NRA Special Review of

Tribufos (DEF)

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FOREWORD

The National Registration Authority for Agricultural and Veterinary Chemicals (NRA) is an independent statutory authority with responsibility for the regulation of agricultural and veterinary chemicals. One of the NRA's regulatory responsibilities is to conduct reviews of registered agricultural and veterinary chemicals to ensure that they continue to do the job that they are supposed to do and that they do not pose unacceptable risks to people, the environment or trade.

The Special Review Program examines urgent or specific concerns about a currently registered agricultural or veterinary chemical, which may require a rapid resolution. It addresses one or more specific aspects of a given chemical, and can be triggered, for example, by the findings of new research, the availability of new scientific data or concerns raised about the use or safety of a chemical.

In undertaking reviews, the NRA works in close cooperation with advisory agencies including the Department of Health and Family Services (Chemicals and Non-Prescription Drug Branch), Environment Australia (Risk Assessment Branch), Worksafe Australia (Chemical Assessment Division) and State Departments of Agriculture.

The NRA has a policy of encouraging openness and transparency in its activities and community involvement in decision-making. When the NRA decides that a review is to be conducted, it consults parties affected by the review (such as applicants, commodity groups, State regulatory agencies) and gives them an opportunity to respond to concerns raised and participate in the review. All participants are notified of the Board's decision and outcomes of special reviews are published in the NRA's Agricultural and Veterinary Chemicals Gazette.

This review report provides an overview of the review that has been conducted by the NRA and advisory agencies. The review findings are based on information collected from a variety of sources, including data packages and information submitted by registrants, information submitted by members of the public, and government organisations and literature searches.

The NRA also makes these reports available to the public and regulatory agencies of other countries which are part of the OECD ad hoc exchange program and as part of bilateral exchange agreements with other countries. Under the OECD ad hoc exchange program, it is proposed that countries receiving these reports will not utilise them for registration purposes unless they are also provided with the raw data from the relevant applicant.

The information and technical data required by the NRA to review both new and existing chemical products must be derived according to accepted scientific principles, as must the methods of assessment undertaken. Details of required data are outlined in various NRA publications.

Other publications explaining the NRA's requirements for registration can also be purchased or obtained by contacting the NRA. Among these are: *Ag Requirements Series*; and the *Vet Requirements Series*.

The NRA welcomes comments on this review and its review program. They can be addressed to Manager, Chemical Review, National Registration Authority for Agricultural and Veterinary Chemicals, PO Box E240, Kingston ACT 2604 Australia.

ABBREVIATIONS AND ACRONYMS

µg	Microgram
AA	Aerial Applicator
AAVCC	Australian Agricultural and Veterinary Chemicals Council
ACAC	Agricultural Chemicals Advisory Council
AChE	Acetylcholinesterase
ACPH	Advisory Committee on Pesticides and Health
ADI	Acceptable Daily Intake
ai	Active ingredient
AMLCS	Closed system mixing/loading
AMLOS	Open system mixing/loading
bodywt	Bodyweight
BUN	Blood urea nitrogen
ChE	Cholinesterase
CHO	Carbohydrate
d	Day
DPCF	Dermal penetration coefficient factor
DPSSC	Drugs and Poisons Schedule Standing Committee
DRL	Dislodgeable residue levels
EC	Emulsifiable Concentrate
EHSU	Environmental Health and Safety Unit
ERG	Electroretinogram
FL	Flagger
GA	Ground applicators
GIT	Gastrointestinal Tract
GLP	Good Laboratory Practice
GMLCS	Ground mixer/loaders
h	Hour
ha	Hectare
HD	High dose
in vitro	outside the living body and in an artificial environment
in vivo	inside the living body of a plant or animal
LD	Low dose
LD50	Dosage of chemical that kills 50% of the test population of organisms
LOEL	Lowest Observed Effect Level
MBO	Module builder operator
MD	Medium dose
mg/kg bw/d	Mg/kg bodyweight/day
min	Minute
mM	Millimolar
MOE	Margin of Exposure
MRL	Maximum Residue Limit
MSDS	Material Safety Data Sheet
NDPSC	National Drugs and Poisons Scheduling Committee
NHMRC	National Health and Medical Research Council
nM	Nanomolar
NOEL	No Observable Effect Level
NOHSC	National Occupational Health and Safety Commission
NTE	Neuropathic Target Esterase
OH&S	Occupational Health and Safety
OP	Organophosphorus Pesticide
OPIDN	Organophosphate Induced Delayed Neuropathy
PACC	Pesticides and Agricultural Chemicals Committee
PNS	Peripheral Nervous System
PO	Picker operator
POEM	Predicted Operator Exposure Model
ppb	Parts per billion
PPE	Personal Protective Equipment
ppm	Parts per million
RBC	Red blood cell/erythrocyte
RK	Rakers
RLC	Registration Liaison Committee
SF	Safety factor
SRL	Safe dislodgeable residue level
SUSDP	Standard for the Uniform Scheduling for Drugs and Poisons
TC	Dermal transfer coefficient
TGAC	Technical Grade Active Constituent
TR	Trampers
USEPA	US Environment Protection Authority
WHP	Withholding Period

EXECUTIVE SUMMARY

Tribufos (s,s,s-tributyl phosphorotrithioate) is a plant growth regulator belonging to the organophosphate group of pesticides. Tribufos was registered for use on cotton as a defoliant.

Background

At their May 1989 meeting, the Drugs and Poisons Schedule Standing Committee (DPSSC) raised concerns on the adequacy of toxicological data for tribufos. The data was considered deficient by current standards. Tribufos was rescheduled from Schedule 6 to Schedule 7 in the Standard for Uniform Scheduling of Drugs and Poisons (SUSDP). Further to this the former Australian Agricultural and Veterinary Chemicals Council (AAVCC) endorsed deregistration of tribufos and accompanying products.

The registrant was advised that approval of tribufos, registration of all products containing tribufos (one) and the approval of the associated product label were to be cancelled, and the MRLs for tribufos recommended for withdrawal. However, this action was not taken as the company agreed to withdraw the product from sale pending clarification of the toxicological issues. The assessment of further toxicological and worker exposure data and representations by the sponsor were the subject of the current special review.

Product details

Label directions for the use of defoliation of cotton requires tribufos to be applied as a spray by aircraft or ground rig at rates of 1.4 to 2.8 L/ha (987 to 1987 g ai/ha) to mature cotton when 50% or more of the bolls are open.

Data submitted

Data which included toxicological and occupational health and safety studies were submitted by the registrant during 1993, 1994, 1995 and 1996 for consideration by the NRA.

Hazard to users

Tribufos has demonstrated the potential to cause organophosphate induced delayed polyneuropathy. Australia has a policy not to register chemicals which have demonstrated this potential. In studies submitted by the registrant to the NRA significant cholinesterase (ChE) inhibition was also evident.

In the OH&S assessment, safe use could not be demonstrated when extensive personal protective equipment was worn and engineering controls were in place.

Outcomes

Following its reconsideration of tribufos the NRA therefore concluded that the continued use of tribufos would be an undue hazard to the safety of people exposed to it during its handling.

Accordingly, key outcomes of the review were:

- that registration of the product Def Defoliant be cancelled;
- that no future applications for registration of products containing tribufos be granted unless the toxicology and worker exposure issues raised in this review can be addressed; and
- that MRLs for tribufos be withdrawn.

Registration of the product Def Defoliant was formally cancelled following a request by the Registrant on 30 June 1997; no TGAC sources for tribufos were ever approved. The NRA will not register a product based on the active constituent, tribufos, without concerns identified in both the Public Health and OH&S assessment reports being adequately addressed.

PART ONE

MAIN REPORT

1. INTRODUCTION

Tribufos is an organophosphate plant growth regulator, with herbicidal (defoliation) activity. Tribufos is registered in Australia in one product, Def Defoliant, for use as a defoliant in cotton. It has not been marketed in Australia since 1990.

1.1 REASONS FOR REVIEW

At its May 1989 meeting the Drugs and Poisons Schedule Standing Committee (DPSSC) considered a toxicological report on tribufos. The available data was considered deficient. The main concerns related to possible adverse effects on the nervous system; in addition, in all studies significant cholinesterase (ChE) inhibition was evident. Inadequate studies were available on tumourigenic effects of this compound, and no developmental or mutagenicity data were provided. Specifically, the data was considered to be deficient in the following areas:

- metabolic/toxicokinetic data;
- sensitisation studies;
- subchronic data demonstrating clear NOELs;
- carcinogenicity studies (2 species);
- developmental studies (2 species); and
- genotoxicity data.

Signficant concerns were expressed about delayed neurotoxicity (with nerve fibre alteration and subsequent nerve degeneration) and ChE inhibition after exposure to low levels of this chemical.

1.2 SCOPE OF THE REVIEW

All registered end-use products containing tribufos, the associated label approvals and approvals of the active constituent tribufos were subject to review. The review focus was on the supplementary toxicity data as they related to occupational exposure, and with an emphasis on delayed neuropathy and cholinesterase inhibition.

1.3 NOTIFICATION OF REVIEW

Bayer Australia Ltd, the registrant of the single registered product containing tribufos and the applicant for TGAC approval, was notified of the proposed reconsideration in March 1989 by the DPSSC and the AAVCC.

The registrant was advised of the inadequacy of toxicology data submitted as part of the TGAC application. The Registration Liaison Committee (RLC) was notified of the review in July 1994. Comments from the State Coordinators were obtained on

whether withdrawal of the product would adversely affect agricultural practices in their state.

1.4 REGISTRATION STATUS

The following product and TGAC were included in the NRA's review with the involvement of one registrant and TGAC approval holder.

Table 1: Products and TGACs included in the review

Product Name	**Registrant**
Def Defoliant	Bayer Australia Pty Ltd (Crop Protection)
Active Constituent	**Applicant**
Tribufos	Bayer Australia Pty Ltd (Crop Protection)

1.5 REGULATORY STATUS IN AUSTRALIA

The Agricultural Chemicals Advisory Committee (ACAC) and the Drugs and Poisons Schedule Standing Committee (DPSSC) considered toxicological data associated with tribufos. Following its consideration, the DPSSC determined that the existing scheduling at Schedule 6 of the SUSDP was not appropriate for tribufos. In August 1989 the DPSSC was advised that alternatives to tribufos were available in Queensland and NSW, the only states where it was registered for use. The committee proposed that tribufos be deleted from Schedule 6 and placed in Appendix M (substances for which registration under agricultural and veterinary chemicals legislation cannot be supported by scheduling until further toxicological information becomes available) because of toxicological data deficiencies.

The registrant requested that the DPSSC review this decision and, after reconsideration of this request, tribufos was rescheduled to Schedule 7.

1.6 REGULATORY STATUS OVERSEAS

The status of tribufos in countries other than the USA has not been ascertained; however, it is not listed on the United Nations Consolidated List of Products whose Consumption and/or Sale Have Been Banned, Withdrawn, Severely Restricted or not Approved by Governments (United Nations 1994).

In the USA, tribufos was considered for re-registration in March 1992. Under the review criterion of neurotoxicity, the USEPA returned a finding of no unreasonable adverse effects and returned tribufos to the registration process. The applicant agreed to protective clothing requirements imposed on the product's use (USEPA 1994).

In the USA, tribufos is marketed by Bayer. The registered product is DEF 6, an emulsion containing 70.5% tribufos and identical to the product formerly registered in Australia. It is used as a cotton defoliant.

1.7 CONSULTATION WITH STATE AUTHORITIES

During the review the States/Territories and other members of the RLC provided input which is summarised below.

Tasmania, South Australia, West Australia, Victoria and the ACT

No product containing tribufos was registered in these states and, therefore, there were no local implications in the event of a withdrawal of the clearance for Def Defoliant.

Queensland (Qld)

Def Defoliant has not been available to cotton growers since 1990 and alternative products have been used. Therefore, in view of the toxicology and worker exposure concerns, Qld supported the cancellation of the clearance of this product.

New South Wales (NSW)

The response from NSW commented on the fact that there was some demand for Def Defoliant because of its lower cost. However, noting that there are alternative products available, NSW recommended the withdrawal of the clearance for this product and confirmed that the withdrawal would have no adverse effects on the cotton industry in that state.

1.8 PUBLIC HEALTH STANDARDS

As part of the review, the Department of Health and Family Services conducted an extensive assessment of the toxicity of tribufos, including evaluation of previously unevaluated data and consolidation of previous toxicology reports. The reports of these assessments are included in Part 2.

The scheduling of tribufos in Schedule 7 of the SUSDP is considered appropriate on toxicological grounds. No ADI has been set for tribufos.

1.9 SAFETY DIRECTIONS AND OCCUPATIONAL HEALTH AND SAFETY STANDARDS

Worksafe Australia conducted the OH&S assessment of the available data on tribufos, concluding that registration of Def Defoliant could not be supported on occupational

health and safety grounds. Safe use could not be demonstrated when extensive personal protective equipment was worn to prevent skin contamination and when engineering controls were in place. Furthermore they did not consider that the addition of respiratory protection was likely to reduce overall exposure to acceptable levels. Therefore, safety directions for this product could not be established. The OH&S assessment report is included in Part 3.

1.10 REVIEW OUTCOMES

Following its reconsideration of tribufos, the NRA concluded that the continued use of tribufos would be an undue hazard to the safety of people exposed to it during its handling.

Accordingly, key outcomes of the review were:

- that registration of the product Def Defoliant be cancelled;
- that no future applications for registration of products containing tribufos be granted unless the toxicology and worker exposure issues raised in this review can be addressed; and
- that MRLs for tribufos be withdrawn.

Registration of the product Def Defoliant was formally cancelled following a request by the registrant on 30 June 1997; no TGAC sources for tribufos were ever approved. The NRA will not register a product based on the active constituent, tribufos, without concerns identified in both the Public Health and OH&S assessment reports being adequately addressed.

PART TWO

TOXICOLOGY ASSESSMENT REPORT

Prepared by

Chemicals and Non-Prescription Drug Branch
Therapeutic Goods Administration

2.0 TOXICOLOGY ASSESSMENT REPORT

SUMMARY

The following assessment report contains only summaries of new studies generated or provided to support the continued marketing of tribufos, following the halt to its marketing in Australia in 1990, due to toxicological concerns. A summary of all available studies on tribufos may be found in the consolidated toxicology summary (see pages 29-34).

Introduction

S,S,S-tributyl phosphorotrithioate (DEF), an organophosphorus plant growth regulator, was used as a defoliant of cotton in NSW and QLD. In May 1989, the NHMRC Chemical Committees considered an application for TGAC clearance. The data package was considered to be inadequate (lack of metabolic/toxicokinetic data, sensitization studies, subchronic data demonstrating clear NOELs, carcinogenicity studies (2 species), developmental studies (two species) and genotoxicity data). Furthermore, significant concerns were expressed about delayed neurotoxicity (with nerve fibre alteration and subsequent nerve degeneration) and potent ChE inhibition after exposure to very low levels. In August 1990, Bayer decided not to continue marketing DEF until such time as new studies had been generated. Additional data have been evaluated in this report. The following is is a summary of the new information only; a consolidated summary of all submitted data may be found at the end of the main body of the report.

Toxicokinetics and Metabolism

'DEF 6', an EC containing 71% active ingredient, appropriately diluted in water, was applied to 15 cm^2 areas of clipped dorsal skin of male SD rats for 10h at levels of 0.121, 0.605 and 6.05 mg/kg. The amount of DEF absorbed after 10h exposure ranged from 38-53%, of which the majority (67-74%) occurred in the first hour. 90% of the absorbed amount was excreted over 168h, primarily in the urine.

Acute Studies

New acute toxicity studies provided with the current submission indicated that DEF had moderate oral toxicity (LD50s of 435 and 234 mg/kg in male and female rats, respectively), moderate dermal toxicity (LD50 of 1093 mg/kg in rabbits), low inhalational toxicity (4h LC50 of 4650 and 2460 mg/m^3 in male and female rats, respectively), was a slight eye irritant (rabbits), and a moderate skin irritant (rabbits) but not a skin sensitizer (guinea-pigs).

Studies with 'DEF 6', a 71% EC formulation, indicated it had moderate oral toxicity (rats), low dermal and inhalational toxicity (rats), and was a severe skin irritant.

Short-Term Repeat-Dose Studies

DEF was applied to the shorn backs of NZ White rabbits at 0, 2, 11 and 29 mg/kg/d for 15 applications of at least 6h/d in 19 days. 1/10 HD males and 4/10 HD females died or were sacrificed.

Clinical signs at the MD and HD included muscle fasciculations, tremors, decreased motor activity and white or clear nasal discharge and, at the HD, anal staining. Erythema, oedema and dried, cracked or flaking skin were seen at the dose site. Apart from tremors and skin lesions in several animals, signs resolved during 14 days recovery. Bodywt gain and food consumption were reduced at the HD, with partial recovery. Reduced WBCs (reversible) and increased BUN (reversible) were noted at the HD. Doserelated inhibition of plasma, red cell and brain ChE was noted; only plasma ChE fully recovered. Hyperkeratosis and moderate acanthosis were seen at and near the dose sites (minimal at the LD).

Wistar rats were exposed (head-nose) to aerosolized DEF in 1:1 PEG 400:water vehicle at 0, 0.27, 2.6, 13.3 and 62.5 mg/m^3 for 6h/d, 5d/week for 2 weeks. At the HD, reversible signs included reduced activity, aggressive behaviour, vocalization when touched, piloerection, exophthalmos, bradypnoea and dyspnoea, and slight hypothermia. At 2.6 mg/m^3 and above, some females were more sensitive to tail-pinch. From 13.3 mg/m^3 in females and 62.5 mg/m^3 in males, plasma ChE was significantly inhibited and at the HD, RBC ChE (both sexes) and brain ChE (females). There were no significant ChE effects at 2.6 mg/m^3. At 13.3 mg/m^3 and above in males there was a dose-related trend to a decrease in relative organ weight of liver and, in HD females, a slight reduction in relative organ wt of spleen.

Subchronic Studies

In a 13-week inhalation study, Wistar rats were exposed (head-nose) to aerosolized DEF at 0, 0.93, 2.43, 12.2 and 59.5 mg/m^3 for 6h/d, 5d/week for 13 weeks. Concentrations up to 12.2 mg/m^3 were tolerated without clinical signs. At the HD, clinical signs (generally more severe in females) included unpreened hair, reduced activity, temporarily-increased aggression and menace reflexes, vocalization when touched, piloerection, exophthalmos, narrowed eyelids, miosis, bradypnoea, dyspnoea, irregular breathing and respiratory sounds, squatting, salivation, convulsions and slight hypothermia. Reflex tests did not indicate any likely neurological or sensorimotor changes. Body weight gains were comparable. Inhibition of plasma ChE inhibition was seen from 12.2 mg/m^3 (2x as severe in females than males), RBC ChE from 12.2 mg/m^3, and brain ChE at 59.5 mg/m^3. In some HD females the fundus was rough, gritty and had uniformly-distributed dark and light zones. Electroretinograms (ERGs) revealed a significant reduction in retinal a- and b-waves at the HD, indicating reduced retinal response but no specific pathological changes were noted in retinae. An increase in adrenal wts and a fine-droplet fatty change in the adrenal cortex of HD rats was noted. There was no effect at 2.4 mg/m^3 based on ChE inhibition at the next higher dose. Based on a respiratory minute volume of 1L/kg, this corresponds to a nominal exposure of about 0.9mg DEF/kg/d.

Chronic Studies

Beagle dogs were given dietary DEF at 0, 3.9, 15.1 and 57.2 ppm for one year. There were no mortalities, clinical signs, bodywt or food consumption changes, palpable masses or ophthalmoscopic findings. There were no deficits in detailed neurological examinations. Apart from ChE effects, there were no noteworthy clinical biochemical or urinalysis parameter changes. At the LD, plasma ChE, and at the LD and MD, erythrocyte ChE, was less than 20% affected. Brain ChE was not significantly affected at any dose. 1/4 MD males and 1/4 HD females had lung discoloration (lung of male also described as having abnormal consistency). No neoplastic lesions were reported. The NOEL was 3.9 ppm (approx. 0.1mg/kg/d) based on plasma ChE inhibition.

In an oncogenicity/neurotoxicity study, Fischer 344 rats were given DEF at 0, 4, 40 and 320 ppm in the diet for up to 2 years. Mean intake of active ingredient was 0.2, 1.8 and 16.8 mg/kg/d (m) and 0.2, 2.3 and 21.1 mg/kg/d (f). Survival was slightly reduced at the HD, and those dying or found moribund were affected significantly earlier. Bodywt gain was reduced such that at term, MD animals were about 5% lighter than controls, HD animals about 15-20%. Clinical signs were most apparent at the HD and included increased incidences of paleness, eye opacities, rough coat, rashes and raised zones, urine staining and diarrhoea. Ophthalmic changes at the HD after 2 years (not at one year) included a very high incidence of cataracts, corneal opacities, corneal neovascularization and irititis/uveitis; increased lens opacity (f); increased numbers of flat (viz. unrecordable) electroretinogram (ERG) responses- in fact, virtually all HD animals examined had unrecordable bilateral ERGs. Clinical pathology changes included anaemia at the MD and HD; reduced protein, globulin, cholesterol and calcium at the HD and cholesterol and calcium at the MD; increased BUN at the HD; reduced PChE and RChE at the MD and HD and BChE at the HD. Pathological examination noted diffuse bilateral retinal atrophy in HD animals (one and 2 years) as well as cataracts and optic nerve atrophy (secondary to the retinal changes?), hyperplasia and vacuolation of the mucosa of the proximal small intestine in MD and HD animals, and increased adrenal cortex vacuolation in HD animals. Pathological examination of neurotoxicity groups (evaluation of axonal and myelin structures in sections of brain, spinal cord and sciatic nerve) did not reveal any changes. There was no reported increases in neoplastic or non-neoplastic changes. The NOEL may be taken as 0.2 mg/kg/d, based on ChE inhibition, clinical pathology changes, reduced spleen and kidney wts, and pathological changes in the small intestine at the next highest dose of 2.3 mg/kg/d.

