] Mixture of avermectin B1a and avermectin B1b.
[b] Higher value determined for the avermectin B1a component only (Moye et al. 1987): 102 d.
[c] Mean value of results from different soils.
[d] Very variable; 10.1 to 102 d are reported (Giesy et al. 2000; Kools et al. 2005).
[e] Lower numbers reported: 2 to 2.6 d (USEPA 2003).
[f] Estimated by dividing the temperate DT50 values by a factor of 2.
[g] Higher numbers reported: 64.5 d (Agarwal et al. 1994).

4 pesticides, for which, in nearly 40% (copper oxychloride) and less than 20% (malathion, metamidophos, parathion-methyl) of the use scenarios, the TER acute trigger was breached. Considerably higher values (between 45% and 67% of scenarios) were found with the TER chronic trigger for these 4 pesticides. The probability that a certain pesticide causes a risk for the soil compartment is loosely correlated with its use type (and mode of action, or both); although herbicides almost never caused a risk, 1 fungicide was among the most toxic substances and another showed just a moderate risk. Out of 7 insecticides, risks for 4 were negligible, and the other 3 were obviously toxic in many use scenarios. Finally, the increase in use scenarios indicating a risk for soil organisms from 10% to 27% shows that the ERA from acute test results can only identify one-third of all scenarios in which risk occurs.

On the basis of the information from the risk assessment, it was also possible to compare the crops independently from the site or pesticide in terms of potential risk (Table 6). Because the different crops are grown in unequal numbers (i.e., several crops at 1 site, only 1 crop at all 4 sites) the number of scenarios per crop also differs considerably and any comparison is difficult. However, a rough classification is possible. The cultivation of 8 of 18 crops (i.e., nearly 50%) never caused an indication of risk (i.e., no trigger was breached). However, these 8 crops covered only 24 of 120 scenarios. In the case of 5 crops (cabbage, cucumber, lettuce, okra, and passion fruit), both TER values (acute, chronic) were breached in 12.5% to 28.6% (TER acute) and 25.0% to 57.1% (TER chronic) of all scenarios with the respective crop. These 5 crops represent together 50 scenarios. For the remaining 5 crops (bunching onion, kale, eggplant, papaya, and tomato), a risk was indicated by the TER chronic value only. The most problematic crop was cucumber, in which 57% of all scenarios (8 of 14) indicated a risk.

Finally, it can be evaluated whether the number of cases in which a risk was identified differs at the 4 sites independent of the individual pesticides or crops (Table 7). When considering the percentage of scenarios compared with the total number, it becomes clear that, on the basis of acute TERs, fewer trigger values were breached at Careiro and Iranduba (3% and 5%, respectively) compared with Manacapuru and Manaus (16%

Table 5. Number of scenarios for each pesticide for which the respective trigger value was breached in the risk assessment (A), refined risk assessment (B) and plausibility check (C)

Pesticide	Nr of scenarios	Acute trigger breached (% of scenarios)			Chronic trigger breached (% of scenarios)		
		A	B	C	A	B	C
Abamectin	3	0.0	0.0	0.0	0.0	0.0	0.0
Copper oxychloride	13	38.5	38.5	0.0	61.5	61.5	61.5
Deltamethrin	22	0.0	0.9	—	0.0	0.0	—
Glyphosate	10	0.0	0.0	0.0	10.0	10.0	0.0
Indoxacarb	2	0.0	0.0	—	0.0	0.0	—
Lambda-cyhalothrin	6	0.0	0.0	0.0	0.0	0.0	0.0
Linuron	3	0.0	0.0	—	0.0	0.0	—
Malathion	6	16.7	16.7	—	66.7	33.4	—
Mancozeb	23	4.4	4.4	0.0	8.7	4.4	4.4
Metamidophos	11	18.2	18.2	—	45.5	54.5	—
Parathion-methyl	21	14.3	14.3	14.3	57.1	52.4	18.4
Total	120	10.0	10.0	—	26.7	24.2	—

and 32%, respectively). The values for the acute TERs were 17% and 23% compared with 32% and 42% for the chronic TER, respectively.