Reproduction Studies

In a rat 2-generation dietary study DEF was administered in the diet to CD Sprague-Dawley rats for two generations at measured levels of 0, 4.0, 30.2 and 260ppm. A marked increase in cannibalization of pups was seen in HD groups. In the F1 HD group there was an indication of reduced fertility index (females pregnant/females inseminated). Lower body wts were observed in the F1 HD animals during the pre-mating period, due to reduced bodywt gain during the lactation period; F2 pups of the HD group also showed reduced bodywt gain during lactation. During the lactation periods, lower bodywts were observed in F0 and F1 HD females, as was food consumption. A slight increase in gestation length and in the number of dams with stillborn pups was seen for F0 and F1 HD groups. During lactation, F1 and F2 pup viability was reduced at the HD. The NOEL based on ChE depression (plasma, RBCs and brain) was 4 ppm DEF in the diet. In 21-day pups, the ChE NOEL was 30.2 ppm.

In a follow-up cross-fostering study, it was shown that the reduced pup bodywt gain, decreased pup viability during lactation, and the cannibalization of pups, were due to a compound-effect on the dams (at a dose causing very significant inhibition of plasma, RBC and brain ChE). In another follow-up study to investigate the equivocal decrease in the fertilityindex (in the HD F1 animals), a smaller reduction (not statistically significant) was found at the same dose; thus the studies do not completely discount the possibility of a small effect of DEF on fertility at high doses.

Genotoxicity Studies

In an Ames *Salmonella*/microsome assay using a reductive modification to the preincubation procedure (bacteria, test article, S9 and reductive cofactors allowed to incubate at 30°C for 30 min prior to the addition of the top agar), there was no evidence of mutagenicity at up to 5000 µg/plate.

CD-1 mice were given DEF by gavage at doses of 0, 60, 125 and 250 mg/kg and sacrificed at 24, 48 and 72 h for preparation of bone marrow smears. DEF did not cause any increase in the number of micronuclei.

In an *in vivo-in vitro* hepatocyte DNA repair assay, CD-1 mice were given gavage doses of DEF at 0, 75, 150 and 300 mg/kg and killed 2 or 16h later for preparation of primary hepatocyte cultures which were labelled with tritiated thymidine prior to fixation, staining and autoradiography. DEF did not cause unscheduled DNA synthesis.

Special Studies

Undiluted DEF was topically applied (5 d/week for 13 weeks) to the combs of adult White leghorn hens at 0, 2.6, 11 or 42 mg/kg. There were no mortalities. Treatment-related signs consistent with delayed neurotoxicity (decreased motor activity and ataxia) were only seen at the HD. Beginning around day 40 with 3/12 hens consistently ataxic, a total of 7/12 hens were affected (2 only transiently) by the end of the study (mildly to severely). Blood ChE was inhibited by 50-60% at all doses. Microscopic examination of neural tissues revealed degenerative changes at the HD, consistent with delayed neurotoxicity (not seen at the LD or MD). TOCP treatment caused a high mortality rate and clear indications of delayed neurotoxicity.

These degenerative lesions included degeneration digestion chambers, axonal degeneration, macrophage accumulation (macrophages or proliferating glial or Schwann cells), and lymphocytic infiltration. Based on extent and distribution, the severity of such lesions was minimal to slight in control, LD and MD hens but increased noticeably in TOCP and 42 mg/kg DEF hens; in general, the mean severity grade for CNS lesions in 42 mg/kg DEF hens was somewhat less than for TOCP hens but the distribution was similar. In peripheral nerves, effects seemed somewhat more prominent in TOCP than 40 mg/kg DEF hens.

DISCUSSION

The acute toxicity of DEF is moderate by the oral and dermal routes, low - moderate by the inhalational route. Clinical signs of toxicity were consistent with ChE inhibition. Dry cracked skin with crusty zones was seen following dermal exposure.

One finding of concern in the new studies was retinal toxicity. Thus, in a 13-week rat inhalation study, ophthalmoscopy noted that in some high-dose females (59.5 mg/m^3) the fundus was rough, gritty and had uniformly-distributed dark and light zones while ERG revealed a significant reduction in a- and b-waves, indicating reduced retinal response. In a 2-year rat dietary study, ophthalmic changes at 16.8-21.1 mg/kg after 2 years (not at one year) included a very high incidence of cataracts, corneal opacities, corneal neovascularization and irititis/uveitis; increased lens opacity and increased numbers of flat (viz. unrecordable) ERG responses - in fact, virtually all HD animals examined had unrecordable bilateral ERGs. Pathological examination noted diffuse bilateral retinal atrophy in HD animals (one and 2 years) as well as cataracts and optic nerve atrophy (secondary to the retinal changes?). However, in a 1-year dog study, there was no evidence of ophthalmoscopic or retinal pathological effects at doses up to 57.2 ppm in the diet.

There was no evidence of treatment-related neoplasms in chronic feeding studies in rats and dogs. In a previously-evaluated mouse study, a significant increase in treatment-related neoplasms was observed at the highest dose of 250 ppm (55mg/kg/day). An increase in liver hemangiosarcomas in males, lung alveolar adenomas in females and small intestine adenocarcinomas in males were observed. These significant changes were only seen at the highest dose level which was greater than the high dose levels in the rat study (16.8 to 21.1mg/kg/day).

Confirming previous negative genotoxicity results, DEF did not show any genotoxic potential in an *in vitro* gene reverse-mutation assay, a chromosomal-effects assay (*in vivo* micronucleus test in mouse), or an unscheduled DNA synthesis assay (*in vivo-in vitro* mouse hepatocyte assay).

The topical application of DEF for 13 weeks to the combs of adult White leghorn hens at 0, 2.6, 11 or 42 mg/kg (ie. 2.5, 10 and 40 µL/kg) caused signs consistent with delayed neurotoxicity (decreased motor activity and ataxia) and degenerative changes of neural tissue at the HD. Thus, this new study does not allay previous concerns about delayed neurotoxicity. Former evaluations noted that delayed neurotoxicity appeared to develop following the administration of DEF by all routes (oral, dermal, inhalation and intraperitoneal); it was noted that, in some of these studies, lesions of the central and peripheral nervous systems occurred without the manifestation of clinical signs, while in others, clinical signs of neurotoxicity were present but there were no detectable lesions. Results of delayed neurotoxicity studies in hens are summarised in Attachment B.

In a 13-week rat inhalation study, reflex tests did not indicate any likely neurological or sensorimotor changes. In a 2-year rat dietary oncogenicity/neurotoxicity study, pathological examination of neurotoxicity groups (evaluation of axonal and myelin structures in sections of brain, spinal cord and sciatic nerve) did not reveal any changes.

Human exposure studies showed that communities in residential areas adjacent to sprayed fields suffered from a significantly increased frequency of fatigue, eye irritation, rhinitis, throat irritation, nausea and diarrhoea (Scarborough ME et al. Arch. Environ. Hlth **44(6)**, 355-360: 1989). DEF is quite stable and has been detected at very low levels in ambient air outside the cotton defoliation season. Butyl mercaptan and dibutyl disulfide are degradation products and impurities produced in the formulation of DEF and 'Folex' (S,S,S-tributyl phosphorotrithioite); the former has a disagreeable odour and is readily noticeable at 0.1-1.0 ppm, detectable as low as 0.0001 ppm. Both can cause nausea at low levels of exposure (around 4 ppm and above?). In 1983, California limited the amount of this mercaptan in DEF and Folex to 0.1%, with a buffer zone of one half a

mile between any application site and nearby residences to be maintained; evidently, complaints about acute health effects continued (ibid).

The exposure calculations in the following paragraph have been considered previously:

Measurements of human exposure showed that highest levels (inhalation and dermal) were observed during ground spraying where applicators were exposed to 43 mg/day, or 0.61 mg/kg/day for a 70 kg man. Aerial spraying results in lower exposure, ranging from 0.1 to 0.2 mg/kg/day depending on the job (eg. flagger, mixer-loader etc.). These exposure levels are high when compared to the NOEL for delayed neurotoxicity of 0.1 mg/kg/day (oral) and 10 mg/kg/day (dermal) and 21 mg/m³/d (inhalation). Minor symptoms of delayed neurotoxicity for dermally administered DEF were seen at 20 mg/kg in a 90-day hen study. Therefore, an appropriate NOEL would be the next lowest dose of 10 mg/kg (obtained in another dermal study at which no symptoms of delayed neurotoxicity were seen). Using this NOEL and a measured maximum exposure level of 0.61 mg/kg/day (ground sprayers), a safety margin of approximately 17 is reached (assuming 100% absorption, which is not unreasonable for an OP ester). This result varies greatly from the company's previously-estimated value of 214.

In a company letter dated 10th August 1990, other estimates of dermal and inhalational exposure were given after aerial application (see Attachment C). A safety factor of >40 was calculated for dermal exposure for the most heavily-exposed flagger, while for inhalational exposure, the safety factor for the most heavily-exposed flagger was estimated as 110. Since exposure is likely to take place by both routes, the wisdom of calculating factors for each route independently is questioned. Combining the highest dermal and inhalational exposures and using the dermal NOEL (viz. 10 mg/kg) would give a safety factor of about 33.

In conclusion, the findings of significant ocular and retinal damage in several rat studies and data confirming previous observations of the induction of delayed neurotoxicity are of concern. Concerns about ocular toxicity are to some extent offset by the fact that they were not reported in a 1-year dietary dog study. With respect to the most-recent delayed neutoxicity study in hens (topical dosing for 5 d/week for 13 weeks), positive findings were only reported at the 42 mg/kg dose, not at the next-lowest dose of 11 mg/kg. However, it should be noted that a number of structurally-similar OPs have ostensibly proved negative in similar tests, albeit using generally less-extensive dosing protocols (see Appendix I). It would be interesting to know whether DEF has sufficient structural similarity to TOCP (see Appendix II) to have a strong affinity for the neuropathic target esterase (NTE).

The company presented arguments (document accompanying a letter dated 10th August 1990) discounting concerns about delayed neurotoxicity findings in hens, as follows (evaluator's comments in parentheses):

(1) The hen is considered the most sensitive species for neurotoxicity studies, whereas no neurotoxicity was detected in longer-term feeding studies in mammalian species. (Comment: It is possible the retinopathic and optic nerve effects in rats may be related to neurotoxic effects of DEF.)

(2) The anticholinesterase dose-range starts below the neurotoxic dose range. (Comment: In tests on exposed workers, peripheral lymphocyte neurotoxic esterase (NTE) was inhibited at exposures at which ChE levels did not change- J Occup Med **25**, 517: 1983.)

(3) Clinical signs of neurotoxicity are the most sensitive parameters and gross symptoms of neurotoxicity would be seen before morphological changes occurred. (Comment: The veracity of this statement would be difficult to check in humans poisoned by OPs.)

(4) There are adequate safety margins between experimental NOELs and occupational exposure. (Comment: See foregoing discussion.)

(5) No neurotoxicity has been detected in people associated with application of DEF over 29 years of marketing in the USA and 25 years in Australia. (Comment: Such statements should be interpreted with caution because of poor reporting schemes for pesticide poisonings. Also, there is at least one literature report of delayed toxicity arising from dermal exposure to 'Folex' - see JAMA **238**, 1950: 1977.)

RECOMMENDATIONS

1. On the basis of toxicological concerns (viz. OPIDN, or (organophosphate-induced delayed neurotoxicity) and inadequate safety factors for users of Def-containing products, it is suggested that TGAC approval for Def Defoliant not be given. Further it is recommended that Def Defoliant not be registered for use in Australia.

2. Another toxicity concern arises from reports that people near fields sprayed with Def Defoliant reported increased incidences of clinical symptoms such as eye irritation, fatigue, rhinitis, throat irritation, nausea and diarrhoea (possibly due to the smell of dibutyl disulfide and butyl mercaptan).

3. It is suggested that Def Defoliant be maintained in its existing Schedule viz. schedule 7, in view of delayed neurotoxicity present at low doses.

4. In view of concerns related to the toxicological profile of DEF and the recommendation that it not be approved for use in Australia, an ADI is not considered appropriate.

5. FIRST AID AND SAFETY DIRECTIONS

In Appendix E Part 2, the T-value for S,S,S-tributylphosphoro-trithioate is listed as 30. On the basis of the lowest LD50 in rats of 150 mg/kg, the T-value should be 10. Also, the chemical name is mispelled in the current entry.

APPENDIX E PART 2 - AMENDMENT

S,S,S-Tributylphosphorotrithioate - amend entry to read:

S,S,S-Tributylphosphorotrithioate.....a, h 10

Since TGAC clearance is not supported, products would not be expected to be marketed. Thus safety directions are not determined.

SUMMARY OF TOXICOLOGICAL HAZARD

Date of preparation: Updated October 1993

Chemical name: S,S,S-Tributyl phosphorotrithioate (DEF)

Worst oral LD50* in rats: 150 mg/kg (female)

Worst oral LD50* in other species: 260 mg/kg in guinea pigs

Worst dermal LD50*: 1000 mg/kg in rats

Worst inhalation LC50*: 1600 mg/m^3 in rats

Skin irritation: moderate in rabbits

Eye irritation: slight in rabbits

Skin sensitisation: Nil in guinea pigs

Remarks: Cholinesterase inhibitor

T-value: 10

NOEL: 0.125 mg/kg/day - chronic dog study

DETAILED TOXICOLOGY ASSESSMENT REPORT

This section contains the detailed assessment reports of the new studies submitted by the registrant following the 1990 decision not to continue marketing tribufos until toxicology concerns had been addressed.

Introduction

In May 1989, the 84th meeting of the PACSC considered an application for TGAC clearance of S,S,S-tributyl phosphorotrithioate (or DEF; previously called tribufos), an organophosphorus compound which was used as a cotton defoliant in NSW and QLD. The data package was considered to be inadequate with respect to metabolic/toxicokinetic data, sensitization studies, subchronic data demonstrating clear NOELs, carcinogenicity studies (2 species), developmental studies (two species) and genotoxicity data. Concerns were expressed about neurotoxicity and potent ChE inhibition.

The 53rd meeting of the DPSSC (May 1989) also considered the TGAC application and concluded that its continued use was unacceptable for the same reasons viz. delayed neurotoxicity (with nerve fibre alteration and subsequent nerve degeneration) and potent ChE inhibition after exposure to very low levels; rescheduling from S6 to S7, with an Appendix J rider ("should be available for research purposes") was foreshadowed. In view of the fact that alternatives to DEF were available, the subsequent meeting of DPSSC (August 1989) recommended an Appendix M entry. In February 1990 the DPSSC (56th meeting) considered further data (on the potential neurotoxicity in animals and exposure levels in humans) and a company appeal against the Appendix M entry; as a result, it was placed in Schedule 7, with a foreshadowed recommendation that it carry an Appendix J rider ("should not be available for use as a pesticide").

In August 1990, Bayer advised that the company would not market DEF as a cotton defoliant, or for any other use in Australia, until the toxicological issues identified by the committees (neurotoxicity and carcinogenicity were specifically noted) had been clarified by further data being generated. Thus, no further regulatory action was taken at that stage and DEF remains listed in Schedule 7.

Toxicology data was submitted in 1993 and this submission covered acute, subchronic, chronic, reproduction, genotoxicity and special studies. This data has been evaluated and reported below. Further studies were submitted in July 1996 after the initial toxiclogy assessment report was finalised. These studies addressed neurotoxicity issues, the assessment of which did not alter the conclusions reached earlier. Accordingly, the initial report does not incorporate these later studies. These are discussed separately in Attachment A (pp 38-40 of this report).

2.1 CHEMISTRY OF THE TGAC

Common name: tributyl phosphorotrithioate ('tribufos')

Chemical name: S,S,S-tributyl phosphorotrithioate (IUPAC)

CAS no: 78-48-8

Trade name: DEF Defoliant

Empirical formula: $C_{12}H_{27}OPS_3$

Molecular weight: 314.5

Chemical and Physical Properties

Appearance:	colourless to pale yellow liquid
Odour:	strong, mercaptan-like odour
Melting point:	less than -25°C
Boiling point:	150°C/0.3 mmHg
Octanol/water partition coefficient:	log P = 3.23
Vapour pressure:	1.8×10^{-5} mbar at 30°C
Solubility:	2.3×10^{-3} g/L in water at 20°C Solubility in N-hexane, dichloromethane, 2-propanol or toluene is greater than 200 g/L
Density:	1.06 g/mL

2.2 TOXICOKINETICS AND METABOLISM

2.1 **Dermal Absorption of Tribufos by Rats from a DEF 6 Emulsifiable Formulation using 14C-Tribufos** (Miles Inc. Agriculture Division Toxicology. Study no. 91-722-KW; Oct. - Dec. 1991) (GLP)

Doses of 2.8, 14 and 140 µg/cm^2 of 'DEF 6', an EC containing 71% active ingredient, (2.02, 10.1 and 101 µg/cm^2 active ingredient) appropriately diluted in water, were applied to 15 cm^2 areas of clipped dorsal skin of male SD rats for 10h. These levels represented total DEF exposure of 0.121, 0.605 and 6.05 mg/kg. (Human dermal exposure has been reported to rangefrom 0.03 to 1.8 mg/kg/d).

The mean amount of DEF absorbed after 10h exposure was 41-50% (estimated by measured amount absorbed) or 38-53% (measured by amount applied less amount recovered in skin wash). Of this, the majority (67-74%) of the absorption occurred during the first 1h. 90% of the absorbed amount was excreted over 168h, primarily in the urine.

2.3 ACUTE STUDIES

The acute studies (GLP) were all performed by the Corporate Toxicology Department of the Mobay Corporation, in Stilwell, Kansas; because of changes in corporate structure/ownership in 1991/1992, these laboratories subsequently became the Agriculture Division Toxicology Test Facility of Miles Incorporated.

Study Type	Species	Outcome		Ref.
Tribufos				
Oral	Rat (SD)	LD50	= 435 mg/kg (m)	
			= 234 mg/kg (f)	1
Dermal	Rabbit (NZ White)	LD50	= 1093 mg/kg (m & f)	2
Inhalation	Rat (SD)	LC50 (4h)	= 4650 mg/m^3 (m)	
			= 2460 mg/m^3 (f)	3
Skin Irritation	Rabbit (NZ White)	Moderate skin irritant		4
Eye Irritation	Rabbit (NZ White)	Slight eye irritant		5
Skin Sensitization	G. pig (Hartley)	Not a sensitizer		6
'DEF 6' Emulsifiable Concencentrate (70% DEF)				
Oral	Rat (SD)	LD50	= 570 mg/kg (m)	
			= 349 mg/kg (f)	7
Dermal	Rat (SD)	LD50	> 2000 mg/kg	8
Inhalation	Rat (SD)	LC50 (4h)	= 3550 mg/m^3 (m)	
			= 2340 mg/m^3 (f)	9
Dermal Irritation	Rabbit (NZ White)	Severe skin irritant		10

Inhalation studies - 4h, nose-only exposure

While the reports are not clear, it appears that the numerical toxicity values for the EC are given in terms of the wt of the product and not in terms of the active ingredient.

Dates given in the following headings are experiment dates, not final report dates.

(1) **Acute Rat PO Study using Technical DEF** (Study no. 90-012-ES; Jan./Feb. 1990)

Technical DEF (clear, pale-yellow liquid; 98.1%) in corn oil was given to SD rats at doses up to 552 (males) and 294 (females) mg/kg; animals were observed for 14 days. Deaths occurred between days 1 to 5, with signs including decreased activity, lacrimation (clear and red), nasal discharge (clear and red), salivation, diarrhoea, perianal and urine staining, decreased reactivity, tremors and convulsions; recovery in surviving animals occurred by day 6.

(2) **Acute Rabbit Dermal study using Technical DEF** (Study no. 90-025-FE; Feb./Mar. 1990)

Technical DEF (98.1%) was applied to the clipped backs (approx. 240 cm^2) of NZ White rabbits for 24h under occlusive dressing at doses of 500 to 2000 mg/kg. Deaths occurred within 5 days and signs included reduced bodywt gain, tremors, muscle fasciculations, decreased motor activity, ataxia, diarrhoea, perianal staining, secretions and stains around the head. In surviving animals, recovery had taken place by day 13. At necropsy, reddened thymus, skin erythema, red fluid in the abdominal cavity, and pale zones in the small intestines, and crusty zones on the treated skin were reported.

(3) **Acute Rat Inhalation Study using Technical DEF** (Study no. 90-042-HQ; Sept./Oct. 1990)

SD rats were exposed (nose only) to technical DEF (98.8%) liquid aerosol for 4h at measured concentrations of 1590 to 6030 mg/m^3. Exposure-related signs included abnormal body position, substantially-reduced wt gains, adipsia, anorexia, apparent paralysis, ataxia, bloody urine, dyspnoea, excitability, hypoactivity, increased vocalization, lacrimation, muscle fasciculations, nasal discharge, red oral discharge, red vaginal discharge, tremors, unthrifty appearance and urine staining. Complete recovery was seen by day 6. Gross necropsy confirmed the above signs as well as reddened lungs and mottled thymus.

(4) **Primary Dermal Irritation in rabbits using Technical DEF** (Study no. 90-325-FS; Mar./April 1990)

Technical DEF (99.7%) was applied to shaved skin of NZ White rabbits (3/sex) under occlusive patch for 4h, with scoring for oedema and erythema for up to 14 days. Well-defined erythema was seen in all animals at 30-60 min after patch removal, resolving at 72h. Dry cracked skin was seen in 2/6 at 48h and 6/6 at 72h, still present at day 14. Slight oedema was seen in 5/6 at 30-60 min after patch removal, increasing to moderate-severe at 24h in 6/6, clearing by day 7.

(5) **Primary Eye Irritation in Rabbits using Technical DEF** (Study no. 91-335-MN; Jan. 1992)

Technical DEF (0.1 mL of a 99.2% pure batch) was placed into the conjunctival sac of one eye of 6 NZ White rabbits, with observation for up to 7 days. Conjunctival redness and chemosis were observed in 6/6 at 1h with no signs of irritation at day 7; there were no corneal or irridal lesions. DEF was classified as a slight eye irritant.

(6) **Skin Sensitization Study in Guinea Pigs using Technical DEF** (Study no. 90-324-GK; June - July 1990)

A Buehler Topical Closed Patch Test was used to assess the potential for technical DEF (99.7%) to cause a dermal sensitization response in male Hartley albino guinea pigs. DNCB served as a positive control. DEF did not cause a dermal sensitization reaction in guinea pigs.

(7) **Acute Rat PO Study Using 71% EC Formulation** (Study no. 92-012-NO;

An emulsifiable concentrate form of DEF (71% ai) in 0.5% w/v methylcellulose/water was given to SD rats at doses up to 970 (males) and 490 mg/kg (females); animals were observed for 14 days. Deaths occurred between days 1 to 5, with signs including decreased activity, lacrimation (clear and red), nasal discharge (clear and red), salivation, perianal and urine staining, increased reactivity, hunched back, laboured breathing, piloerection, muscle fasciculations,and tremors; dose-related reductions in bodywt gain occurred over the first 7 days, with recovery in surviving animals by day 14. Findings at gross necropsy included discoloured stomach zones, red fluid in bladder, gas/fluid in intestines, and fluid in abdomen.

(8) **Acute Rat Dermal Study using a 71% EC Formulation** (Study no. 92-022-NZ; Mar. 1992)

An emulsifiable concentrate form of DEF (71% ai) was applied to clipped areas of the backs of SD rats for 24h under occlusive dressing at doses of 500, 1000 and 2000 mg/kg. Deaths occurred between days 1 to 4, only at the HD. Signs included reduced bodywt gain, tremors, muscle fasciculations, decreased motor activity, increased reactivity, irritation at the dose site, ataxia, hunched back, ventral staining, secretions and stains around the head. In surviving animals, recovery had taken place by day 14 (apart from dose-site irritation). At necropsy, crusty red zones on the treated skin of HD animals were noted.

(9) **Acute Rat Inhalation Study using a 71% EC Formulation** (Study no. 91-042-MD; Oct. - Dec. 1991)

SD rats were exposed (nose only) to a liquid aerosol of 'DEF 6' (a 71% EC formulation) for 4h at measured concentrations of 540 to 5160mg/m^3. Exposure-related signs included reduced wt gains, apparent paralysis, dyspnoea, excitability, hypoactivity, lacrimation, muscle fasciculations, red nasal staining, tremors, morbidity, unthrifty appearance and urine staining. Complete recovery was seen by day 8. Gross necropsy confirmed many of the above signs as well as reddened turbinates and lungs.

(10) **Primary Skin Irritation Study in Rabbits using a 71% EC Formulation** (Study no. 91-325-MG; Nov. 1991)

'DEF 6', an EC formulation of DEF (71% ai) was applied to shaved skin of NZ White rabbits (6 males) under occlusive patch for 4 h, with scoring for oedema and erythema for up to 14 days. Slight to well-defined erythema and slight oedema were seen in all animals at 30-60 min after patch removal. By day 14 all animals still had erythema but no oedema. Dry cracked skin was seen in 2/6 at 48 and 72h, 6/6 at 7 days, and 1/6 at 14 days. Examination of skin of the latter rabbit showed multifocal ulcerations of the epidermis, with moderate acanthosis and hyperkeratosis. 'DEF 6' was classified as severely irritating and corrosive to the skin.

2.4 SHORT-TERM REPEAT-DOSE TOXICITY

4.1 **Rabbit 21-Day Dermal Study using Technical DEF** Sheets LP & Phillips SD. Mobay Corporate Toxicology Dept, Kansas. Study no. 90-125-FP. Report no. 5899, dated Aug. 1991. Expt date April - June 1990 (GLP)

DEF (98.1-99.7%) in PEG 400 was applied to the shorn backs (approx. 240 cm^2) of NZ White rabbits (5/sex/dose) at 0, 2, 11 and 29 mg/kg/day for 15 applications of at least 6h/day in 19 days. Additional control and HD recovery groups were included.

1/10 HD males and 4/10 HD females died or were sacrificed in extremis (between days 12-19). Clinical signs in MD and HD animals included muscle fasciculations, tremors, decreased motor activity and white or clear nasal discharge and, at the HD only, anal staining. Erythema, oedema and dried, cracked or flaking skin were also seen at the dose site. Apart from tremors which persisted in 1 male and skin lesions in several animals, signs resolved during recovery. Bodywt gain and food consumption were reduced at the HD, with partial recovery evident. Ophthalmoscopy was negative. Reduced lymphocyte counts (reversible) and increased BUN (reversible) were noted at the HD. Doserelated inhibition of plasma, red cell and brain ChE was noted. At the end of the recovery period only plasma ChE had fully recovered. There were no treatment-related gross lesions. Microscopic lesions were confined to the skin at and near the dose site of MD and HD animals, with hyperkeratosis and moderate acanthosis; these findings were minimal at the LD. There were no noteworthy effects at 2 mg/kg/d, if accepted that a ChE inhibition not exceeding 20% at this dose was not biologically significant.

4.2 **Rat Inhalation Range-Finding Study** Pauluhn J. Bayer AG. Fachbereich Toxicologie, Germany. Study no. T 1039694, Report no. 19903, dated Jan. 1991. Expt date Nov. 1990 (GLP)

In this well conducted and reported study, Wistar rats (10/sex/gp) were exposed (head-nose) to aerosolized DEF (97.9%) in 1:1 PEG 400:water vehicle at measured concentrations of 0, 0.27, 2.6, 13.3 and 62.5 mg/m^3 for 6h/d, 5d/week for 2 weeks.

At the HD, signs included reduced activity, aggressive behaviour, vocalization when touched, piloerection, exophthalmos, bradypnoea and dyspnoea, and slight hypothermia. At 2.6 mg/m^3 and above, some females were more sensitive to tail-pinch. Clinical signs were reversed by the

following day and were not cumulative. Body weight gains were comparable. From 13.3 mg/m^3 in females and 62.5 mg/m^3 in males, plasma ChE was significantly inhibited and at the HD, RBC ChE was also inhibited (both sexes). Brain ChE was inhibited in HD females. There were no significant ChE effects at 2.6 mg/m^3. At 13.3 mg/m^3 and above in males there was a dose-related trend to decrease in relative organ weight of liver and, in HD females, a slight reduction in relative organ wt of spleen.

2.5 SUBCHRONIC STUDIES

5.1 **Rat 13-Week Inhalation Study** Pauluhn J. Bayer AG Fachbereich Toxicologie, Germany. Study no. T 7039744. Report no. 102697, dated June 1992. Expt date Jan.-May 1991 (GLP)

In this well-conducted and reported study, Wistar rats (10/sex/gp) were exposed (head-nose) to aerosolized DEF (97.9%) in 1:1 PEG 400: water vehicle at measured concentrations of 0, 0.93, 2.43, 12.2 and 59.5 mg/m^3 for 6h/d, 5d/week for 13 weeks.

Concentrations up to and including 12.2 mg/m^3 were tolerated without clinical signs. At the HD, clinical signs included unpreened hair, reduced activity, temporarily-increased aggression and menace reflexes to external stimuli, vocalization when touched, piloerection, exophthalmos, narrowed eyelids, miosis, bradypnoea, dyspnoea, irregular breathing and respiratory sounds, squatting, salivation, convulsions and slight hypothermia; these signs tended to be more severe in females than males. Reflex tests did not indicate any likely neurological or sensorimotor changes. Body weight gains were comparable.

Statistically significant, concentration-related plasma ChE inhibition was seen from 12.2 mg/m^3 (25-50% inhibition at this dose; 2x as severe in females than males), a RBC ChE inhibition from 12.2 mg/m^3 (approx. 60%) and a brain ChE inhibition at 59.5 mg/m^3 (about 40%). Ophthalmoscopy noted that in some HD females the fundus was rough, gritty and had uniformly-distributed dark and light zones. Electroretinography revealed a significant reduction in a- and b-waves at the HD, indicating reduced retinal response. No specific changes were noted in retinae at histopathological examination. There were no organ wt changes apart from an increase in adrenal wts at the HD. Apart from a fine-droplet fatty change in the adrenal cortex of HD rats, there was no evidence of other histopathological changes. There was no observable effect at 2.4 mg/m^3, with ChE inhibition at the next higher dose. Based on a respiratory minute volume of 1 L/kg, 2.4 mg/m^3 was reported to correspond to a nominal exposure of about 0.9mg DEF/kg/d.

2.6 CHRONIC TOXICITY

6.1 **Dog 1-Year Dietary Study** Christenson WR. Mobay Corporate Toxicology Dept, Kansas. Study no. 88-274-AB. Report no. 5899, dated Aug. 1991. Expt date April- June 1990 (GLP)

Beagle dogs (4/sex/gp) were given DEF (batch 85R26-39; 98.7% pure) in the diet at mean measured concentrations of 0, 3.9, 15.1 and 57.2 ppm for one year.

Body weights and food consumption were not significantly affected by exposure to DEF. Observation for clinical signs, palpation for masses and ophthalmoscopy did not reveal any compound-related effects. Examination of the eye included pupil reflexes, conjunctiva, cornea, vitreous humor, and retina. There were no mortalities. There were no deficits in detailed neurological examinations.

Apart from ChE effects, there were no noteworthy clinical biochemical or urinalysis parameter changes. At the LD, plasma ChE, and at the LD and MD, erythrocyte ChE, was less than 20% affected. Brain ChE was not significantly affected at any dose. It was claimed (summary and results sections) that a transient non-significant decrease in RBCs, Hct and Hb occurred at the HD at the initiation of dosing; this was not evident on perusal of the actual data.

Organ wts and gross and microscopic pathology apparently were unaffected; 1/4 MD males and 1/4 HD females had lung discoloration (lung of male also described as having abnormal consistency) but the numbers of animals in the groups were too small to make any conclusions about whether this was a compound-related effect. No neoplastic lesions were reported. The NOEL for this study was 3.9 ppm (approx. 0.1 mg/kg/d) based on significant plasma ChE inhibition.

6.2 **Rat 2-Year Dietary Oncogenicity/Neurotoxicity Study** Christenson WR. Miles Inc. Agriculture Division Toxicology, Kansas. Study no. 88-271-AA. Report no. 6590, dated April 1992. Expt date June 188 - July 1990 (GLP)

Fischer 344 rats (50/sex/gp) were given DEF (98.1-99.7%) at dietary levels of 0, 4, 40 and 320 ppm for 2 years; another 10/sex/gp (20/sex for controls and HD) were sacrificed at one year. Another 2 lots of 10/sex/gp were included as oneyear and 2-year neurotoxicity groups. Mean intake of active ingredient was 0.2, 1.8 and 16.8 mg/kg/d (males) and 0.2, 2.3 and 21.1 mg/kg/d (females).

Survival was claimed not to be significantly affected. However, in females, while the numbers dying/killed prior to term were 14/50, 13/50, 17/50 and 20/50 in the respective groups (control to HD), the mean times to death were 690, 704, 676 and 615 days ie. those dying/moribund were affected significantly earlier. Bodywt gain was reduced such that at term MD animals were about 5% lighter than controls, HD animals about 15-20%. At the HD, average daily food consumption was slightly reduced, although increased on a bodywt basis. Clinical signs were most apparent at the HD and included increased incidences of paleness, eye opacities, rough coat, rashes and raised zones, urine staining and diarrhoea. Body temperature was not affected.

Ophthalmic changes attributable to dosing at the HD after 2 years (not at one year) were:

- very high incidence of cataracts, corneal opacities, corneal neovascularization and irititis/uveitis at 2 years;
- increased lens opacity in females;
- increased numbers of flat (viz. unrecordable) electroretinographic (ERG) responses at 2 years. In fact, virtually all HD animals examined had unrecordable bilateral ERGs.

There was little evidence of a treatment-related effect on ERGs at the LD or MD; although data indicated some increase in unrecordable ERGs at the LD cf. controls (right eyes of animals

examined), this was not supported by data from recordings from left eyes and nor was there any indication of an effect at the MD. In general, retinal examination was not possible in HD animals since the light from the ophthalmoscope did not penetrate through the high density of the opaque/cataractic lens.

Clinical pathology changes included:-
- Reduced RBCs, Hb and Hct in MD and HD animals and altered MCV, MCHb and MCHbC at the HD;
- reduced total protein, globulin, cholesterol and calcium at the HD and in cholesterol and calcium at the MD;
- increased BUN at the HD;
- decreased ALP and ALT in MD males and HD animals (of unknown significance);
- reduced PChE and RChE activity at the MD and HD and BChE at the HD.

Organ wt changes included increased testes wts and reduced spleen and kidney wts (HD males), and increased adrenal wts (absolute and relative) in HD animals of both sexes (at one and 2 years -also observed grossly). Gross pathological examination noted discoloured small intestines of abnormal consistency at the MD and HD (one and 2 years) as well as the eye changes noted above. Microscopic pathology noted diffuse bilateral retinal atrophy in HD animals (one and 2-year animals) as well as cataracts and optic nerve atrophy (considered secondary to the retinal changes), hyperplasia and vacuolation of the mucosa of the proximal small intestine in MD and HD animals, and increased adrenal cortex vacuolation in HD animals. Pathological examination of neurotoxicity groups (confined principally to evaluation of axonal and myelin structures in sections of brain, spinal cord and sciatic nerve) did not reveal any compound-related changes. There was no reported increase in the incidence of neoplastic or non-neoplastic changes.

The eye lesions, prominent retinal atrophy, were extensive in HD animals by one year and characterised by diffuse loss of most of the outer layers of the retina (including the layer of rods and cones), outer limiting membrane, outer nuclear layer, outer plexiform layer and sometimes portions of the inner nuclear layer. The pigment epithelium was present but contained increased eosinophilic granular to flocculent cytoplasmic material which distorted the cells. The choroid was reduced in thickness and the layer of optic nerve fibres and the ganglion cell layer were sometimes reduced in thickness. In HD animals which died at the 3-month bleeding interval, there was no evidence of retinal changes.

The NOEL may be taken as 0.2 mg/kg/d, based on ChE inhibition, clinical pathology changes, reduced spleen and kidney wts, and pathological changes in the small intestine at the next highest dose (2.3 mg/kg/d).

2.7 REPRODUCTION STUDIES

2.7.1 **Rat 2-Generation Dietary Study** Eigenberg DA & Elcock LE. Mobay Corporate Toxicology Dept, Kansas. Study no. 88-671-AK. Report no. 5889 dated Sept. 1991. Expt date June 1988 - April 1989 (GLP)

DEF (98.5-99.7%) was administered in the diet to CD Sprague-Dawley rats for two generations (30/sex/gp for F0 and F1 parents), at measured levels of 0, 4.0, 30.2 and 260 ppm. Dosing commenced at 8 weeks of age for F0 parents with dosing for 10 weeks prior to mating, while F1 parents were dosed from weaning (also for 10 weeks prior to mating). Randomly selected offspring from F1 litters were mated to produce F2 litters.

A marked increase in cannibalization of pups was seen in HD groups but no other treatment-related clinical signs were reported in adults or pups. Lower body wts were observed in the F1 HD animals during the pre-mating period, due to reduced bodywt gain during the lactation period; F2 pups of the HD group also showed reduced bodywt gain during lactation. During the lactation periods, lower bodywts were observed in F0 and F1 HD females, as was food consumption. A slight increase in gestation length and in the number of dams with stillborn pups was seen for F0 and F1 HD groups. During lactation, F1 and F2 pup viability was reduced at the HD.

For the F1 HD group there was a reduction in the fertility index (no. females pregnant/no. females sperm positive) although this did not attain statistical significance; the fertility indices were 97%, 93%, 90% and 76% in the control - HD groups respectively. This finding was investigated in more detail (see 7.3 below).

Plasma ChE in adult animals was reduced at the MD and HD. At study termination, LD females had PChE depression (26%) but as this was not seen at earlier times or in F1 females at term, it was not considered significant. Red cell and brain ChE depression occurred at the MD and HD. The ChE NOEL was 4 ppm DEF in the diet. In 21-day pups, the ChE NOEL was 30.2 ppm.

Apart from the cannibalization and related findings, no treatment-related pathological effects were ascribed to dietary administration of DEF. There was a slight increase in discoloured livers/liver zones in HD F1 pups but since this was not seen in F2 pups and histopathology was not performed, it is not possible to assess this finding.

2.7.2 **Rat Dietary Cross-Fostering Study** Eigenberg DA. Mobay Corporate Toxicology Dept, Kansas. Study no. 88-971-BZ. Report no. 4890, dated Aug. 1991. Expt date Feb.- June 1989 (GLP)

In a previous 2-generation reproductive toxicity study (see Section 7.1 above), an increase in the cannibalization of pups, decreased birth wts, and a reduction in pup bodywt gain during the lactation period were observed in the HD group (260 ppm in the diet). This study was designed to see if the cannibalization and growth retardation were due to an effect on the dams or the pups (and to determine if the decreased birthwt reported was real or due to the fact that the pups were weighed at 24h after birth rather than as soon as possible after birth).

CD Sprague-Dawley rats (15M and 30F per group) were given 0 or 240 ppm DEF (98.1-99.7%) in the diet from 7 weeks of age; two groups received control diet, 2 the test diet. Following a 10-week pre-mating exposure period, the rats were bred (1:2 mating) for up to 21 days. After

birth, pups were culled to 4/sex/litter and, within approx. 36h were cross-fostered ie. one group of 0 ppm dams reared one group of 240 ppm pups and vice versa, while the other groups of pups were reared by dams which were in the same dosage group (ie. 0 or 240 ppm DEF) but which were not their birth mothers (to control for the non-specific effects of cross-fostering).

No treatment-related signs were seen (excluding cannibalization of pups). This primarily occurred in those groups in which the dams received 260 ppm DEF, indicating that cannibalization was due to an effect on the dams. There was no difference in the birth wt of pups from control and treated groups. A reduction in pup bodywt gain was only seen in litters nurtured by dams receiving 260 ppm DEF. Thus the reduced wt gain was due to an effect of DEF on the dams. A reduction in pup viability (not solely due to cannibalization) was observed in groups in which pups were reared by treated dams, and groups in which pups were born to treated dams and nurtured by control dams; the extent of this reduced viability was:- pups born to and fed by treated dams >> control pups fed by treated dams > pups born to treated dams and fed by control dams. The reduced viability in the latter group appears to be due to the nuturing of the pups by treated dams within the period after birth before the cross-fostering. Marked plasma, RBC and brain ChE depression were observed in treated animals (average depression being 90%, 57% and 81% respectively).

Reduced pup bodywt gain, decrease in pup viability during lactation, and the cannibalization of pups, were due to a compound-effect on the dams (at a dose causing very significant inhibition of plasma, RBC and brain ChE).

2.7.3 **Rat Dietary Reproductive Toxicity Study** Eigenberg DA Mobay Corporate Toxicology Dept, Kansas. Study no. 89-971-DC. Report no. 5502, dated Aug. 1991. Expt date July - Oct. 1989 (GLP)

In a 2-generation reproduction study (see section 7.1 above), an equivocal decrease in the fertility index was seen in the HD F1 animals. To verify this, DEF (98.1-98.7%) was given in the diet to F1 generation CD Sprague Dawley rats (30/sex/gp) at 240 ppm (obtained from the previous cross-fostering study - see section 7.2 above). Dams were necropsied on day 20 of pregnancy.

No treatment-related clinical signs were observed. A significant depression of plasma, RBC and brain ChE was observed. The fertility index (no. females pregnant/no. females sperm positive) was 93% and 83% in the control and dosed groups, respectively. This was not statistically significant. Thus the magnitude of the decrease of the fertility index in HD F1 females noted in the previous 2-generation study (viz. 97% to 76%) was not confirmed in this report, although some treatment-related effect cannot be discounted.

2.8 DEVELOPMENTAL STUDIES

None provided with the current submission.