Second step: Refined ERA

It should be noted that the same temperate toxicity values (earthworm LC50s and NOECs (=LC50/10), but tropical DT50 values, were used for the calculation of refined TERs because the number of toxicity values from tropical areas was not sufficient for this purpose.

The outcome of this comparison did not differ considerably from the results of the risk assessment (Table 5). In both steps a risk was indicated in 10% of all scenarios with the use of TER acute values, whereas the number of risk scenarios decreased from 26.7% to 24.3% of all scenarios with TER chronic values. In addition, the same pesticides were identified as being of concern. The same is true for the comparison of crops, in which individual numbers changed slightly; again, cucumber was identified as the crop with the highest rate of concern: 50% of all scenarios (7 of 14; Table 6). Not surprisingly, when comparing the 4 sites, the distribution of scenarios in which risk was identified is also nearly identical with that found in the risk assessment (Table 7).

Third step: Plausibility check

In the risk assessment approach presented, the outcome of the refined assessment was checked in terms of plausibility by calculating new TERs with the tropical PECs and with the effect values from earthworm tests performed under tropical conditions. Unfortunately, only LC50 and NOEC values for 6 pesticides (abamectin, copper oxichloride, glyphosate, lambda-cyhalothrin, mancozeb, and parathion-methyl) can be used for this purpose. Therefore, only the TER calculations for those pesticides are presented in Table 5.

Use of the tropical earthworm LC50 values did not lead to a breach of the TER acute trigger values for 5 of the 6 pesticides. For copper oxychloride, no risk was identified compared with the risk assessment and the refined ERA. In contrast, the percentage of risky scenarios for parathion methyl did not change in the risk assessment, the refined ERA, and the plausibility check. With the use of actual NOEC values determined in tropical tests, the percentage of scenarios in which TER chronic values were breached remained (almost) the same for copper oxychloride and mancozeb after the plausibility check. In the case of parathion-methyl, the plausibility check indicated risk for approximately one third of the scenarios, with the TER chronic value breached on the risk assessment and the refined ERA. Finally, no risk was indicated for the other scenarios with abamectin, glyphosate, or lambda-cyhalothrin.

DISCUSSION

The environmental risk assessment of pesticides in soils of the Amazon described here was based on the regulations of the European Union. However, because of the scarcity of soil fate and effect data under tropical conditions, we could not use the EU approach in detail. In addition, we modified this EU approach with the use of PECs calculated on the basis of real local conditions (e.g., application rates and frequencies, crops, environmental conditions). This approach has been used successfully in Thailand and Sri Lanka for the aquatic compartment (Van den Brink et al. 2003). This way of assessing the risk of pesticides to soil organisms leads to differentiated results that could be (unfortunately only for a few substances) confirmed in a plausibility check.

In the refinement step of the ERA, the PECs have been modified with the use of tropical DT50 values. These values are on average a factor of 2 lower than those known from temperate regions. This change of PECs did not affect the TERs considerably: The number of use scenarios showed only a very slight potential risk of decrease. Therefore, it seems that temperate DT50 values can be used for the calculation of PECs in tropical regions, preferably with a safety factor of 2.

Table 6. Number of scenarios for each crop for which the respective trigger value has been breached in the risk assessment (A) and refined risk assessment (B)

Crop	Nr of scenarios	Acute trigger breached (% of scenarios)		Chronic trigger breached (% of scenarios)	
		A	B	A	B
Banana	6	0.0	0.0	0.0	0.0
Bunching onion	18	0.0	0.0	16.7	16.7
Kale	10	0.0	0.0	20.0	20.0
Cabbage	7	28.6	28.6	42.9	42.9
Long coriander	3	0.0	0.0	0.0	0.0
Cucumber	14	28.6	28.6	57.1	50.0
Eggplant	3	0.0	0.0	33.3	33.3
Gherkin	5	0.0	0.0	0.0	0.0
Lettuce	16	25.0	25.0	43.8	37.5
Melon	1	0.0	0.0	0.0	0.0
Okra	5	20.0	20.0	40.0	20.0
Papaya	8	0.0	0.0	12.5	25.0
Paprika	2	0.0	0.0	0.0	0.0
Passion Fruit	8	12.5	12.5	25.0	25.0
Pepper	4	0.0	0.0	0.0	0.0
Sweet Potato	1	0.0	0.0	0.0	0.0
Tomato	7	0.0	0.0	43.0	28.6
Watermelon	2	0.0	0.0	0.0	0.0
Total	120	10.0	10.0	26.7	24.2