2.9 GENOTOXICITY STUDIES

2.9.1 Gene Mutation

9.1.1 ***Salmonella typhimurium* (Histidine reversion) Test with Preincubation under Reductive Conditions** Holmes RM. SRI International, Menlo Park, California. Study no. 8446-A01-89, Report no. 5459, dated Jan. 1991, Expt date Dec. 1989- Jan. 1990 (GLP)

The Ames *Salmonella*/microsome assay was used to evaluate the ability of DEF Technical to cause gene mutations in strains TA1535, TA1537, TA1538, TA98 and TA100, using a reductive modification to the preincubation procedure in which the bacteria, test article, S9 (prepared from uninduced hamster livers) and reductive cofactors were allowed to incubate at 30°C for 30 min prior to the addition of the top agar. On the basis of a preliminary toxicity study with TA98, duplicate studies in all tester strains were conducted with 6 concentrations of DEF in the range 10 to 5000 µg/plate. There was no evidence of mutagenicity under these conditions. Positive controls (9-aminoacridine, 2-anthramine) gave the expected results.

2.9.2 Chromosomal Effects

9.2.1 **Micronucleus Test - *in vivo* Mouse Study** O'Loughlin KG SRI International, Menlo Park, California. SRI Study no. 8446-C01-89, Report no. 5642, dated March 1991, Expt date Dec. 1989 - Jan. 1990 (GLP)

Groups of CD-1 mice were given DEF (98.1%) by gavage at doses of 0, 60, 125 and 250 mg/kg and sacrificed at 24, 48 and 72h for preparation of bone marrow smears. Doses were based on a range-finding assay at 6 dose levels in the range 10 to 2000 mg/kg; most animals at 500 mg/kg and above died. Benzene served as a positive control.

There was no indication that DEF caused an increase in the number of micronuclei in mouse bone marrow erythrocytes whereas benzene was active.

2.9.3 Other Genotoxic Effects

9.3.1 **Unscheduled DNA Synthesis in Mouse Hepatocytes** Hamilton CM & Steinmetz KL. SRI International, Menlo Park, California. Study no. LSC 8446-A01-89, Report no. 5641, dated March 1991, Expt date Nov. - Dec. 1989 (GLP)

In this 'in vivo-in vitro' hepatocyte DNA repair assay, groups of CD-1 mice were given gavage doses of DEF (98.1%) in corn oil at 0, 75, 150 and 300 mg/kg and killed 2 or 16h later; doses were based on a range-finding study at 200, 500, 1000 and 2000 mg/kg in which most animals at 500 mg/kg and above died. Positive control animals received 10 mg/kg dimethylnitrosamine. Primary hepatocyte cultures were prepared and labelled with tritiated thymidine prior to fixation, staining and autoradiography.

DEF did not cause UDS (measured by net grains/nucleus or percentage of cells in repair).

2.10 SPECIAL STUDIES

2.10.1 **Subchronic Delayed Neurotoxicity Study in Hens** Sheets LP. Mobay Corporation Corporate toxicology Dept, Kansas. Study no. 89-428-CS. Report no. 5465, dated Feb. 1991. Expt date Sept. - Dec. 1990 (GLP)

Undiluted DEF (97.7%) was topically applied (5 d/week for 13 weeks) to the combs of adult White leghorn hens (12/gp) at 0, 2.5, 10 or 40 µl/kg viz. 0, 2.6, 11 or 42 mg/kg. Another group was dosed with 18 mg/kg triorthocresyl phosphate (TOCP) as positive control. Hens were monitored for clinical signs, mortality, body wt, ataxia, blood ChE, and the incidence of gross and histopathological lesions at necropsy.

Application was topical to the comb rather than oral because: (a) previous literature reports suggesting the induction of delayed neurotoxicity in hens by topical but not oral exposure; (b) DEF produces 'late acute' toxicity (ataxia and death) when given orally but not dermally, apparently arising from metabolism after PO dosing to n-butyl mercaptan; (c) the comb is highly vascular, permeable to OPs, and readily accessible.

There were no mortalities in DEF hens. Treatment-related signs consistent with delayed neurotoxicity (decreased motor activity and ataxia) were only seen at the HD. Beginning around day 40, 3/12 hens were consistently ataxic, 2 more became ataxic around day 60. A total of 7/12 hens had been affected (2 only transiently) by the end of the study, graded from mild to severe. Eye irritation was observed in some because of DEF draining into the eyes. Blood ChE was inhibited by 50-60% throughout the study at all doses. Gross observations at necropsy only included the comb which was reduced in size and, in 1 hen, granular in appearance. Microscopic examination of neural tissues revealed degenerative changes at the HD consistent with delayed neurotoxicity (not seen at the LD or MD). TOCP dosing was discontinued after 6 weeks (30 applications), with clear clinical and histopathological indications of delayed neurotoxicity; 6/12 TOCP hens were sacrificed between days 39 to 57 because of severe ataxia.

Lesions included degeneration digestion chambers (focal to focally disseminated nervefibre degeneration characterised by vacuolar change, lysis or fragmentation of the axon and/or myelin), axonal degeneration (the presence of single to multiple swollen eosinophilic hyaline to granular axons), macrophage accumulation (subtle to large aggregates of large mononuclear cells - macrophages or proliferating glial or Schwann cells; the most severe accumulations paralleled the presence of extensive degeneration digestion chambers associated with the "cleanup" or sequelae to nerve fibre damage), and lymphocytic infiltration. Such 'lesions' were minimal to slight in control, LD and MD hens (based on extent and distribution) but increased noticeably in TOCP and 42 mg/kg DEF hens. In general the mean severity grade for 42 mg/kg DEF hens was less than for TOCP hens but the distribution was similar. There was a characteristic location for the lesions within specific nerve fibre tracts. In the cerebellar/brainstem sections, the dorsolateral tracts of the brainstem were symmetrically affected, in cervical spinal cord sections the dorsolateral tracts and tracts along the dorsal fissure were usually affected, while in thoracic and lumbar spinal cord sections, tracts along the ventral fissure were affected. In peripheral nerves (tibial and fibular), degeneration digestion chambers, axonal degeneration and/or macrophage accumulation occurred in the distal branches; PNS effects seemed somewhat more prominent in TOCP than 40 mg/kg DEF hens.

CONSOLIDATED SUMMARY

This section includessummaries of studies provided with the most recent submission by the sponsor to maintain use of tribufos (see pages 8-29) plus summaries of all studies on tribufos previously submitted to Australian regulatory authorities ie it is a comprehensive summary of all studies provided on the toxicology and toxicokinetics of tribufos.

Introduction

S,S,S-tributyl phosphorotrithioate (DEF), an organophosphorus compound, has been used as a defoliant of cotton. No NOEL or ADI has been set because its use in Australia has been suspended. It is in Schedule 7 of the SUSDP. It has an MRL of at or about 0.1mg/kg for cottonseed.

Toxicokinetics and Metabolism

'DEF 6', an EC containing 71% active ingredient, appropriately diluted in water, was applied to 15 cm^2 areas of clipped dorsal skin of male SD rats for 10h at levels of 0.121, 0.605 and 6.05 mg/kg. The amount of DEF absorbed after 10h exposure ranged from 38-53%, of which the majority (67-74%) occurred in the first hour. 90% of the absorbed amount was excreted over 168h, primarily in the urine.

Acute Toxicity

DEF has moderate acute oral toxicity, with LD50 values of about 150 mg/kg in rats (up to 234-435 mg/kg in other experiments) and 260 mg/kg in guinea pigs. Dermal toxicity was moderate, with an LD50 value in rats about 1000 mg/kg for the technical and formulated (50%) DEF. DEF technical was of moderate to low inhalational toxicity; LC50 values for DEF technical were 1600 (m) and 4000 mg/m^3(f) rats, (after 4 hr exposure); in a further study, equivalent LC50 values in rats were 4650 and 2460 mg/m^3. The chemical was a moderate skin and slight eye irritant in rabbits and the effects were quickly reversible. Signs of acute toxicity consisted of effects on the nervous system. DEF technical was not a skin sensitizer in guinea pigs.

Studies with 'DEF 6', a 71% EC formulation, indicated it had moderate oral toxicity (rats), low dermal and inhalational toxicity (rats), and was a severe skin irritant.

Short-Term Repeat-Dose Studies

DEF administered dermally to rats for 3 weeks at 170 mg/kg caused body weight loss, mild cholinergic symptoms, and inhibition of brain ChE (to 12% of control). The only other effect identified was increased size of adrenal glands. Data on blood chemistry or urinalyses were not reported.

DEF was applied to the shorn backs of NZ White rabbits at 0, 2, 11 and 29 mg/kg/d for 15 applications of at least 6h/d in 19 days. 1/10 HD males and 4/10 HD females died or were sacrificed. Clinical signs at the MD and HD included muscle fasciculations, tremors, decreased motor activity and white or clear nasal discharge and, at the HD, anal staining. Erythema, oedema and dried, cracked or flaking skin were seen at the dose site. Apart from tremors and

skin lesions in several animals, signs resolved during 14 days recovery. Bodywt gain and food consumption were reduced at the HD, with partial recovery. Reduced WBCs (reversible) and increased BUN (reversible) were noted at the HD. Doserelated inhibition of plasma, red cell and brain ChE was noted; only plasma ChE fully recovered. Hyperkeratosis and moderate acanthosis were seen at and near the dose sites (minimal at the LD).

A 21-day inhalation study in Wistar rats (15 exposures of 6h each), using doses of 0, 2, 7 or 32 mg/m³, revealed clinical signs of toxicity such as lethargy, unkempt fur and isolation behaviour only at the HD. Plasma ChE was significantly depressed at 7 and 32 mg/m³. Increased absolute and relative adrenal weights were observed at 32 mg/m³, as well as an increased frequency in inflammatory changes of the respiratory tract. No effects were seen at 2 mg/m³.

Wistar rats were exposed (head-nose) to aerosolized DEF in 1:1 PEG 400:water vehicle at 0, 0.27, 2.6, 13.3 and 62.5 mg/m^3 for 6h/d, 5d/week for 2 weeks. At the HD, reversible signs included reduced activity, aggressive behaviour, vocalization when touched, piloerection, exophthalmos, bradypnoea and dyspnoea, and slight hypothermia. At 2.6 mg/m^3 and above, some females were more sensitive to tail-pinch. From 13.3 mg/m^3 in females and 62.5 mg/m^3 in males, plasma ChE was significantly inhibited and at the HD, RBC ChE (both sexes) and brain ChE (females). There were no significant ChE effects at 2.6 mg/m^3. At 13.3 mg/m^3 and above in males there was a dose-related trend to a decrease in relative organ wt of liver and, in HD females, a slight reduction in relative organ wt of spleen.

Subchronic Studies

Two subchronic studies in mice and rats concentrated largely on the anticholinesterase properties of the chemical (neither included data on blood chemistry, haematology or urinalyses). DEF administered to mice for 8 weeks at doses up to 270 ppm had no effect on body weight, food consumption or mortality rates. Plasma ChE was inhibited significantly, even at the LD (5 ppm), while blood ChE was significantly inhibited at 10 ppm and above. Brain ChE was inhibited at 270 ppm. Mice survived with plasma ChE levels about 10% of control values. In rats, DEF up to 500 ppm for 16 weeks had no effect on mortality while growth rates were affected at 500 ppm only. Levels as low as 10 ppm caused significant inhibitions of ChE of erythrocytes and plasma. No gross pathological lesions could be attributed to DEF in either of the studies. Inhibition of ChE was seen at the lowest doses tested in both species.

In a 13-week inhalation study, Wistar rats were exposed (head-nose) to aerosolized DEF at 0, 0.93, 2.43, 12.2 and 59.5 mg/m^3 for 6h/d, 5d/week for 13 weeks. Concentrations up to 12.2 mg/m^3 were tolerated without clinical signs. At the HD, clinical signs (generally more severe in females) included unpreened hair, reduced activity, temporarily-increased aggression and menace reflexes, vocalization when touched, piloerection, exophthalmos, narrowed eyelids, miosis, bradypnoea, dyspnoea, irregular breathing and respiratory sounds, squatting, salivation, convulsions and slight hypothermia. Reflex tests did not indicate any likely neurological or sensorimotor changes. Body weight gains were comparable. Inhibition of plasma ChE inhibition was seen from 12.2 mg/m^3 (2x as severe in females than males), RBC ChE from 12.2 mg/m^3, and brain ChE at 59.5 mg/m^3. In some HD females the fundus was rough, gritty and had uniformly-distributed dark and light zones. Electroretingrams revealed a significant reduction in retinal a- and b-waves at the HD, indicating reduced retinal response but no specific pathological changes were noted in retinae. An increase in adrenal wts and a fine-droplet fatty change in the

adrenal cortex of HD rats was noted. There was no observable effect at 2.4mg/m^3, with ChE inhibition at the next higher dose. Based on a respiratory minute volume of 1L/kg, 2.4 mg/m^3 corresponds to a nominal exposure of about 0.9mg DEF/kg/d.

Chronic Studies

A proposed 2-year feeding study in mice using doses of 0, 10, 50 or 250 ppm DEF, was terminated at 90 weeks because of high mortality at thge HD. Plasma ChE was significantly depressed at all dose levels, throughout the study. Erythrocyte ChE was depressed in all dosed females while brain ChE was depressed in all dosed males. Treatment-related non-neoplastic lesions were detected in the adrenals, liver and GIT of animals treated at 250ppm. Extramedullary hematopoiesis occurred in the spleen of 50 and 250 ppm males. Neoplastic lesions of the liver (hemangiosarcoma) of male mice, lung (alveolar adenoma) of female mice and small intestine (adenocarcinoma) of male mice were found to be significantly elevated at 250 ppm. A NOEL could not be established since ChE was significantly depressed at all doses.

In a 2-year rat dietary study, DEF up to 250 ppm had no effect on mortality or food consumption. Haematology and blood chemistry studies were not done. Organ weight analyses and histological examination did not indicate any treatment-related effects, with the exception of an increased incidence of vacuolar cytoplasm of the liver cells at 100 and 250 ppm. ChE was significantly inhibited at 25 ppm and above, giving a NOEL of 5 ppm or 0.25 mg/kg/day. This study was inadequate for assessing the tumourigenic potential of DEF because very few animals were examined.

In a 2-year dietary study in dogs, DEF up to 50 ppm had no observable effect on general wellbeing, haematological parameters, organ weights or on any tissue. Significant inhibition of ChE in plasma and erythrocytes was noted at doses of 10 ppm and above, giving a NOEL of 5 ppm or 0.125 mg/kg/day.

Beagle dogs were given dietary DEF at 0, 3.9, 15.1 and 57.2 ppm for one year. There were no mortalities, clinical signs, bodywt or food consumption changes, palpable masses or ophthalmoscopic findings. There were no deficits in detailed neurological examinations. Apart from ChE effects, there were no noteworthy clinical biochemical or urinalysis parameter changes. At the LD, plasma ChE, and at the LD and MD, erythrocyte ChE, was less than 20% affected. Brain ChE was not significantly affected at any dose. 1/4 MD males and 1/4 HD females had lung discoloration (lung of male also described as having abnormal consistency). No neoplastic lesions were reported. The NOEL was 3.9 ppm (approx. 0.1mg/kg/d) based on plasma ChE inhibition.

In an oncogenicity/neurotoxicity study, Fischer 344 rats were given DEF at 0, 4, 40 and 320 ppm in the diet for up to 2 years. Mean intake of active ingredient was 0.2, 1.8 and 16.8 mg/kg/d (m) and 0.2, 2.3 and 21.1 mg/kg/d (f). Survival was slightly reduced at the HD, and those dying or found moribund were affected significantly earlier. Bodywt gain was reduced - at term, MD animals were about 5% lighter than controls, HD animals about 15-20%. Clinical signs were most apparent at the HD and included increased incidences of paleness, eye opacities, rough coat, rashes and raised zones, urine staining and diarrhoea. Ophthalmic changes at the HD after 2 years (not at one year) included a very high incidence of cataracts, corneal opacities, corneal neovascularization and irititis/uveitis; increased lens opacity (f); increased numbers of flat (viz. unrecordable) electroretinographic (ERG) responses - in fact, virtually all HD animals examined

had unrecordable bilateral ERGs. Clinical pathology changes included altered red cell parameters indicative of anaemia at the MD and HD; reduced protein, globulin, cholesterol and calcium at the HD and cholesterol and calcium at the MD; increased BUN at the HD; reduced PChE and RChE at the MD and HD and BChE at the HD. Pathological examination noted diffuse bilateral retinal atrophy in HD animals (one and 2 years) as well as cataracts and optic nerve atrophy (secondary to the retinal changes?), hyperplasia and vacuolation of the mucosa of the proximal small intestine in MD and HD animals, and increased adrenal cortex vacuolation in HD animals. Pathological examination of neurotoxicity groups (evaluation of axonal and myelin structures in sections of brain, spinal cord and sciatic nerve) did not reveal any changes. There was no reported increases in neoplastic or non-neoplastic changes. The NOEL may be taken as 0.2 mg/kg/d, based on ChE inhibition, clinical pathology changes, reduced spleen and kidney wts, and pathological changes in the small intestine at the next highest dose of 2.3 mg/kg/d.

Reproduction Studies

DEF had no effect on the reproductive parameters in a 3-generation study in mice. The NOEL reported was the highest dose tested, 100 ppm or 15 mg/kg/day; however cholinesterase activity was not measured.

In a rat 2-generation dietary study DEF was administered in the diet to CD Sprague-Dawley rats for two generations at levels of 0, 4.0, 30.2 and 260 ppm. In the F1 HD group there was an indication of reduced fertility index (females pregnant/females inseminated). A marked increase in cannibalization of pups was seen in HD groups. Lower body wts were observed in the F1 HD animals during the pre-mating period, due to reduced bodywt gain during the lactation period; F2 pups of the HD group also showed reduced bodywt gain during lactation. During the lactation periods, lower bodywts were observed in F0 and F1 HD females, as was food consumption. A slight increase in gestation length and in the number of dams with stillborn pups was seen for F0 and F1 HD groups. During lactation, F1 and F2 pup viability was reduced at the HD. The ChE NOEL (plasma, RBCs and brain) was 4 ppm DEF in the diet. In 21-day pups, the NOEL based on ChE depression was 30.2 ppm. In a follow-up cross-fostering study, it was shown that the reduced pup bodywt gain, decreased pup viability during lactation, and the cannibalization of pups, were due to a compound-effect on the dams (at a dose causing very significant inhibition of plasma, RBC and brain ChE). In another follow-up study to investigate the equivocal decrease in the fertility index (in the HD F1 animals), a smaller reduction (not statistically significant) was found at the same dose; thus the studies do not completely discount the possibility of a small effect of DEF on fertility at high doses.

Developmental Studies

Teratology studies were carried out in the rat and the rabbit. In the rat (0, 1, 7 or 28 mg/kg/day) there was no evidence of treatment-related teratogenicity at any dose level used in this study. Maternal toxicity (depressed cholinesterase activity) was evident at all dose levels, but fetal brain cholinesterase activity was unchanged at day 20 of the study.

In the rabbit (0, 1, 3 or 9 mg/kg/day) a significant reduction in maternal body weight gain and a slight reduction in food consumption were evident at the highest dose level. Plasma cholinesterase activity was significantly depressed at day 20, but not at day 28; erythrocyte

cholinesterase activity was depressed at all dose levels at all sampling times during the study. There were no treatment-related effects on reproductive parameters or teratogenic activity.

Genotoxicity Studies

A sister-chromatid assay was negative for DEF.

A data package included a reverse-mutation assay in 'S. typhimurium' (667 to 10000 µg/plate), an unscheduled DNA synthesis assay in rat primary hepatocytes (0.0001 to 0.006 µg/mL) and a chromosomal aberration assay using CHO cells (0.004 to 0.1 µL/mL). Preliminary dose-ranging studies found that higher dose levels were cytotoxic. All these assays produced negative results for the genotoxicity activity of DEF.

In an Ames 'Salmonella'/microsome assay using a reductive modification to the preincubation procedure (bacteria, test article, S9 and reductive cofactors allowed to incubate at 30°C for 30 min prior to the addition of the top agar), there was no evidence of mutagenicity at concentrations up to 5000 µg/plate.
CD-1 mice were given DEF by gavage at doses of 0, 60, 125 and 250mg/kg and sacrificed at 24, 48 and 72h for preparation of bone marrow smears. DEF did not cause any increase in the number of micronuclei.

In an 'in vivo-in vitro' hepatocyte DNA repair assay, CD-1 mice were given gavage doses of DEF at 0, 75, 150 and 300 mg/kg and killed 2 or 16h later for preparation of primary hepatocyte cultures which were labelled with tritiated thymidine prior to fixation, staining and autoradiography. DEF did not cause unscheduled DNA synthesis.

Special Studies

The administration of DEF to hens, regardless of the route, resulted in delayed neurotoxicity. The following NOEL's were established for delayed neurotoxicity by the stated route of administration; inhalation - 21 mg/m^3, dermal - 10 mg/kg, and oral - 0.1 mg/kg.

In another topical administration study, Undiluted DEF was applied (5 d/week for 13 weeks) to the combs of adult White leghorn hens at 0, 2.6, 11 or 42 mg/kg. Treatment-related signs consistent with delayed neurotoxicity (decreased motor activity and ataxia) were only seen at the HD. Beginning around day 40 with 3/12 hens consistently ataxic, a total of 7/12 hens were affected (2 only transiently) by the end of the study (mildly to severely). Blood ChE was inhibited by 50-60% at all doses. Microscopic examination of neural tissues revealed degenerative changes at the HD, consistent with delayed neurotoxicity (not seen at the LD or MD). TOCP treatment caused a high mortality rate and clear indications of delayed neurotoxicity. These degenerative lesions included degeneration digestion chambers, axonal degeneration, macrophage accumulation (macrophages or proliferating glial or Schwann cells), and lymphocytic infiltration. Based on extent and distribution, the severity of such lesions was minimal to slight in control, LD and MD hens but increased noticeably in TOCP and 42 mg/kg DEF hens; in general, the mean severity grade for CNS lesions in 42 mg/kg DEF hens was somewhat less than for TOCP hens but the distribution was similar. In peripheral nerves, effects seemed somewhat more prominent in TOCP than 40 mg/kg DEF hens.

Numerous human exposure studies have been carried out. Airborne levels were measured on a flagger carrying a personnel air sampler. Peak levels of 13.5 mg/m³, for a 20 minute sampling period, were registered. Dermal exposure as measured by cloth patches showed DEF residues of 108 µg/cm^2 were reached using standard spraying practice. The maximum total exposure (inhalation and dermal) level was observed during ground spraying where applicators were exposed to 43 mg/day.

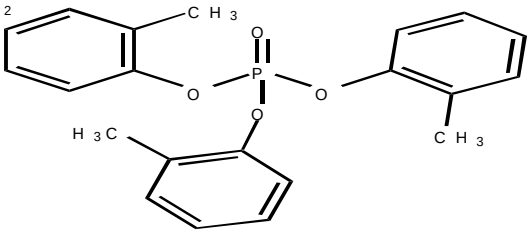
DEF levels, measured at 10 to 1000 metres from the sprayed field (aerial spray), decreased with distance from the field. Air levels of DEF were almost to low to be detected (0.2 ng/L/hr) at 60 to 180 minutes after spraying. Air sampling at adjacent residential areas found an average DEF concentration of 110 ng/m³ during cotton harvesting season. People living or working near a sprayed field were shown to have an elevated incidence of clinical symptoms such as fatigue, eye irritation, rhinitis, throat irritation, nausea and diarrhoea.

APPENDIX I: RESULTS OF DELAYED NEUROTOXICITY EXPERIMENTS IN HENS WITH SOME ORGANOPHOSPHORUS COMPOUNDS OF SIMILAR STRUCTURE

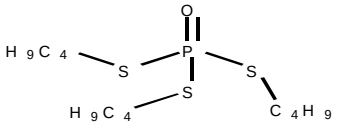
COMPOUND	FINDING*	ORAL DOSE & EXPERIMENTAL PROTOCOL
acephate	-	2 x 785 mg/kg, given 21 days apart
cadusafos	-	2 x 8 mg/kg, " " "
demeton-S-methyl	-	2 x 200 mg/kg " " "
dichlorvos (1)		
dimethoate (2)		
ethoprofos	-	5.3 & 6.5 mg/kg, " " "
methamidofos	-	6.7 mg/kg (60% EUP), " " "
	-	1000 ppm in diet for 4 weeks
	-	0.3 mg/kg PO for 78 days
naled	-	117 mg/kg PO
omethoate	-	20 - 80 mg/kg
trichlorfon	-	185 & 167 mg/kg, given 21 days apart
vamidothion	-	up to 100 mg/kg for 21 days

Information taken from from Chemicals Safety Unit files.
* '-' means a negative finding
(1) "very limited neurotoxic potential"
(2) "methodological difficulties in delayed neurotoxicity study"

APPENDIX II: STRUCTURES OF TRIBUTYLPHOSPHOROTRITHIOATE (tribufos) AND TRI-ORTHO-CRESYL-PHOSPHATE (TOCP)



T O C P



D E F

ATTACHMENT A - Chronology of toxicological assessment of tribufos

Tribufos is an organophosphorus compound used previously as a defoliant of cotton in Qld and NSW. DEF DEFOLIANT is an emulsifiable formulation containing 705 g/L tribufos. It was applied as a spray by aircraft or ground rig at rates of 1.4 to 2.8 L/ha (987 to 1974 g ai/ha) to mature cotton when 50% or more of the bolls were open.

The Technical Grade Active Constituent (TGAC) clearance of tribufos was initially examined in May 1989 by the former Pesticides and Agricultural Standing Committee (PACSC) and the Drugs and Poisons Schedule Standing Committee (DPSSC). Both PACSC and DPSSC considered the data package to be inadequate.

In 1993 Bayer Australia Ltd submitted additional toxicology studies which were considered at the Committee's 3rd (January 1994) meeting. The Committee considered that there were a number of toxicological concerns relating to organophosphate-induced delayed polyneuropathy (OPIDP) following a single exposure, and that this was irreversible; significant ophthalmic effects; and inadequate safety margins for users of the formulated DEF products. Furthermore, members considered that it would not be appropriate to estimate an acceptable daily intake (ADI) of tribufos under such circumstances. Accordingly, the Committee recommended that tribufos not be registered for sale and use in Australia.

On 18 June 1996 the National Registration Authority for Agricultural and Veterinary Chemicals (NRA) provided an extension of time for Bayer Australia Ltd to provide further input into the NRA's consideration of the proposal to withdraw the registration and use of tribufos in Australia. Bayer submitted three pages of comments from an expert consultant in the neurotoxicity of organophosphates, Dr Marcello Lotti, Professor of Occupational Medicine, University of Padua Medical School, responding to some points made in the Department's most recent (October 1993) assessment of tribufos. In addition, Bayer submitted a number of published papers on the issue of the delayed neurotoxicity of tribufos, and some of Prof Lotti's laboratory notes.

Prof Lotti's paper was succinct, but primarily addressed scientific issues related to the causation of OPIDP and the role of organophosphates in general. Professor Lotti's main thesis was that the human risk assessment for tribufos should not be based on the classical ChE inhibition end-point and he proposed an alternative risk assessment paradigm. The paper did not dispute that tribufos is neurotoxic and potentially capable of causing OPIDP and acknowledged that, if inhibition of Neuropathy Target Esterase (NTE) is an important precursor to OPIDP, tribufos may have the potential to cause OPIDP at doses which have little effect on Acetylcholinesterase (AChE).

In addition to Prof Lotti's hypothesis, the Company argued that:

- in 1992 the USEPA returned a finding of no unreasonable adverse effects in considering the re-registration of tribufos;
- there is no evidence of any adverse effects on workers from several decades of use (except for acute effects);
- the exposure model as used by Worksafe was inappropriate, as by far the majority of defoliant application occurs aerially;
- the use of flaggers is almost entirely eliminated in Australia by the use of technology.

Bayer made arrangements to allow Prof Lotti to present this scientific argument about the more appropriate approach to human risk assessment for OP neurotoxicity to the Committee.

DISCUSSION

Professor Lotti provided an interesting presentation on the general aspects of OPIDP, the complications and the intrinsic problems in risk assessment. A short video presentation on a poisoning case study gave a general overview of a patient with OPIDP. Some of the key clinical symptoms were detailed by Professor Lotti and included episodes of paralysis and the accompanying neurophysiological symptoms. It was outlined how the clinical symptoms with severe polyneuropathy lasted up to 15 months following poisoning, and the patient is presently experiencing ongoing symptoms with a change in condition not expected.

Various slides were presented on the mechanism of initiation of OPIDP, and the change in clinical presentation from a flaccid to spastic paralysis with progression of neuropathy. The presentation highlighted the molecular events (chemistry of molecules, affinity constants etc), and in particular, the mechanism of initiation and events in the pathogenesis of OPIDP. Aspects such as Neuropathy Target Esterase (NTE) inhibition, was presented as NTE is considered to be the molecular target for OPIDP caused by several esterase inhibitors. The potency of a particular NTE inhibitor to cause OPIDP is related to the chemistry of the residue left attached to NTE, in addition to the affinity for the enzyme. The capability of inhibited NTE to undergo an ageing process (loss of an alkyl group from the phosphoryl residue attached to NTE leaving a negatively charged phosphorylated NTE) distinguishes inhibitors with high potency from those with negligible or low potency to cause OPIDP. Professor Lotti indicated that OPIDP is usually induced in most cases when there is 70 to 80% inhibition of NTE and that the use of the hen model to predict OPIDP in humans may not be suitable for some OPs.

Four chemicals which were problematical in risk assessment were presented (Methamidophos, Chlorpyrifos, Tribufos and mixtures). With regard to tribufos Professor Lotti considered that the ratio of AChE/NTE inhibition provides a useful prediction of a compound's likelihood to cause OPIDP. According to this theory tribufos would be considered to have a high potential to cause OPIDP in humans as it has negligible cholinergic toxicity. However, whilst DEF caused OPIDP in the hen at high doses, a 1985 study [1] on occupational exposure to tribufos reported inhibition of lymphocytic NTE but no clinical or electrophysiological signs of OPIDP were detected. Professor Lotti indicated that this phenomenon may be attributable to the active metabolite of tribufos not being capable of reaching the target organ due to its high chemical reactivity.

Other difficulties in the risk assessment process were highlighted including the unpredictability of combined exposures to either protect or promote OPIDP pathogenesis. Molecules such as phosphates, phosphoroamidates and phosphonates cause OPIDP by inhibiting large amounts of NTE; whereas, phosphinates, carbamates, and sulfonyl halides cause either protection from or promotion of OPIDP when administered before or after OP, respectively.

In summary, Professor Lotti suggested that OPIDP has been found to be initiated by interactions of several esterase inhibitors with NTE, and the clinical expression of OPIDP was related to high

[1] Lotti M. et al. (1983) J. Occup. Med., 25, 517-522

inhibition/aging of NTE in the peripheral and central nervous system. Recent research has indicated that the ageing reaction, thought to be a key reaction for triggering OPIDP, may not be a necessary step. Exceptions to the aging theory as the cause of OPIDP were outlined, namely, that certain organophosphates do not cause OPIDP, and that repeated high doses of what might be considered protective compounds can cause a mild neuropathy. Very little is known about the proceeding cascade of biochemical and physiological events which follow NTE inhibition, however, selective reduction of retrograde axonal products is affected.

The Chairman thanked Professor Lotti for his informative presentation and members were then provided with the opportunity to question him following which Professor Lotti withdrew. The Committee discussed Professor Lotti's presentation and believed that an even more cautious approach may be needed in risk assessment of this particular class of chemicals, and suggested that in the future, regulatory agencies should interpret any new data on OPIDP very cautiously.

Additionally, the Committee agreed that the NRA be formally advised of emerging issues in relation to OP neuropathies and considered that the NRA may need to undertake a special review of OP pesticides outside of the Existing Chemical Review Program. The Committee agreed that on the basis of currently available information (toxicology information and Professor Lotti's presentation) tribufos should not be registered for use, particularly as there are a number of alternative compounds available for this use that are likely to have a greater degree of safety.

ATTACHMENT B - SUMMARY OF DELAYED NEUROTOXICITY STUDIES- HENS

Reference	Route of Administration (Length of Exposure)	Dose Rate	Results
Bayer 5948	Oral (single treatment)	300 mg/kg bw	No neurotoxic effects
	(two treaments)	300 mg/kg bw	No neurotoxic effects
Bayer 6440	Oral Feeding (30 days)	6.03 mg/kg bw	Neurotoxic threshhold level
		10.9 mg/kg bw	Nerve fibre damage*
		18.5 mg/kg bw	Nerve fibre damage*
			*brain,spinal cord, PNS
Bayer 6941	Oral Feeding (30 days)	1.7 mg/kg bw	No neurotoxic effects
		3.5 mg/kg bw	No neurotoxic effects
		6.3 mg/kg bw	No neurotoxic effects
		6.1 mg/kg bw	No neurotoxic effects
		10.9 mg/kg bw	No neurotoxic effects
		18.5 mg/kg bw	No neurotoxic effects
Abou-Donia	Oral (90 days)	0.1 mg/kg bw	
		0.5 mg/kg bw	Mild ataxia
		1.0 mg/kg bw	Gross ataxia
		2.5 mg/kg bw	Gross ataxia
		5.0 mg/kg bw	Gross ataxia
		10.0 mg/kg bw	Gross ataxia
		20.0 mg/kg bw	Ataxia with near paralysis
		40.0 mg/kg bw	Ataxia with near paralysis
		80.0 mg/kg bw	Ataxia with near paralysis
			Histol. CNS lesions 0.5 to 80 mg/kg. Little evidence of PNS damage.
Gaines	Subcutaneous	200 mg/kg bw	Leg weakness detected 14-28 days after dosing
Bayer 6255	Dermal (24 hours)	ca 530 mg/kg bw	No neurotoxic effects.
		ca 1060 mg/kg bw	Mild neurotoxic symptoms in week 3.
		ca 2120 mg/kg bw	Typical neurotoxicity in week 2
Bayer 8031	Dermal	1067 mg/kg bw	
	(15x6 Hours)	320 mg/kg bw	
		107 mg/kg bw	
		32 mg/kg bw	NOEL
		11 mg/kg bw	
Abou Donia	Topical (90 days)	20 mg/kg bw	Gross ataxia.
		40 mg/kg bw	Severe ataxia.

Reference	Route of Administration (Length of Exposure)	Dose Rate	Results
Mobay	Dermal (5 days/week for 13 weeks)	2.65 mg/kg bw 10.6 mg/kg bw 42.4 mg/kg bw	Whole blood ChE reduced. >55% in all treatments. Ataxia from week 6.
Bayer 6444	Inhalation (1 x 4 hours)	391 mg/m^3 878 mg/m^3 1585 mg/m^3	No neurotoxic effects. No neurotoxic effects. Neurotoxic effects.
	(5 x 4 hours)	62 mg/m^3 145 mg/m^3 256 mg/m^3	No neurotoxic effects. Mild delayed neurotoxic effects. Typical neurotoxic effects.
Bayer 7649	Inhalation (15 x 6 hours)	2 mg/m^3 7 mg/m^3 32 mg/m^3	No neurotoxic effects. No neurotoxic effects. No neurotoxic effects.
Bayer 7614	Inhalation (15 x 6 hours	8 mg/m^3 21 mg/m^3 84 mg/m^3	No neurotoxic effects. No neurotoxic effects.
Biochem Pharm 12, 73:1963	Intraperitoneal (10 days)	100 mg/kg/day	Clin signs of n/t 10-18 days after end of dosing.
Biochem J 120, 523: 1970	Subcutaneous (single dose)	220 mg/kg 1100 mg/kg	Not neurotoxic. Neurotoxic.
Brit J P'Col 23, 295: 1964	Oral (3-15 days)	50 mg/kg 100 mg/kg 150 mg/kg	Poorly tolerated. Severe lesions in brain, spinal cord and sciatic nerve.
	Intraperitoneal (3-15 days)	50 mg/kg 100 mg/kg	Neurotoxicity seen 10-60 days after 1st injection; Pathol damage CNS and PNS.

ATTACHMENT C - OCCUPATIONAL EXPOSURE DATA AFTER AERIAL SPRAYING - BAYER

Occupational exposure

From field measurements the daily dermal exposures for workers were measured as below.

Daily Dermal Average Exposure Values (mg/kg bw/day)

	Average	Range
Pilot	0.14	0.07 - 0.25
Flagger	0.13	0.02 - 0.26
Mixer/loader	0.13	0.06 - 0.20

These values compare with the NOEL's of 32 (15 x 6 hour exposure), and 10.6 mg/kg bw (5 days x 13 week exposure) - Bayer Report 8031 and Mobay Interim Report. Thus, a safety factor of >40 for the most heavily exposed flagger may be calculated.

Similarly, the exposure levels in air are summarised below.

	Concentration of DEF mg/m³ air	
	Average	Range
Pilot	0.0394	0.0056 - 0.1556
Flagger	0.0729	0.0182 - 0.1912
Mixer/loader	0.0618	0 - 0.171

These values compare with a NOEL of 21 mg/m³ in Bayer Report 7614; thus, the most conservative safety factor of 109.8 may be calculated.

PART III

OCCUPATIONAL HEALTH & SAFETY ASSESSMENT REPORT

Prepared by

Agricultural and Veterinary Chemicals Section
Worksafe Australia

3.0 OCCUPATIONAL HEALTH AND SAFETY ASSESSMENT REPORT

3.1 Introduction

S,S,S-tributyl phosphorotrithioate (DEF, tribufos) is an organophosphate (OP) compound previously registered for use in Australia as a cotton defoliant. The product marketed in Australia was DEF Defoliant (EC 705 g/L). DEF was withdrawn from the market by Bayer Australia Ltd in 1990, following their failure to provide further toxicity data requested by NHMRC.

In 1993, Bayer Australia Ltd provided additional toxicity data for assessment by the Environmental Health and Safety Unit (EHSU). The EHSU Report (1993) (No 4460, 9632 and 10192) for DEF recommended that TGAC approval for DEF should not be given. Reasons cited included the toxicological concerns of organophosphate-induced delayed neurotoxicity (OPIDN), potent ChE inhibition at very low exposure levels and inadequate safety factors for the use of DEF-containing products. An occupational health and safety assessment was not conducted in conjunction with the assessment of new toxicity data.

In January 1994 the Advisory Committee on Pesticides and Health recommended that DEF should be precluded from use as a cotton defoliant because of its toxicological profile, including OPIDN and ophthalmic effects.

In September 1994, worker exposure studies were provided by Bayer Australia Ltd in order that reconsideration should take into account exposure as well as toxicology. Receipt of these new studies initiated an occupational health and safety review.

In December 1995, Bayer provided additional information concerning ocular effects of OPs and implications for exposure to DEF. These studies are under evaluation by EHSU.

This report summarises the new and existing information (public and industry data) concerning worker exposure to DEF. Emphasis is given in the report to evaluation of the worker exposure studies submitted in 1994 by Bayer Australia Ltd and the consequent assessment of risk for Australian workers. Recommendations are made based on the occupational health and safety risk assessment concerning the future use of DEF products in Australia.

3.2 REGULATION OF DEF PRODUCTS IN OTHER COUNTRIES

Def is not sold only sold in the USA. In the USA, DEF was considered for re-registration in March 1992. Under the review criteria Neurotoxicity, the US EPA returned a finding of no unreasonable adverse effects and returned DEF to the registration process. The applicant agreed to protective clothing requirement (US EPA 1994).

In the USA, tribufos is marketed by Bayer. The registered product is DEF 6, an emulsion containing 70.5% tribufos and identical to the product formerly registered in Australia. It is used as a cotton defoliant. The US EPA label has the following safety directions and PPE requirements:

May be fatal if swallowed, inhaled or absorbed. Do not get in eyes, on skin, on clothing or breathe vapour or spray mist. Causes substantial but temporary injury. Causes skin irritation. Wear protective clothing, rubber gloves, and goggles or face shield. Wear a mask or pesticide respirator jointly approved by the Mine Safety and Health Administration and the National Institute for Occupational Safety and Health. Wash thoroughly with soap and water after handling and before eating, drinking or using tobacco. Remove contaminated clothing and wash before reuse.

3.3 NATURE OF TRIBUFOS

Tribufos is a colourless to pale yellow liquid with a strong mercaptan-like odour. The odours relate to butyl disulfide and butyl mercaptan that are formulation impurities and environmental degradation products of tribufos.
Tribufos has a very low vapour pressure at 1.7×10^{-6} mm Hg, 20C.

Tribufos is soluble in most organic solvents but insoluble in water.

3.4 HEALTH EFFECTS INFORMATION

A detailed toxicity assessment is given in EHSU (1993). The toxicology endpoints relevant to the occupational health and safety risk assessment are given below.

Tribufos is a cholinesterase inhibitor.

3.4.1 Summary of experimental toxicology

Absorption
Tribufos is rapidly absorbed, metabolised and excreted after oral dosing. Following the administration of a single dose of radiolabelled Tribufos in rats, 96% of the label was excreted within 72 hours. Excretion was via the urine (55-80%) and faeces (15-42%). The major metabolite in the urine was (3-hydroxy) butylmethyl sulfone (HBM sulfone), however 18 metabolites were detected. Tribufos was the major compound detected in faeces.

High dermal absorption of 38-53% was found in rats after 10h exposure to DEF 6. Most absorption (67-74%) occurred in the first hour.

Acute toxicity
Tribufos has an oral LD_{50} of 435 and 234 mg/kg in male and female rats, respectively (moderate), dermal LD_{50} of 1093 mg/kg in rabbits (moderate) and inhalation LC_{50} (4 h) of 4650 and 2460 mg/m^3 in male and female rats, respectively (low).

Tribufos is a slight eye irritant (rabbits) and moderate skin irritant (rabbits) but not a skin sensitiser in guinea-pigs.

Short-term repeat dose and subchronic toxicity

The proposed use pattern for tribufos indicates the use of NOELs derived from short term studies. Four such NOELs are relevant to the risk assessment. These concern effects related to cholinesterase inhibition and acute toxicity and delayed neurotoxicity in hens.

Cholinesterase inhibition and selected NOELs

Short-term repeat dose and subchronic toxicity studies showed clinical signs primarily related to cholinesterase inhibition. There was an interspecies difference in the dose needed to inhibit plasma ChE, erythrocyte ChE and brain ChE.

Studies selected for NOELs for the risk assessment relate to the most likely routes of exposure for end users, ie dermal and inhalation. Those related to cholinesterase inhibition are:

(1) Dermal NOEL of 2 mg /kg bw/day (cholinesterase inhibition of < 20% at this dose) obtained in 19 day dermal study in rabbits (Sheets LP and Phillips SD, Mobay Corporate Toxicology Dept, Kansas, study no 90-125-FP, report no 5899, August 1991) (EHSU 1993).