However, this observation is based on only a few (11) pesticides, meaning that much more data are needed before a recommendation can be given as how to proceed. In addition, it should be kept in mind that this result might have been confounded by the influence of soil properties. In the literature is a consensus that DT values can easily differ by a factor of 10 for the same compound applied in the same climatic zone but on different soils (e.g., Racke et al. 1997). This influence could not sufficiently be considered here because of a lack of data.

Probably more important than the problems discussed so far for the exposure situation is the lack of data on the effect side: Although it was possible to find temperate LC50 values for all pesticides, only one-third of such values were determined under tropical conditions. The number of values is even lower if only data from peer-reviewed literature is considered. So far, it seems that there is no systematic difference between temperate and tropical earthworm LC50 values. However, the number of available data from soil species does not allow us to propose a certain relationship concerning the sensitivity of temperate and tropical species as has been proposed recently for aquatic ecosystems (Kwok et al. 2007). Therefore, it is extremely important to increase the number of earthworm (and arthropod) toxicity values

Table 7. Number of scenarios for each site for which the respective trigger value has been breached in the risk assessment (A) and refined risk assessment (B)

Site	Nr of scenarios	Acute trigger breached		Chronic trigger breached	
		A	B	A	B
Careiro da Várzea	29	3.4	3.4	17.2	17.2
Iranduba	47	2.1	2.1	23.4	19.1
Manacapuru	25	16.0	16.0	32.0	32.0
Manaus	19	31.6	31.6	42.1	36.8
Total	120	10.0	10.0	26.7	24.2

determined under standardized conditions. The necessary methodological modifications have already been proposed (Garcia 2004) but need to be validated and standardized.

When comparing the use scenarios that have been identified as causing a potential risk for earthworms with the respective PECs (and the application rates and frequencies), it becomes clear that, in some cases, the exposure is extremely high (in particular for glyphosate and mancozeb), indicating that these pesticides are problematic because of misuse. Even though such high application rates, application frequencies, and shorter intervals are not recommended on the label, it is not known how often such a misuse is happening at a site, meaning that the extent of this problem cannot be assessed. On the other hand, the insecticides metamidophos, parathion-methyl, and malathion, as well as the fungicide copper oxychloride, already caused risks at low concentrations of between 0.3 and 2.0 mg/kg (insecticides) and 4.7 mg/kg (fungicide). This high toxicity of copper compounds and parathion-methyl to earthworms is in agreement with results from temperate regions (Römbke et al. 1994; Frampton et al. 2006). Effects at such low concentrations confirm the observation that, for these pesticides, many use scenarios cannot be supported.

One possible way to reject or confirm the potential risk of these 4 compounds would be to investigate their effects in the field (e.g., by performing standardized field tests with modifications of existing tests) (ISO 1999; Römbke et al. 2003) or to conduct monitoring studies (preferably by measuring the concentration of the pesticides in the soil and the number, biomass, and diversity of earthworms and other soil organisms at the same sites in agricultural regions), thus following a triad approach (Chapman 2000; Rutgers et al. 2000). With 1 recent exception (Garcia 2004), such field investigations are not known from the Amazon. The only indication that this could be a problem for wide areas is the observation that, in the water of several river sites located in the Pantanal area, malathion was found in about 26% of all samples taken. This pesticide is regularly used for cash crops (mainly soybean) of the highland regions of northern Mato Grosso state (Laabs, Amelung, Pinto, Wantzen, et al. 2002).