Tribufos (98.1-99.7%) was applied to the shorn backs of rabbits (5/sex/dose) at 0, 2, 11, and 29 mg/kg/day for 15 applications of at least 6h per day over 19 days. Controls and higher dose recovery groups were also included. The length of the recovery period is not stated. Systemic signs in MD and HD animals included muscle fasciculations, tremors and decreased motor activity. Skin irritation was observed at the dose site. Apart from persistent tremors in one male and skin lesions in several animals, signs resolved during recovery. Ophthalmoscopy was negative. Eighteen percent inhibition of plasma cholinesterase (males only) and 20% inhibition of RBC cholinesterase (females only) was observed at **2 mg/kg bw/day**. This is accepted as an appropriate dermal NOEL, with a ChE inhibition of $\leq$ 20% taken as not biologically significant. Dose-related inhibition of plasma, red blood cell and brain ChE was also noted (levels not provided). At the end of the recovery period only plasma ChE had fully recovered. No treatment-related gross lesions were observed.

(2) Inhalation NOEL of 2.4 mg/m^3 (higher doses demonstrated inhibition of plasma and RBC cholinesterase) obtained in a 13 week inhalation study in rats (Pauluhn J, Bayer AG Fachbereich Toxicologie, Germany study no T7039744 Report no 102697, June 1992) (EHSU 1993).

Rats (10/sex/gp) were exposed to aerosolized tribufos (97.9%) at 0, 0.93, 2.43, 12.2 and 59.5 mg/m^3 for 6 h/d, 5d/week for 13 weeks. Clinical signs were seen only at the highest dose and included reduced activity, exophthalmos, miosis, bradypnoea, dyspnoea, irregular breathing, salivation and convulsions. No neurological or sensorimotor changes were detected. ChE inhibition (25-50%) was seen from 12.2 mg/m^3, RBC ChE inhibition (~ 60%) from 12.2 mg/m^3 and brain ChE inhibition (~ 40%) at 59.5 mg/m^3. Plasma ChE inhibition was concentration-related and statistically significant. Electroretinography showed reduced retinal response. There were no observable effects at **2.4 mg/m^3** (the level of ChE inhibition is not reported), with statistically significant ChE inhibition seen at the next highest dose.

Organophosphate induced delayed polyneuropathy and selected NOELs

OPIDN develops about 2-3 weeks after exposure to a neurotoxic OP. The mechanism of OPIDN is not related to cholinesterase inhibition and is only caused by certain OPs. Tribufos is known to induce OPIDN in hens, the animal model shown so far to be predictive of OPIDN in humans (Johnson 1987). EHSU (1993) summarises many studies on delayed neurotoxicity of tribufos in hens. Administration of tribufos to hens resulted in delayed neurotoxicity irrespective of the route of administration. It can be seen after single doses. Delayed toxicity of tribufos appears to be cumulative; toxicity is observed at lower doses as the number of doses are increased.

Delayed neurotoxic effects in humans following accidental or long-term exposure to OPs may be permanent (Johnson 1987). There are no reports of OPIDN in humans caused by tribufos in the scientific literature.

NTE is the target enzyme in the development of OPIDN. A correlation has been shown between NTE inhibition and the development of clinical signs of OPIDN (Barrett and Oehme 1985), however the exact mechanism is unclear. A 70-80% depression of brain NTE is usually needed to induce neurotoxic effects in the hen. *In vitro* experiments have not demonstrated differences in hen and human NTE (Lotti 1987).

Brain and lymphocyte NTE have functional similarities (various references cited by Lotti 1987), leading to the proposal that measurement of lymphocyte NTE may act as an indicator for the development of OPIDN (Lotti, Becker, Aminoff, Woodrow, Seiber, Talcott and Richardson 1983). As yet, the threshold for NTE inhibition and OPIDN has not been established. Depressed lymphocyte NTE (by 65%) was measured by Lotti *et al* (1983) in cotton field workers exposed to tribufos and merphos, an OP of similar structure to tribufos that converts to tribufos in the environment, at 3-4 weeks after the start of the exposure period. No subclinical effects on the peripheral nervous system or changes in blood cholinesterase were seen in any worker.

Work is continuing to define the predictive ability of lymphocyte and other,eg platelet, NTE in OPIDN (for example, Mutch, Blain and Williams 1992).

For ultimate use in the risk assessment, it is preferable to select a dermal rather than an oral NOEL for delayed neurotoxicity, if available. Early investigations by Abou-Donia and colleagues

(cited by Lapadula, Carrington and Abou-Donia 1984), in comparing the effects of oral and topical single and repeated doses of tribufos in hens suggested that the pattern of response was different between the two exposure routes. This was further investigated by (Lapadula *et al* 1984) who demonstrated that NTE was selectively inhibited in hens following topical application but not oral administration of the same single dose of tribufos. Plasma butyl cholinesterase, involved in acute cholinergic toxicity, was significantly inhibited by both oral and dermal doses. The difference in response was linked to the more rapid metabolism and excretion of tribufos after oral as opposed to dermal, dosing. Abou-Donia, Abdo, Timmons and Proctor (1986) pursued investigations on the dose response of single dermal doses of tribufos on neuropathology and specific brain and plasma enzyme activity. Tribufos induced OPIDN in a dose-dependent fashion, with clinical signs accompanied by histopathological changes in the spinal cord and peripheral nerves. OPIDN occurred at topical doses lower than those required to induce acute cholinergic toxicity.

NOELs for delayed neurotoxicity given in the EHSU Toxicity Report are based on the demonstration of clinical signs. In the dermal study described below, delayed neurotoxicity is seen in hens at a dose that initiated a decrease of 50-60% in blood cholinesterase throughout the treatment period. No clinical signs of toxicity relating to depressed blood cholinesterase are mentioned. No indications are given that acute cholinergic effects were blocked by administration of atropine. This indicates that in the hen, delayed neurotoxicity may occur after topical doses without any prior indications of acute toxicity relating to cholinesterase inhibition.

(3) Dermal NOEL of 11 mg/kg bw/day (delayed neurotoxicity atthe next dose (HD)) for 65 applications in 13 weeks in hens (Sheets LP, Mobay Corporation Corporate Toxicology Dept, Kansas, study no 5465, February 1991) (EHSU 1993).

Tribufos (97.7%) was topically applied to the combs of adult white leghorn hens (12/group) at 0, 2.6, 11 or 42 mg/kg at 5 d/week for 13 weeks. Another group was dosed with 18 mg/kg TOCP as positive control. Hens were observed for clinical signs, mortality, body weight changes, ataxia, blood ChE and the incidence of gross histopathological lesions at necropsy. There were no mortalities in tribufos-treated hens. Treatment-related signs consistent with delayed neurotoxicity (decreased motor activity and ataxia) were only seen at the HD. Signs commenced at day 40 with 3/12 hens affected; 7/12 hens were affected by the end of the study. Microscopic examination of neural tissues revealed degenerative changes at HD consistent with delayed neurotoxicity. These changes were not seen at the LD or MD.

TOCP treatment caused clear clinical and histopathological effects of delayed neurotoxicity. Blood ChE was inhibited by 50-60% at all doses. The dermal NOEL was **11 mg/kg bw/day**.

(4) Inhalation NOEL of 21 mg/m^3 (neurotoxic effects at the next dose (HD)) obtained in a 3 week repeat dose study in chickens (Thyssen and Schilde 1978a, Unpublished report no 7614 (HES No 1767, Miles Tox Rep No B7614) June 15 1978) (EHSU 1990)

In this subacute inhalation study, mature female chickens were exposed to tribufos aerosol at 8, 21 and 84 mg/m^3 for 6 hours per day, 5 days per week for three weeks, with a 5 week observation period. Effects were observed at HD, where 5 animals died and the remaining 5 developed signs of delayed neurotoxicity during the first week of the observation period. Histological changes

indicative of neurotoxicity were seen in the HD animals. There is no indication that effects on ChE were measured. The inhalation NOEL was **21 mg/m^3**.

Other effects

There was no evidence of treatment-related neoplasms in chronic feeding studies in rats or dogs but a significant increase in treatment-related neoplasms was observed in mice.

In long term studies, the lowest NOEL was 0.1 mg/kg/d (3.9 ppm) based on significant plasma ChE inhibition at higher doses in rats fed dietary tribufos for one year. No clinical signs were observed including neurological or ocular effects. In a long term study in Fischer 344 rats, the most significant findings besides cholinesterase inhibition, were ophthalmic changes, including cataracts, corneal opacities, corneal neovascularisation and iritis/ uveitis and unrecordable electroretinographs at the highest dose. No neurotoxic potential was observed in this species.

Boyes, Tandon, Barone and Padilla (1994) summarise current awareness of the effects of OPs on the visual system of rats. They indicate that a number of OPs, including tribufos, reportedly produce ocular toxicity. The EHSU indicates that there is no new information upon which to reach a conclusion on the relationship between tribufos and ocular effects.

In a two generation dietary reproductive toxicity study in rats, some effects on reproductive parameters were seen.

Tribufos was not embryotoxic, fetotoxic or teratogenic in rabbits at doses of $\leq$ 9 mg/kg/day. In the rat, tribufos was not teratogenic at $\leq$ 28 mg/kg/day.

Results of *in vitro* and *in vivo* mutagenicity assays for genotoxic potential were negative.

3.4.2 Summary of human health effects

There is little information available on the human health effects of tribufos. Scarborough, Ames, Lipsett and Jackson (1989) (California Department of Health Services) investigated community complaints about health effects and application of cotton defoliants, including DEF and Folex (merphos, which degrades to tribufos). They found an increased frequency of fatigue, eye and throat irritation and gastrointestinal effects in residents of cotton growing communities compared to residents in agricultural communities that did not grow cotton. The mercaptan degradation product of tribufos has an offensive odour readily detectable by humans. Mercaptan and dibutyl disulphide degradation products can cause nausea in humans at low levels of exposure. Odour problems and low airborne concentrations of tribufos and degradation products have been detected in cotton growing areas in the USA. Ames and Gregson (1995) (California Environmental Protection Agency) recently reported on the relationship between mortality and use of the cotton defoliants DEF and merphos over 21 years in a cotton growing area of California. Their results suggested that total suspended particulates, not defoliants, may be related to the increased proportion of respiratory causes mortality found during the defoliation season.

3.4.3 Conclusions on toxicity of the EUP

Acute toxicity studies with DEF 6 (equivalent to DEF Defoliant) show it has moderate oral toxicity (rats), low dermal and inhalation toxicity (rats) and is a severe skin irritant.

3.4.4 Hazard classification

Tribufos

Tribufos is not included in the List of Designated Hazardous Substances (NOHSC 1994b).

A complete health effects classification for tribufos has not been undertaken but consideration of the acute oral, dermal and inhalation toxicity and the effects of repeated exposure indicate that tribufos will be a hazardous substance on these grounds.

Butyl mercaptan, a degradation product of tribufos, is included on the list (NOHSC 1994b). Health effects classification is not provided.

DEF DEFOLIANT

DEF Defoliant will be a hazardous substance based on the concentration of tribufos in the product.

3.5 OCCUPATIONAL EXPOSURE

This report considers exposure to end users and those entering treated areas.

3.5.1 Use pattern of the end use product

The draft product label is included in the 1994 Bayer Australia Ltd submission.

DEF Defoliant (70.5% Tribufos) is to be applied at 1.4-2.8 L/ha (corresponding to 1-2 kg ai/ha) in 20-30 L water/ha by aerial application when 50% or more of the cotton bolls are open. The maximum concentration of spray contains 14% DEF Defoliant and 9.9% Tribufos. Sufficient spray should be applied to treat all the leaves. The draft label indicates that workers should not handle crops for seven days after spraying unless wearing protective clothing. The product should not be applied within 7 days of picking.

The label specifies a 20 L container.

Defoliation is temperature dependent and may take between four and fourteen days.

The mode of action of DEF Defoliant indicates that it will be used for a discrete period only at the end of the growing season. Contract workers would handle DEF Defoliant repeatedly over this period of time.

Following DEF Defoliant application, there should be a much reduced need for workers to enter treated areas as the time for weed and insect control would have passed.

3.5.2 Exposure information

3.5.2.1 Existing reports of worker exposure - summary

Studies under this section are not included in the Bayer Australia Ltd 1994 submission. They originate from the scientific literature and other sources. These summaries are included for background information only. Original studies could not always be obtained and even when this was possible, the data was frequently of limited use in the assessment of tribufos in Australia. Overall, studies in this section have minimal influence on the occupational health and safety risk assessment. The recent studies provided by Bayer Australia Ltd in support of DEF Defoliant **(5.2.2)** are the basis of the risk assessment.

The studies in this section consider mainly end users of tribufos. Categories of workers include:

ground applications-mixers/loaders, applicators
aerial applications-mixers/loaders, applicators (pilots) and flaggers

Workers are also required to clean up spills and maintain spraying equipment.

Limited exposure information is given for workers in cotton gin factories.

(a) Maddy KT (1976) Summary of efforts in California to resolve alleged health problems associated with use of cotton defoliants. Agricultural Chemicals and Feed, Department of Food and Agriculture, State of California, Sacremento, California.

This unpublished study was not available for assessment. The summary is taken from US EPA (1981) (DEF: Decision Document PB87-186789 Nov 81). Field studies were conducted in 1975 to investigate the decline in tribufos residues on cotton plants up to 9 days after application. Tribufos was applied by air at standard rates to three fields. Residues on cotton foliage had declined by 35% (to 185 ppm) after 5 hours, 89% (to 24 ppm) after 3 days and 91% (to 15 ppm) after 9 days.

(b) Wilson *et al* (1980) Organophosphate risk assessment: Field testing of DEF with scaleless chicken. *Bulletin of Environmental Contamination and Toxicology 24* pp 921-928.

US EPA (1981) summarised this study but as it applies to one applicator the risk was not estimated. This study is not considered further in this report.

(c) Cox (1980a, b, c) Industrial hygiene report of mechanical cotton picker operator exposure to DEF at Valley Picking Company/Schletz Brothers/Harold O'Banion Farming, Hazard Evaluations and Field Studies National Institute for Occupational Safety and Health, Cincinnati, Ohio.

The original studies were not seen for this report. This summary derives from NIOSHTIC 1994 (CD ROM database).

Mechanical cotton picker operator exposure to Tribufos was measured at various workplaces in California **(Table 1).** Samples were collected after a period of heavy rain; the author believed that residues may have been washed away, resulting in lower than anticipated exposures.

Table 1: Inhalation and dermal exposures of mechanical cotton picker operators

Study	Inhalation Exposure		Dermal Exposure	
	No of samples	Amount measured ug/m^3	No of samples	Amount measured mg/8 hr
1980a	23	0.02-0.26	8	0.02-0.09
1980b	10	0.02-0.23	2	0.32-1.02
1980c	not known	0.02-0.20	1	2.8

No further information is available.

(d) The US EPA (1981) reports on accidental exposure to tribufos as indicated through the Pesticide Incident Monitoring Scheme. Thirty incidents were recorded from 1966 to June 1981, 12 of which involved tribufos alone. This is not an exhaustive reporting scheme but serves as an indicator of field problems. Acute symptoms of pesticide poisoning (headache, nausea and skin rash) were observed but victims recovered fully within a few days. The reported accidents were caused by accidental exposure to drift, spillage, unsafe behaviour and/or not having proper protective clothing.

(e) Peoples, Maddy, Datta, Johnson, Smith, Conrad and Cooper (1981) Monitoring of potential exposures of mixer-loaders, pilots, and flaggers during application of tributyl phosphorotrithioate (DEF) and tributyl-phosphorotrithioite (Folex) to cotton fields in the San Joaquin Valley of California in 1979. California Department of Food and Agriculture, Publication HS-676, Feb 10.

This study has not been assessed by Worksafe Australia. The summary derives from the US EPA (1981) and EHSU (1990).

The study measured exposure of mixer/loaders, pilots and flaggers during DEF 6 application on cotton fields in the San Joaquin Valley. Mixer/loaders and flaggers wore socks, shirts, pants, washable cap, clean cotton long-sleeved and long legged coveralls and rubber boots. Mixer/loaders also wore neoprene gloves. Pilots wore shoes and socks, a helmet and long-sleeved shirts and long-legged cloth pants. All workers worked a 7-hour day.

DEF 6 was aerially applied by certified applicators at 1.5-1.7 kg ai/ha (1.32-1.5 lb ai/acre) in 100 L of water. This is similar to Australian application rates. Closed systems were used for mixing and loading.

Dermal and inhalation exposure were measured.

Dermal exposure was estimated from dermal patches on the arms, legs, neck and face and hand washes. The calculations for dermal exposure assume workers are wearing long trousers and long-sleeved open-neck shirt and no gloves. The face, back of neck and the "V" of chest are considered as exposed areas. Exposures assume that all the tribufos measured from the bottom layer of patches worn on the clothing will be absorbed into the skin.

Inhalation exposure was measured by personal air samplers.

Table 2 shows estimated dermal and inhalation exposures, extrapolated over a 7-hour day.

Table 2: Concentration of DEF 6 in breathing zone and on the skin of workers during aerial application of DEF 6 for a 7-hour period of measurement

Job	No of workers	DEF in the breathing zone mg/m^3 Average (range)	Dermal exposure mg/person/day Average (range)
Mixer-loader	5 (6 for dermal exposure)	0.06 (0.0-0.2)	9.1 (4.2-14)
Pilot	7	0.04 (0.01-0.16)	9.8 (4.9-17.5)
Flagger	7	0.07 (0.02-0.2)	9.1 (1.4-18.2)

(f) Lotti, Becker, Aminoff, Woodrow, Seiber, Talcott and Richardson (1983) Occupational exposure to the cotton defoliants DEF and merphos A rational approach to monitoring organophosphorus-induced delayed neurotoxicity. ***Journal of Occupational Medicine 25*** **pp 517-522.**

The study group comprised seven workers exposed to tribufos and merphos during cotton defoliation. Workers included mixer/loader-ground (1), mixer/loader-flagger (3), ground rig operator (1), ground rig operator and mixer/loader (1) and pilot (1). Exposure was measured via cloth patches, hand rinsing and air sampling in the breathing zone.

Following total exposure periods of 25-294 hr, erythrocyte and plasma acetylcholinesterase and butyl cholinesterase activities were not significantly affected and there were no electrophysiological changes or adverse effects on the peripheral nervous system.

Lymphocyte NTE activity was inhibited by 40% to 65% in exposed workers, between days 25 and 30 of exposure to tribufos. Three weeks after the termination of exposure lymphocyte NTE activity tended to return to pre-exposure levels. No deterioration of motor conduction velocity and sensory action potential was noted in any of the workers.

The authors concluded that if blood lymphocyte activity is a mirror of nervous system NTE activity, high levels of inhibition of NTE activity (70% to 80%) might be required in humans to trigger the neurotoxic response, as has been shown in hens.

(g) Kilgore, Fischer, Rivers, Akesson, Wicks, Winters and Winterlin (1984) Human exposure to DEF/merphos. *Residue Reviews* 91 pp 71-101.

This study was conducted in a cotton growing area of California where tribufos and merphos are used as cotton defoliants. Exposure was monitored in workers from one aerial spraying firm and two cotton farms during harvest.

Dermal and inhalation exposure to tribufos was measured for workers involved in application (mixer/loader, pilot, flagger) and harvest and at the cotton gin. Dermal exposure was also measured on flagger mannequins. Flagger mannequins are dummies that remained in the line of flight during spray operations; normal flagging procedure is to move out of the direct line when the plane is correctly lined up. In addition, airborne tribufos residues were measured in close proximity to harvest operations, inside cotton gins and in residential areas. Mixer/loaders used enclosed mixing.

The study did not attempt to quantify dermal exposure. Residues on external cloth patches were used to compare the amount and body site of contamination for the various groups of workers. Workers also carried personal air samplers for inhalation residue measurements. They used these samplers for varying lengths of time.

Biological monitoring was done for workers and selected residents. Tribufos was measured in a morning urine sample. Application workers underwent pre- and post-exposure medical and psychological testing. This included analysis of total and RBC cholinesterase.

For all analyses, only tribufos was measured, as merphos converts to tribufos in the environment.

Results for airborne residues indicated that most residues were associated with particulate material. Flaggers were subjected to highest airborne residues, followed by mixer/loaders and workers at the cotton gin.

Results from personal air monitoring also showed that flaggers were exposed to high tribufos concentrations, in comparison with harvesters and pilots **(Table 3).** Harvesters would be exposed for longer periods than flaggers, however.

Table 3: Tribufos residue in personal air samples (at 17 L/min)

Site	DEF (mg/m^3)
Inside harvester cab	0.2-0.9
Outside harvester cab	0.5-13.3
Cockpit spray aircraft	0.8-6.9
Flagger	7.4-13.5

A comparison of tribufos residues on cloth patches indicated that flagger mannequins then flaggers had much the highest concentrations, followed by mixer/loaders, trampers and harvester operators. For example maximum residues were 6.6 ug/cm^2 for a flagger mannequin (chest), 0.2 ug/cm^2 for a flagger (chest), 0.13 ug/cm^2 for a mixer/loader (chest), 0.1 ug/cm^2 for a harvester operator (thigh) and 0.06 ug/cm^2 for a tramper (thigh).
Workers in the cotton gin had minor or trace residues.

Maximum airborne tribufos residues measured in drift at 300 feet from cotton harvesting sites was 0.19 ug/m^3. Airborne residues in 10 residential areas during application ranged from below or at the limit of detection of 0.0027 ug/m^3 to 0.0874 ug/m^3. After application but during harvest, one site had trace residues. Most airborne residues were likely to be adsorbed onto particulate material.

Urinary tribufos was not detected for workers or residents; the authors propose several reasons for this, including that the biotransformation products of tribufos are difficult to analyse.

Over the almost 7 week exposure period, no significant medical differences, including cholinesterase levels, were noted in pre-and post exposure estimations.

(h) Winterlin, McChesney, Schoen and Seiber (1986) Chemical residues during screening, composting, and soil incorporation of cotton gin waste. *Journal of Environment Science and Health* B21 pp 507-28.

This study investigates the fate of pesticides and defoliants, including DEF, in cotton waste generated during the ginning process. Residues in waste have implications for the subsequent use or disposal of such waste. The study also investigated air contamination with residues.

Samples collected from the vicinity of the rotating screen cleaner contained residues associated with particulate matter. Air samples collected here had the highest tribufos residues compared with subsequent points in the cotton waste processing operation. The author concluded that workers should be provided with respiratory protection because of the marked generation of dust during the screening process.

This study is not assessed further. More information would be needed to define the respiratory risk to these workers.

(i) EHSU (1990, 1993) summary: Mobay Corp Study, January 1989, RD Knarr.

Estimated Tribufos exposures were:

Ground spraying	
mixer/loaders	1.7 - 8.7 mg/day
applicators	9.3 - 43.0 mg/day
Aerial spraying	
mixer/loaders	17.0 mg/day
applicators	9.2 mg/day
flaggers	13.1 mg/day

No further information was available for assessment. For this reason, the study is not considered further in the report.

3.5.2.2 New exposure data submitted by Bayer Australia Ltd

Two major worker exposure studies were submitted by Bayer Australia Ltd to the NRA in 1994. One study deals with worker exposure during ground and aerial spraying of DEF 6. The other deals with worker exposure during cotton harvesting and aims to establish a re-entry period.

Results obtained from these studies are applicable to DEF Defoliant use under Australian conditions. DEF Defoliant will be applied using similar concentrations of Tribufos. The degree of expertise of workers, mechanisation of pesticide application and harvest will be similar between the USA and Australia.

Both studies were performed under the data requirements of US EPA Pesticide Assessment Guidelines Subdivision U.

3.5.2.2.1 Mixers, loaders and applicators

Evaluation of worker exposure to tribufos during aerial and ground application of DEF 6 to cotton, Miles Inc Missouri (Study Number DE202301) Report No 103889, January 25 1993.

Additional explanation was provided by Bayer Australia Ltd in November 1995.

Method

- All aspects of the study were in accordance with Good Laboratory Practice Standards (40 CFE Part 160), except for determination of blood cholinesterase. These analyses were performed in a manner consistent with GLP in a certified chemical laboratory.

- Three sites in California (2) and Mississippi (1) were used. Test subjects were all male volunteers. They came from 4 commercial crews (2 aerial and 2 ground applicators) in California and mixer/loaders in Mississippi.

- Tribufos application and the use of PPE were in accordance with proposed DEF 6 label directions, actual use practices and US state and federal regulations. The study was done to determine actual exposure to tribufos (DEF) during various operations and applications.

- DEF 6 was applied by ground to mature cotton at ~1.9 lb ai/acre (~2 kg ai/ ha) and by air at ~1.9 lb ai/acre (~2 kg ai/ha) and ~1.1 lb ai/acre (~1.3 kg ai/ha). These are comparable with proposed Australian application rates.

- Dermal exposure was measured using whole-body dosimeters (long-sleeved T-shirts and footless tights), solvent handwashes and gauze patches. The whole body dosimeters were worn over the worker's underwear and under a set of cotton/polyester coveralls. They were used to estimate exposure through covered parts of the body, ie upper and lower torso including arms and legs. Dermal gauze patches were used to estimate exposure to the exposed regions-face, neck and head. Hand washes were used to estimate hand exposure.

- Inhalation exposure was measured using personal air-sampling pumps.

- Blood cholinesterase was monitored weekly (on Fridays) during the application season. Pre-exposure blood samples were taken at the end of a 2 week period in which the workers did not work with anticholinesterase compounds.

- Protective clothing and equipment were worn in accordance with proposed label directions. All workers including pilots (AA (aerial applicators)) wore long trousers, long-sleeved shirt (or overalls as a substitute to trousers and shirt) and hat or helmet. In addition, mixer/loaders (ML) used goggles or face shield and chemical-resistant gloves. Ground applicators (GA) used chemical-resistant gloves but not inside closed cabs. Additional protective clothing was recommended for flaggers (FL) and AA if there was increased potential for exposure.

The number of workers involved in the study and the number of replicates are provided in **Table 4**.

Table 4: Workers and replicates used in Miles Inc Missouri (Study Number DE202301) Report No 103889, January 25 1993.

Application	Job category	Number of workers	Total samples[(1) (2)]
Aerial			
	Mixer/loader		
	Closed system (AMLCS)	2	8
	Open system (AMLOS)	2	8
	Pilot (AA)	2	8
	Flagger (FL)	4	16
Ground			
	Mixer/loader		
	Closed system (GMLCS)	2	8
	Applicator (GA)	2	8
Total		**14**	**56**

(1) Total for each of dermal and inhalation replicates.

(2) There were 4 replicates for each worker

Work Practices

Aerial spraying

The closed system mixing/loading (AMLCS) used 500-gallon containers with automatic transfer of product to a holding tank. One to three planes were serviced. The open system (AMLOS) used 5-gallon containers and contents were manually poured into the holding tank. One plane was serviced. The mixer/loaders connected and disconnected hoses to the plane. They did not perform routine maintenance on the planes. The mixing site was located away from the treatment site.

AA covered an average of 1076 acres (436 ha) per day.

The Australian 20 litre container is equivalent to the US 5-gallon size. There is no indication that the 20 litre container is reusable or can be incorporated in enclosed mixing systems. It could be emptied by an equivalent gravity feed system as used by mixer/loaders for ground spraying. On the basis of available information, Australian mixer/loaders are equivalent to AMLOS.

Ground spraying

Ground mixer/loaders (GMLCS) used a closed system to mix DEF 6 from commercial 5-gallon and 30-gallon-containers. They removed the product via a gravity feed system (5-gallon containers) or a suction probe (30-gallon containers). GMLCS triple-rinsed and disposed of containers. They connected and disconnected hoses to the spray-rig and performed routine maintenance. Mixing sites were located next to fields being treated.

Applicators used closed cabs with air conditioning. The effectiveness of the air conditioning and air filtration was not assessed.

GA covered an average of 200 acres (81 ha) per day.

The Australian draft label mentions only aerial spraying for DEF Defoliant. In the event of ground spraying, use of closed cabs with air conditioning would be likely. The methods used by mixer/loaders in the study to add the concentrate to the tank could be used by Australian workers. Alternatively, the concentrate could be dispensed manually.

Results

Exposure was calculated as individual or group geometric means $\pm$ standard deviation. Exposure was presented as µg/lb ai handled, µg/hr worked, or µg/exposure replicate. For the purposes of this assessment, lb ai handled has been converted to kg ai handled. Estimates of amounts ai handled per day for job were made. Exposures are for workers performing dedicated tasks only. They were not measured in ground spray workers performing combined tasks of mixing/loading and applying. Exposures and shift times are summarised in **Table 5.**

Results show that dermal exposure accounted for the bulk of total exposure, with inhalation exposure a maximum of 3.4%. None of the mixer/loaders or applicators in this study wore a respirator. Flaggers had the option of wearing a respirator if there was an obvious risk of inhalation exposure.

For dermal exposure, considering exposure per amount handled, the tasks in order of highest to lowest exposure are:

GMLCS
FL
AMLOS
AMLCS ≈ GA ≈ AA.

On a discrete task basis the operations involving most exposure are mixing/loading and flagging, followed by aerial mixer/loaders in the open system. Lastly aerial mixer/loaders in the closed system and ground and aerial applicators had least exposure.

Table 5: Standardised dermal and inhalation exposure for each job category derived from Miles Inc Missouri (Study Number DE202301) Report No 103889, January 25 1993.

Job	**Shift time (hour)**	**lb handled/ shift**	**Dermal ug/lb handled GM**	**Dermal ug/kg bw/8h day(1) GM (range)**	**Inhalation ug/kg bw/8h day(1) (% total)**
AMLCS	4.54	1248	3.10	94.6 (28.3 - 257.6)	2.2 (2.5)
AMLOS(2)	**2.50**	**231**	**6.5**	**73.9 (22.2 - 196.4)**	**0.4 (0.6)**
AA(2)	**4.6**	**1248**	**3.0**	**88.5 (24.8 - 567.4)**	**1.2 (1.4)**
FL(2)	**4.74**	**1248**	**10.6**	**310.2 (84.3 - 1360.1)**	**5.7 (1.9)**
GMLCS	4.29	125	45.9	188.2 (62.3 - 500.8)	2.4 (1.4)
GA	4.29	125	3.1	12.5 (4.3 - 20.6)	0.4 (3.4)

GM: geometric mean.

(1) Extrapolated to the maximum amount of product that could be handled in a single day (8h), standardised for average body weight of 70 kg.

(2) **Bold results** indicate equivalent Australian use pattern.

When results are standardised over a typical working day, the exposure ranking changes because the amount handled depends upon the nature of the spraying operation (ie acres able to be treated over the day). Taking into account the working day, the ranking becomes:

FL
GMLCS
AMLCS ≈AA ≈ AMLOS
GA

Flaggers suffer the highest exposure over the typical working day. These are followed by ground mixer/loaders using the closed system, who have higher exposure than any mixer/loaders for aircraft or aerial applicators. GA appear to be the most protected workers.

The distribution of dermal exposure on covered skin, exposed skin and hands and inhalation exposure was provided. Results are presented in **Table 6**.

Table 6: Percent distribution of exposure for each job category derived from Miles Inc Missouri (Study Number DE202301) Report No 103889, January 25 1993.

Body site	**Job Category** **% Distribution**					
	AMLCS	**AMLOS(1)**	**AA(1)**	**FL(1)**	GMLCS	GA
Covered skin	48.6	**38.8**	**14.0**	**17.0**	39.8	68.5
Exposed skin	24.5	**14.9**	**9.4**	**50.7**	21.1	5.4
Hands	24.5	**45.6**	**75.2**	**30.4**	37.7	22.8
Inhalation	2.5	**0.6**	**1.4**	**1.9**	1.4	3.4

(1) **Bold results** indicate the equivalent Australian use pattern.

Distribution of exposure varies between job categories. Hand exposure is high for all categories, including the mixer/loaders who wore gloves. The automated mixing system for aerial operations AMLCS, offers the most hand protection for mixer/loaders. The high proportion on hands for AA is attributed by Bayer Agriculture Division (USA) to the fact that AA washed the windscreens of the planes. For mixer/loaders using all systems, ~ 80% exposure is through covered skin (long trousers and long-sleeved shirt or overalls as a substitute to trousers and shirt) plus hands (gloves). For ground sprayers, almost 70% of exposure is through covered skin alone. For flaggers, most exposure is through exposed skin and hands.

The blood cholinesterase activity of 1-5 workers in each job category was monitored for the duration of the DEF application season. Workers participated if they could be monitored over this time and were not potentially exposed to other anticholinesterase compounds. Biological monitoring did not necessarily include the personnel involved in the exposure study. Plasma and

erythrocyte cholinesterase of 5 AMLCS, 3 GMLCS, 1 AMLOS, 5 AA, 3 GA and 5 FL were monitored on a weekly basis for a 3-4 week period. No individuals needed to be removed from the trial with an erythrocyte cholinesterase of < 70% of their baseline, the cut-off level adopted in the study protocol. Group mean erythrocyte cholinesterase ranged from 99.4% ± 6.5% of pre-exposure baseline values for GMLCS to 106.9% ± 2.5% of pre-exposure baseline values for AMLCS. Group mean plasma cholinesterase values ranged from 95.9% ± 4.8% of pre-exposure baseline values for AA to 107.5% ± 3.5% of pre-exposure baseline values for AMLOS.

3.5.2.2.2 Re-entry workers

Evaluation of worker exposure to tribufos during harvesting of cotton treated with DEF 6 Eberhart DC and Ellisor GK (Miles Inc. Study No DE202302), Report No 105103, March 15 1993.

Method

- The field and laboratory components of the study were conducted according to Good Laboratory Practice. A quality assurance review of the study was conducted by Mobay Corporation. The study was conducted under USEPA guidelines for re-entry (40 CFR Part 158.390, 1984).

- The study determined dermal and inhalation exposure for workers harvesting cotton treated with DEF 6 and monitored blood cholinesterase activity over the harvest season. Fields were harvested by either the module harvesting system at days 15 and 17 post-application or the trailer-harvesting system at day 20 post application. The trailer harvesting technique is now a minor method and used mainly by smaller operators.

- Five commercial cotton-harvesting crews were monitored for inhalation and dermal exposure. Job categories involved in the module harvesting system were picker operators (PO) (those operating the mechanical harvester), module builder operators (MBO) (those operating the machine that compresses harvested cotton into modules) and rakers (RK) (those raking up spilt cotton round the MBO). Job categories involved in the trailer harvesting technique included PO, trampers (TR) (those physically tramping down harvested cotton to compact the load) and RK.

- Workers monitored were 5 PO (10 replicates), 3 MBO (6 replicates), 6 RK (10 replicates) and 2 TR (4 replicates).

- Dermal exposure and dislodgeable residue data were used to calculate a dermal transfer coefficient for each job category. The worst case dermal transfer coefficient was used to determine a "safe" residue level and ultimately a "safe" re-entry interval.

- All applications to cotton and use of PPE were in accordance with the proposed (US) label directions, actual use practices and state and federal regulations. A single application of DEF 6 was made by ground or air at the maximum label rate of 1.9 lb ai/acre (equivalent to 2 kg ai/ha). The study was conducted in three locations in California and Mississippi.

- The timing of harvest was determined by the extent of cotton defoliation.

- Actual dermal exposure was measured using whole-body dosimeters (long-sleeved T-shirts and footless tights), solvent handwashes, and gauze patches. The whole body dosimeters were worn over the worker's underwear and under a set of cotton/polyester coveralls. Inhalation exposure was measured by personal air sampling.

- The monitoring of one worker for one work cycle (approximately 4-5 hours) constitutes an exposure replicate. Thirty dermal and inhalation exposure replicates were collected. Erythrocyte and plasma cholinesterase of 15 workers (5 PO, 5 MBO/TR, 5 RK) were monitored on a weekly basis during the 5-6 week cotton harvesting season. Pre-exposure samples (3) were collected from each participant to establish baselines following a 2-week period without exposure to anticholinesterase compounds.

- Dislodgeable residues were measured in cotton balls collected prior to DEF 6 spraying and at approximately daily intervals up to and including the day of harvest. Samples (50 g) were collected in triplicate, from upper, middle and lower portions of the plant.

Results

Dermal exposures were monitored at harvest on days 15, 17 and 20 after DEF 6 application **(Table 7).**

Table 7: Dermal and inhalation exposure for each job category derived from Miles Inc. Study No DE202302, Report No 105103, March 15 1993.

Job Category	Dermal GM mg/hr (range)	Inhalation GM mg/hr	Inhalation % of total
PO	137.3 (61.7-245.9)	4.6	3.2
MBO	38.9 (20.6-128.7)	4.8	11.0
TR	172.8 (64.1-451.0)	6.9	3.8
RK	83.6 (27.5-195.6)	4.4	5.0

GM: geometric mean

Trampers had the highest dermal and inhalation exposure, followed by for dermal exposure, PO, RK and MBO. PO, MBO and RK had similar inhalation exposure.

The proportion of dermal exposure on covered and exposed body regions and hands and inhalation exposure was calculated for each worker group. Between 39% and 57% of exposure was on covered body regions. Hand exposure accounted for 45-46% of total exposure on PO and TR. RK and MBO had 27% and 18% hand exposure, respectively.

Biological monitoring indicated that individual mean and group mean values for erythrocyte and plasma cholinesterase were within the normal range of biological variation (± 20%). Compared with pre-exposure values, erythrocyte cholinesterase post-exposure ranged from 96.0% ± 6.7% (POs) to 98.4% ± 4.1% (MBOs and TR). Plasma cholinesterase ranged from 95.8% ± 2% (POs) to 98.3% ± 6.4% (RK).

Dermal transfer coefficients for cotton harvesters

The worker exposure data was used to calculate dermal transfer coefficients relating work exposure during cotton harvesting to dislodgeable residue levels (DRLs) on cotton balls collected on the same days. A dermal transfer coefficient (g/hr) is an estimate of contact with pesticide-treated plant material during normal work activity.

The dermal transfer coefficient (TC) is calculated for each group of workers as follows:

$$TC(g/hr) = Exposure(\mu g/hr) / DRL(\mu g/g)$$

TC were calculated for each job category. TC were highest for TR (3086 g/hr) followed by PO (1914 g/hr), RK (1166 g/hr) and MBO (459 g/hr). TC for the PO was used by **Miles Inc** in calculating the safe re-entry interval for workers harvesting Tribufos -treated cotton. The TC for TR was not used because trailer-harvesting is seldom used commercially nowadays.

A safe dislodgeable residue level (SRL) for Tribufos -treated cotton is calculated from the following equation:

$$SRL\ (\mu g/g) = (NOEL)(BW)(DPCF) / (SF)(TC)(HR)$$

NOEL = No Observable Effect Level (µg/kg), BW = the average body weight in kg and DPCF = the dermal penetration correction factor used when dermal exposure is compared with an oral NOEL. DPCF = 1 when a dermal NOEL is used. SF = safety factor, TC = the dermal transfer-coefficient (g/hr) for the activity being considered and HR = the estimated amount of hours worked per day.

The SRL is used in conjunction with the Tribufos -dislodgeable residue decay curve to determine a safe re-entry interval. The most conservative SRL was calculated for both dermal toxicity and neurotoxicity.

Miles Inc calculated the SRL of 0.61µg/g using the dermal NOEL of 2 g/kg bw/day (cholinesterase inhibition), SF = 10, TC of 1,914 g/hr for PO, BW = 70 kg and HR of 12 for harvesting Tribufos -treated cotton. The SRL was 0.34 µg/g using the parameters for neurotoxicity, ie the dermal NOEL of 11 mg/kg/day (neurotoxicity) and SF = 100 (other parameters unchanged). Using the TC of 3086 g/hr for TR, the SRL was 0.38 61 µg/g using cholinesterase inhibition parameters.

Re-entry interval

The re-entry interval for harvesting tribufos-treated cotton was determined by comparing the most conservative SRLs with the tribufos dislodgeable residue degradation curve derived from all 4 study sites, according to the Allowable Exposure Level Method in USEPA Subdivision K guidelines for Re-entry Protection. Tribufos dislodgeable residue levels (µg/g) declined with an average half-life over all sites of 3.7 days. Residues approximated pre-application levels in about 14 days post-application.

The combined tribufos degradation curve is described by $Y=1.166e^{-0.188x}$.

From the curve, the interval required to fall to a SRL of 0.61 µg/g is 3.5 days for dermal toxicity (0.38 µg/g is 6-7 days) and 6.5 days for neurotoxicity.

3.6 RISK ASSESSMENT

Workers will be exposed to the products and the diluted spray via the skin and respiratory tract, when mixing and applying the spray and during flagging and clean-up operations. Some secondary oral ingestion may occur via respiratory ingestion. The risk assessment concentrates on dermal exposure, as the worker studies indicate that this route accounts for most exposure to tribufos.

There is no information on human toxic end points for tribufos to use in the risk assessment.

3.6.1 Dermal absorption

High dermal absorption of 38-53% was found in rats after 10h exposure to DEF 6. Most absorption (67-74%) occurred in the first hour. Where a dermal absorption factor is needed in the risk assessment, 53% is used.

3.6.2 Acute toxic potential

Tribufos has moderate acute dermal toxicity (LD_{50} = 1093 mg/kg). Considering this, a 60 kg person would need to be contaminated with 93 mL of the product and 664 mL of the diluted spray (containing 9.9% of Tribufos) to receive a dose equivalent to the LD_{50}.

DEF Defoliant has moderate oral toxicity, low dermal and inhalation toxicity and is a severe skin irritant.

Consideration of the acute toxicity of the product alone indicates that skin protection is needed for those handling the concentrate and working strength solutions.

3.6.3 Repeat dose toxic potential

Dermal NOELs for cholinergic effects including (significant) depressed cholinesterase and delayed neurotoxicity are relevant to assess repeat dose toxic potential. Using the subacute dermal NOEL of 2 mg/kg bw/day (depressed ChE and cholinergic effects at 11 mg/kg bw/day), a 60 kg worker would need to be contaminated with more than 120 mg DEF, 0.170 mL of DEF Defoliant and 1.21 mL of spray to exceed the NOEL.

For clinical signs of neurotoxicity, using the subchronic dermal NOEL of 11 mg/kg bw/day (signs at 42 mg/kg bw/day), a 60 kg worker would need to be exposed to more than 660 mg of DEF, 0.94 mL of DEF Defoliant and 6.7 mL of spray to exceed the NOEL.

The lowest NOEL for chronic exposure is 0.1 mg/kg bw/day for plasma cholinesterase inhibition from a dog dietary study. A 60 kg worker would need to be contaminated with more than 11 mg of DEF, 0.029 mL of DEF Defoliant or 0.21 mL of spray to exceed the NOEL. This incorporates 53% dermal absorption.