The number of use scenarios for which a risk has been identified differs only very slightly between the risk assessment (26.7%) and the refined risk assessment (24.2%) when compared with the total number of use scenarios. This small difference was not expected because, on average, the dissipation was quicker by a factor of 2 under tropical conditions. This means that in about one fourth of all cases, a risk for soil organisms has been found.

The 4 sites investigated in this study are representative of the agricultural practice in the region of Manaus (for details, see Waichman and Nina 2003). Certainly, the risk of pesticides is different in the crops grown in this region. The cultivation of 8 of 18 crops (i.e., nearly 50%) never indicated risk. However, these 8 crops covered only 24 of 120 scenarios. In a further 5 crops (papaya, bunching onion, kale, okra, and passion fruit), a risk could occur after pesticide application in 12.5% to 25% of all use scenarios. Even higher was the percentage of use scenarios with a potential risk in the last 5 crops: Cabbage, cucumber, eggplant, lettuce, and tomato. The most problematic crop was cucumber, for which a risk was indicated in 50% of all scenarios (7 of 14).

Differences between sites are also apparent: The number of such use scenarios is lowest in Careiro and highest in Manaus, with Iranduba and Manacapuru in between. Factors such as the availability of pesticides and cultural needs play an important role in explaining these differences. For example, if mainly banana or gherkin are planted—crops that usually do not require a lot of pesticides—the probability of breaching a TER trigger is much smaller than at sites where cucumber or tomato are planted. A 2nd related factor influencing this difference could be the distance to the nearest market, and thus the marketability of the fruits and vegetables. This might be the main reason for the high percentage in Manaus, assuming that the city population preferentially selects higher quality produce.

All of the 11 compounds presented in this study are registered in Brazil, but when looking at the registration status in temperate regions, for example in the European Union, the situation is different. Out of the 11 pesticides used in the study area, 2 compounds, parathion-methyl and malathion, have already been banned there. In the case of 2 other pesticides (abamectine and copper oxychloride), the decision is pending (partly for several years). The remaining 7 pesticides are registered in the European Union. Interestingly, copper oxychloride, with a decision pending in the European Union, is most often the cause of potential risk to earthworms (in addition to the 2 banned pesticides) according to the data presented here.

Unfortunately, this does not mean that restricting the use of the risky compounds in the Amazon would protect the soil organism community because the ERA is based only on earthworm data so far. Also knowing that arthropods are ecologically relevant in the Amazon, Garcia (2004) tested the effects of lambda-cyhalothrin on the isopod species *Porcellionides pruinosus*. When comparing his results (e.g., the LC50 of 0.2 mg/kg) with the PECs of lambda-cyhalothrin, the TER acute trigger was breached in 5 of 6 use scenarios, whereas no risk was identified in any scenario when using earthworm LC50 values. Garcia (2004) confirmed the risk of this insecticide to the soil ecosystem by performing semifield and field studies at the EMBRAPA site close to Manaus. Assuming that similar results would be found when testing other insecticides with arthropods as test species (e.g., abamectin for collembolans; see Kolar et al. 2007), the use of earthworms as the sole representatives of the soil invertebrate community is certainly not sufficient (the same conclusion was drawn for temperate regions as well; Jänsch et al. 2006).

As has been confirmed in this study, the ERA for tropical soils is strongly affected by the existence of only very few fate and, even more pronounced, effect data. Therefore, it is suggested that the following studies should be conducted.

- Measure the concentration of the most relevant pesticides in the soil (and, if possible, in organisms like earthworms).
- Test the effects of the same pesticides on selected (preferably native) species of different soil invertebrates.
- Perform (fate and effect) field tests and monitor studies in appropriate local areas.

In fact, comparable suggestions have already been made for tropical African countries (Wiktelius et al. 1999).

The long-term aim of these activities is to perform an ERA according to the TRIAD approach for the most important pesticides in the Amazon region. Such a test program considering the specific regional conditions would also be important for other tropical countries.

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