3.6.4 Delayed neurotoxicity upon accidental dermal exposure

An estimation of the dermal dose required for an accidental exposure to progress to delayed neurotoxicity is made using the results from a single dose study in hens (Abou-Donia *et al* 1986). Hens receiving a single topical dose of 250 mg/kg developed severe ataxia, progressing in some cases to near-paralysis or paralysis, plus histopathological changes in nervous tissue. There were no effects at the next lowest dose of 100 mg/kg.

A single topical neurotoxic dose of 250 mg/kg for a 60 kg worker corresponds to 15 g of DEF, 21 mL of DEF Defoliant or 150 mL of spray.

The average exposures found in the **Miles Inc** worker study suggests that this degree of exposure is unlikely to occur normally, however it could occur under accidental conditions or where occupational hygiene practices were poor. The exposure ranges indicate that those replicates on the upper end of exposure would not be near this accidental single dose **(Table 5).** Flaggers had the highest upper exposure, with the highest replicate equivalent to 1.36 mg/kg bw/day.

3.6.5 Assessment of end use exposure studies

Margins of exposure (MOE) were calculated from the worker exposure data provided in the **Miles Inc** Missouri (Study number DE202301) Report No 103889, January 25 1993.

Exposure was standardised for an 8 hour working day (incorporating the typical work practices of the study) and for an average worker body weight of 60 kg.

MOE are given for dermal and inhalation exposure separately. It is not possible to estimate the risk of combined exposure using the individual NOELs. However, inhalation absorption is assumed to be 100%; in comparison with maximum dermal absorption of 53%, inhalation exposure could be assumed to contribute up to 6% of the risk (maximum of 3.1% inhalation exposure in the worker study). MOE for chronic exposure are not given as workers only need to handle tribufos for a defined period during the season. Results for dermal and inhalation risk are shown in **Table 8**.

Table 8: Dermal exposure and estimations of Margins of Exposure

Job category	Dermal Exposure [1]			Inhalation exposure [2]		
	mg/kg bw/d [3]	MOE (cholinergic effects and ChE inhibition)[4] (range)	MOE (neuro-toxicity) [5] (range)	mg/kg bw/day [3]	MOE (ChE inhibition) [6]	MOE neuro-toxicity) [7]
FL	361.9	6 (1-20)	30 (7-112)	6.6	63	549
GMLCS	219.6	9 (3-28)	50 (19-151)	2.8	150	1304
AMLCS	110.4	18 (7-60)	100 (37-333)	2.6	162	1404
AA	103.2	19 (3-69)	107 (17-380)	1.4	300	2607
AMLOS	86.2	23 (9-77)	128 (48-425)	0.47	894	7766
GA	14.9	134 (85-399)	738 (469-2193)	0.47	894	7766
GMLCS/GA[8]	191.1	11 (3-25)	58 (18-138)			

(1) Personal protective equipment worn was according to the product label. All workers wore long trousers, long-sleeved shirt (or overalls as a substitute to trousers and shirt) and hat or helmet. In addition, mixer/loaders (ML) used goggles or face shield and chemical-resistant gloves. Ground applicators (GA) used chemical-resistant gloves but not inside closed cabs.
(2) Respiratory protection was not used
(3) Average body weight of 60 kg; 8 hour day
(4) MOE based on NOEL of 2 mg/kg bw/d
(5) MOE based on NOEL of 11 mg/kg bw/d
(6) MOE based on NOEL of 2.4 mg/m^3 or 0.42 mg/kg bw/day (converted to human exposure, 29 LPM)
(7) MOE based on NOEL of 21 mg/m^3 or 3.65 mg/kg bw/day (converted to human exposure, 29 LPM)
(8) Combined tasks, assuming treatment of 50 hectares over an 8 hour day.

Discussion

The discussion considers ground and aerial operations separately. Ground spraying is not included on the draft label, but is included in the assessment against future applications for this use. In the first instance, risk is discussed with respect to workers developing acute cholinergic symptoms. The risk with respect to neurotoxicity is discussed later for all operations.

Biological monitoring

The finding that no workers exhibited significantly altered plasma or RBC cholinesterase over the season, suggests that there was not significant uptake of the contaminating chemical under the conditions of the study. Some workers involved in the biological monitoring program were not involved in the exposure study. They were selected to supplement the numbers in each group and were not exposed to other anticholinesterase compounds during the study. The rate at which DEF 6 was applied was discretionary, but a weekly record of the amount of Tribufos handled was kept. Differences in the amount of Tribufos handled by individuals or between groups, for instance aerial operators handled more that ground operators, were not reflected in cholinesterase measurements. As workers performing separate tasks were monitored, the biological monitoring results are not applicable for ground operations where workers may perform combined tasks. The amount of exposure indicates what is available for uptake should circumstances be favourable.

Inhalation MOE

Apart from flaggers, discussed separately below, worker risk from inhalation is acceptable **(Table 8).** MOE demonstrate that considering inhalation exposure alone, there is little to be gained by reducing exposure by using respirators.

Ground operations

Mixer/loaders (GMLCS)

Mixer/loaders for ground operations wore extensive personal protective equipment over the skin. They used an enclosed mixing system involving the addition of concentrate by gravity feed or a suction probe. These workers triple rinsed the containers and arranged their disposal. The method of rinsing for the 5-gallon containers within the gravity feed system is not described; rinsing was via the suction probe for the 30-gallon containers.

Despite these controls, ~ 80% of dermal exposure was through protective clothing including gloves.

This demonstrates that there is little scope to reduce further mixer/loader exposure during transfer and mixing of the concentrate, by means of additional protective clothing or engineering controls.

For these workers, only 1.4% of total exposure was via the respiratory tract. The addition of respiratory protection would not impact substantially on exposure or risk.

The MOE derived from the subacute dermal NOEL (rat; cholinergic symptoms at the next dose), for these workers range from 3 to 28. This range is unacceptable on the basis of exposure (considering extensive exposure controls are in place) and toxicity (there is a 5.5-fold difference to the next dose demonstrating clinical cholinergic toxicity (11 mg/kg bw/day). If ground spraying of DEF Defoliant were conducted in Australia, transfer of the concentrate to the tank could involve procedures similar to those described in the study or the container could be manually handled. In any case, the risk would be at least as great as estimated here.

Applicators (GA)

Ground applicators were located in closed cabs with airconditioning. The effectiveness of the airconditioning and air filtration was not assessed in the worker studies. However ground applicators had the highest MOE (range 85-399) for any worker group and this range is acceptable for workers performing this task alone.

Ground applicators also wore extensive personal protective equipment. Despite this, only 5% of exposure was on exposed skin, 22% on (mostly) uncovered hands, with ~ 70% through protected skin.

Inhalation exposure is only a small portion of the total (3.4%). Respiratory protection is of little value to these workers.

Combined tasks (GMLCS & GA)

The worker study did not cover those doing combined tasks. However some extrapolations can be made for workers performing combined tasks. This scenario would be applicable to Australian workers if ground spraying of DEF Defoliant were permitted.

Firstly, workers performing combined tasks would be unable to cover the same area in a typical day (8 hour) as those where tasks are shared. Assuming an average coverage of 50 hectares per day and using the worker study application rate and exposure per amount handled, the combined MOE gives a range of 3-25. Values in this range are low and unacceptable.

Summary

For mixer/loaders in ground operations the MOE are low and unacceptable. There are few options for substantially reducing exposure. Australian workers involved in mixing/loading operations would be exposed to risks at least as great as the workers in the study. Ground applicators in closed cabs demonstrated an acceptable risk. It is likely that the majority of ground sprayers in Australia would use closed cabs, but their use cannot be enforced. For workers performing combined operations, the risk is unacceptable.

Aerial operations

Mixer/loaders

Mixer/loaders for aerial operations wore extensive personal protective equipment over the skin. Mixer/loaders connected and disconnected hoses to the plane. Taking into account the 8 hour day, aerial mixer/loaders using both closed and open systems had similar exposures and similar risk.

Closed system (AMLCS) This involved automatic transfer of the product from bulk containers to a holding tank. The product was mixed with water as it was pumped from the holding tank to the plane. Bulk containers are likely to be refillable, so rinsing should not be required. Despite engineering controls and protective clothing, ~ 73% of dermal exposure was through protective clothing including gloves.

This demonstrates very little scope to reduce further mixer/loader exposure by means of additional protective clothing or engineering controls for transfer and mixing of the concentrate.

For these workers, 2.5% of total exposure was via the respiratory tract. The addition of respiratory protection would not impact substantially on exposure or risk.

Open system (AMLOS) This involved manual pouring of the contents of the 5-gallon container into the holding tank. Workers would have rinsed the containers manually. Workers were protected by protective clothing only. They had twice the proportion of hand exposure (45.6%) as aerial mixer/loaders using the closed system (24.5%). Approximately 84% of total dermal exposure was through protective clothing, including gloves.

Workers using the open system would need to convert to a closed system in order to reduce exposure, as there is little scope through the use of additional protective clothing. Less than 1% of exposure is respiratory so the addition of a respirator would have little impact on exposure.

The MOE calculated for workers using the closed and open system are much the same (18 (7-60) and 23 (9-77), respectively). This is despite the fact that mixer/loaders using the open system have about twice the exposure on an amount handled basis. In the worker studies presented, the closed mixing system was used by larger operators, where more planes (up to 3) were serviced, therefore more active handled, over an 8 hour day. Under Australian conditions only the 20 litre container is proposed. Therefore it is likely that either the open system or other (various) less manual but not totally enclosed systems would be used. Small operators could expect a similar MOE to that shown here for the open system (23). If more than one plane were serviced using the open system, exposure and risk would increase.

The MOE for mixer/loaders using both systems are not acceptable. For the closed system they are derived from conditions where engineering controls are exhaustive and extensive protective clothing is worn. There are few options to reduce exposure. Workers in small operations would have more acceptable MOE if they had the option of using an enclosed transfer and mixing system. In Australia a bulk container is not proposed and the 20 Litre container is not described as suitable for an enclosed transfer and mixing system.

Aerial applicators

The MOE of 19 (range 3-69) for aerial sprayers is similar to those of the aerial mixer/loaders and is unacceptable. Pilots wore long trousers and long sleeves. For activities outside the plane they were to wear face, eye and hand protection. A large proportion of dermal exposure (75%) is on the hands. The study authors attributed this to the fact that pilots cleaned the plane windscreen without wearing gloves.

Combined operations for mixing/loading and application

This is not covered as tasks would normally be separated in aerial operations.

Flaggers

Flaggers had the lowest dermal MOE at 6 (range 1-20) of any of the workers in the study. They wore long trousers and long sleeves, with options for face, eye, hand and respiratory protection under unfavourable environmental conditions. One flagger of the four is documented as wearing nitrile gloves and goggles. Inhalation exposure was highest for flaggers, but proportionally inhalation exposure accounted for 1.9% of total exposure. MOE considering inhalation exposure alone (63) are barely acceptable for flaggers. The bulk of dermal exposure was on exposed skin (51%) and hands (30%, possibly uncovered).

Exposure for flaggers is high and with the low MOE indicates that control measures are inadequate. Manual flagging is unacceptable for DEF Defoliant.

Summary

For mixer/loaders, similar MOE are calculated using both mixing/loading systems. This reflects the combined factors of the amount of chemical handled and the degree of engineering control. Larger operations handling more chemical had a more controlled system. In Australia, only a 20 L container is proposed, therefore either the open system or other less manual but not entirely enclosed mixing systems could be used. Under these circumstances, a MOE similar to that of the open system (23) could be expected. This does not demonstrate an adequate exposure margin, given that these operators also wear extensive protective clothing.

The MOE of aerial sprayers is similar to that of the mixer/loaders and does not demonstrate an adequate exposure margin. Conditions for Australian spray pilots would be similar to those in the study.

Manual flaggers had the lowest exposure margins of any workers in the study. This indicates that manual flagging is not acceptable for Tribufos.

Repeat exposure risk in relation to delayed neurotoxicity (all operations)

MOE calculated using the dermal NOEL for delayed neurotoxicity (hens) (Table 8) are greater than those calculated for cholinergic toxicity. However the fact that the clinical effects of delayed neurotoxicity can be permanent in humans needs to be accounted for in the assessment of risk.

MOE ranges indicate concern for certain individuals (those at the lower end of the range) conducting normal tasks. This applies to all worker categories except ground applicators.

Studies of single topical doses of tribufos in the hen have shown that inhibition of enzymes related to the development of OPIDN and damage to nervous tissue as revealed by histological examination, occur at doses that do not demonstrate an acute cholinergic response. OPIDN is caused by smaller doses as the frequency of exposure is increased.

The neurotoxic NOELs used in this assessment relate to clinical neurotoxicity at the next dose. There are suggestions that depression of NTE may act as an early indicator of OPIDN. NTE was not measured in the studies ultimately selected for the NOEL. In the study of worker exposure to tribufos and merphos conducted by Lotti *et al* (1983), depression of NTE by 65% was observed without registering any change in blood cholinesterase or the peripheral nervous system or health effects. Current health surveillance techniques to monitor and control exposure may not be adequate for OPIDN. Work is continuing to define the use of NTE as a predictive enzyme marker for OPIDN. It is possible that this work may ultimately result in assigning lower NOELs than those used at present.

3.6.6 Assessment using exposure calculation model

The UK Predictive Operator Exposure Model (POEM) was used for the DEF Defoliant risk assessment to calculate risk for ground operations, including an open system for mixing/loading not covered in the **Miles Inc** study. The container for use in Australia is not described as able to be used for enclosed or automated mixing. Therefore it is appropriate to test exposure through an open mixing model. POEM printouts are provided as **Attachment 1**. The model was used for boom spray, ie Vehicle mounted with cab hydraulic nozzles, Vehicle mounted with cab rotary disc atomisers. POEM does not cover practices used in aerial operations.

POEM parameters:

Product name	DEF Defoliant
Active ingredient	Tribufos
Concentration	705 mg/mL
Formulation type	EC
Max in use concentration	66 mg/mL
Container size	20 L
Application dose	2.8 L prod/ha
Work rate	50 ha/day. A lower value was selected than found in the Miles Inc study as here workers perform combined tasks of mixing/loading and application
Application volume	30 L/ha. The lower amount specified for aerial spraying was used to account for differences in equipment.
Duration of exposure	6 hour. Selected as here workers perform combined tasks of mixing/loading and application
Percent absorbed -dermal	100%
Percent absorbed - inhalation	100%
NOEL (dermal)	2 mg/kg bw/day (cholinergic effects and ChE inhibition)
NOEL (dermal)	11 mg/kg bw/day (neurotoxicity)

MOE from POEM are very low **(Table 9)** and indicate that the risk for workers performing individual or combined tasks is unacceptable, based on the toxic endpoints of cholinergic and neurotoxic effects.

Table 9: Margins of exposure for ground spraying derived from POEM for mixer/loaders and applicators wearing gloves.

Job	**Vehicle mounted with cab hydraulic nozzles**		**Vehicle mounted with cab rotary disc atomisers**	
	MOE (cholinergic effects and ChE inhibition) (1)	**MOE (neuro-toxicity)** (2)	**MOE (cholinergic effects and ChE inhibition)**(1)	**MOE (neuro-toxicity)** (2)
mixing	< 1	3	< 1	3
applying	< 1	2	2	9
mixing and applying	< 1	1	< 1	2

(1) MOE based on NOEL of 2 mg/kg bw/d
(2) MOE based on NOEL of 11 mg/kg bw/d

3.6.7 Re-entry assessment

The Miles Inc report on exposure to workers at cotton harvest covers similar situations as would exist in Australia and the results are applicable to the Australian situation.

Actual exposure measured during harvesting on days 15, 17 and 20 provided adequate MOE for neurotoxic effect for harvest workers. For dermal exposure, TR are at highest risk, but MOE are still adequate. The extent of manual tramping in Australia is not known. Margins may be low on occasion for individuals, but overall the risk from dermal exposure is acceptable. Inhalation risk for all workers is low. Results for cholinesterase monitoring support the MOE (cholinergic effects) **(Table 10)**, however they do not allow any conclusions to be drawn about early effects upon NTE or implications for OPIDN (see above).

Table 10: Dermal exposure and estimations of Margins of Exposure

Job category	Dermal Exposure [1]			Inhalation exposure [2]		
	mg/kg bw/d [3]	MOE (cholinergic effects and ChE inhibition)[4] (range)	MOE (neuro-toxicity) [5] (range)	mg/kg bw/day [3]	MOE (ChE inhibition)[6]	MOE neuro-toxicity) [7]
PO	18.3	109 (61-244)	601 (335-1341)	0.61	689	5984
MBO	5.2	385 (116-741)	2115 (640-4074)	0.64	656	5703
TR	23.1	87 (33-235)	476 (183-1294)	0.92	457	3967
RK	11.2	179 (77-540)	982 (421-2973)	0.59	712	6186

(1) Workers wore normal work clothing
(2) Respiratory protection was not used
(3) Average body weight of 60 kg; 8 hour working day used
(4) MOE based on NOEL of 2 mg/kg bw/d
(5) MOE based on NOEL of 11 mg/kg bw/d
(6) MOE based on NOEL of 2.4 mg/m^3 or 0.42 mg/kg bw/day (converted to human exposure, 29 LPM)
(7) MOE based on NOEL of 21 mg/m^3 or 3.65 mg/kg bw/day (converted to human exposure, 29 LPM)

Calculations of transfer coefficients, tribufos residue decay curves and safe residue limits (incorporating a safety factor of 10), showed that a re-entry interval of 6-7 days was required for harvesting operations. In Australia harvest would be possible within 1-2 weeks of treatment with defoliants. The need for crop maintenance is reduced after defoliation treatment, however workers entering treated areas within the re-entry interval would need to wear overalls and gloves. The tribufos residue decay curve showed that baseline levels were achieved 7 to 9 days after application.

3.7 OCCUPATIONAL CONTROLS AND RECOMMENDATIONS

3.7.1 Statement of hazardous nature

Tribufos and Def Defoliant are classified as hazardous substances according to NOHSC criteria. Hazardous substances are subject to the workplace controls outlined in the Control of Workplace Hazardous Substances (NOHSC 1994a).

3.7.2 Recommendation to the NRA on the use of DEF Defoliant

Use of DEF Defoliant cannot be supported on occupational health and safety grounds under the end use conditions outlined in the Bayer Australia submission and current Australian agricultural practice. Reasons for this decision are given below.

Toxicity

The toxicity of tribufos is of major concern for worker health. OPIDN can be permanent in humans. Although Tribufos is not reported to have caused this condition in humans, it is known to reliably produce it in hens (the model of choice for this condition), irrespective of the route of administration and after single and repeated doses. Delayed toxicity of Tribufos appears to be cumulative, with toxicity observed at lower doses as the number of doses are increased. NTE is the target enzyme in the development of OPIDN. In hens the threshold for NTE inhibition and OPIDN is 70-80%. In humans the threshold has yet to be established.

Workers handling tribufos and merphos (a related OP) during the cotton defoliation season, demonstrated a 65% inhibition of NTE, but no subclinical effects on the peripheral nervous system or significant changes in blood cholinesterase. A single dermal exposure to tribufos in hens resulted in OPIDN at lower doses than those required to cause acute cholinergic toxicity. OPIDN was dose-dependent and clinical signs were accompanied by histopathological changes in the spinal cord and peripheral nerves.

Ground operations - Summary
For mixer/loaders in ground operations the MOE are low and unacceptable. There are few options for substantially reducing exposure. Australian workers involved in mixing/loading operations would be exposed to risks at least as great as the workers in the exposure study assessed. It is likely that the majority of ground sprayers in Australia would use closed cabs, but their use cannot be enforced. For workers performing combined operations, the risk is unacceptable as extrapolated from the worker exposure study and the exposure model POEM.

Aerial operations - Summary
For mixer/loaders, similar MOE are calculated using both mixing/loading systems. This reflects the combined factors of the amount of chemical handled and the degree of engineering control. In Australia, only a 20 L container is proposed, therefore either the open system or other less manual but not entirely enclosed mixing systems could be used. Under these circumstances, a MOE similar to that of the open system could be expected. This does not demonstrate an adequate exposure margin, given that these operators also wore extensive protective clothing.

The MOE of aerial sprayers is similar to that of the mixer/loaders and does not demonstrate an adequate exposure margin. Conditions for Australian spray pilots would be similar to those in the study.

Manual flaggers had the lowest exposure margins of any workers in the study. This indicates that manual flagging is not acceptable for tribufos.

Accidental exposure and delayed neurotoxicity
A comparison of the estimated single dose on the skin needed to induce delayed neurotoxicity with occupational exposure under routine conditions indicates that the required accidental dose is unlikely to occur normally. However the possibility cannot be excluded that it could occur under accidental conditions or where occupational hygiene practices are poor, resulting in irreversible health effects.

Safety Directions
Safety directions cannot be established for this product. Safe use cannot be demonstrated when extensive personal protective equipment is worn to prevent skin contamination and when engineering controls are in place. Addition of respiratory protection is unlikely to reduce overall exposure to acceptable levels.

Health Surveillance
Guide-lines for monitoring of Organophosphate Pesticides have been endorsed by NOHSC (NOHSC 1995).

The guidelines include an estimation of red blood cell and plasma cholinesterase as predictors of cholinergic effects. Specific measurement of enzymes predictive of OPIDN are not included in the NOHSC guidelines. Further scientific investigations are required to define the role of target enzymes in OPIDN before their routine use could be endorsed.

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