Section 4
EVALUATION OF THE MAMMALIAN TOXICOLOGY AND METABOLISM/TOXICOKINETICS

1. INTRODUCTION

1.1 Regulatory History of Health Considerations in Australia

Monocrotophos is an organophosphorothioate insecticide used in agriculture to control a range of pests in a range of horticultural and agricultural crops. Residue levels of monocrotophos were set for apples, pears and cotton seed in 1968, with uses extended into potatoes, tomatoes, sweet corn, bananas, beans and cereals throughout the 1970s and 1980s.

In Australia, public health standards for agricultural and veterinary chemicals, such as the poison schedule, first aid and safety directions and an acceptable daily intake (ADI), are set by the Department of Health and Family Sevices. Poison schedules are set by the National Drugs and Poisons Schedule Committee (NDPSC) or the Australian Health Ministers' Advisory Council (formerly the Drugs and Poisons Schedule Committee (DPSC) or the National Health and Medical Research Council (NHMRC). In the case of maximum residue limits (MRLs), these were formerly established by the Pesticide and Agricultural Chemicals Committee (PACC) of the NHMRC, however in 1992, the Department of Health became directly responsible for establishing MRLs, a function subsequently transferred to the National Registration Authority (NRA) in June 1994.

Health Standards

NOEL/ADI

The PACC established an acceptable daily intake (ADI) of 0.0003 mg/kg bw/day (December 1990). This ADI was set on a No Observable Effect Level (NOEL) of 0.003 mg/kg bw/day based on plasma ChE inhibition in a human study.

Poisons Schedule

Monocrotophos is in Schedule 7 (S7) of the Standard for the Uniform Scheduling of Drugs and Poisons (SUSDP).

MRLs

Monocrotophos has MRLs set for fruits, vegetables, meats, milks and cereal grains. The MRLs for monocrotophos at detailed in the MRL standard (June 30 1994) are outlined in Table 1 below.

Table 1. Australian Maximum Residue Limits for Monocrotophos.

Commodity	MRL (mg/kg)
Apple	0.5
Banana	0.5
Beans, except broad beans and soya been	0.2
Broad bean (green pods and immature seeds)	0.2
Cereal grains	*0.02
Cotton seed	0.1

Edible offal (mammalian)	*0.02
Eggs	*0.02
Meat (mammalian)	*0.02
Milks	*0.002
Pear	0.5
Potato	0.1
Poultry, edible offal off	*0.02
Poultry meat	*0.02
Sweet corn (corn on the cob)	*0.01
Tomato	0.5
Vegetable oils, edible	*0.05

Existing Chemical Review Program

Monocrotophos is one of some 80 agricultural and veterinary chemicals identified as candidates for priority review under the ECRP. Following data call-in processes, a number of additional studies on the toxicology of monocrotophos have been received from industry. These data, together with all previously submitted data have been evaluated and are detailed in the report below. The data submission details covering toxicological and public health aspects of monocrotophos are summarised in Appendix 1.

1.2 International Toxicology Assessments

Monocrotophos has been evaluated by the Joint FAO/WHO Expert Committee on Pesticide Residues (JMPR) in 1972, 1975, 1991, 1993 and 1995. An ADI of 0.0006 mg/kg bw/day was allocated by JMPR in 1993, and confirmed in 1995.

This ADI was based on the following levels causing no toxicological effects.

Mouse: <1 ppm in the diet, equivalent to <0.15 mg/kg bw/day (2 year study)
Rat: 0.1 ppm in the diet, equivalent to 0.005 mg/kg bw/day (2 year study)
Human: 0.006 mg/kg bw/day (30 day study)

1.3. Chemistry

Chemistry details for monocrotophos are contained in Section 3 of this report

2. METABOLISM AND TOXICOKINETICS

2.1. Rat

Lee PW (1987) Rat Metabolism Study of ^{14}C-DPX-Y2034 Lab: EI du Pont de Nemours & Co. Inc. Lab Project ID AMR-653-87 RTI-3852 GLP:USEPA

Wistar rats (Crl(W1)BR, Charles River Labs, Kingston NY) (7/sex) were given single gavage doses of ^{14}C-monocrotophos (radiochemical purity 98.4%, batch E-48043-32, Shell Chemical Co.) at 2 mg/kg bw. Animals were individually housed in metabolism cages and urine, faeces, and CO_2 were collected throughout the study. The study was terminated when either 90% of the radioactive dose had been recovered or at 7 days following dosing. Levels of radioactivity were determined in urine, faeces, expired CO_2 and selected tissues. Urinary metabolites were isolated and identified using TLC, HPLC and MS.

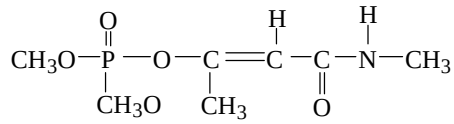
Thirty minutes after dosing, common signs of organophosphate poisoning were evident (trembling, twitching, salivation, chromodachyorrhoea, and piloerection) for up to 3 h.

82% of the administered radioactive dose was detected in the urine after 96 h (76% was detected in the urine after 12 h). After 96 h, excretion of radioactivity in the faeces and expired air accounted for approximately 3% and 6% of the radioactive dose respectively. The rate and route of excretion were independent of sex.

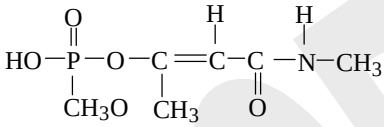
Only low levels of radioactivity were detected in the tissues after 96 h, the highest level being found in adipose tissue (<0.08 ppm). The liver contained <0.05 ppm, while the skin contained approximately 0.04 ppm. Although widely distributed, radioactivity detected in the tissues accounted for less than 1% of the radioactive dose. The distribution of radioactivity was independent of sex.

Unchanged monocrotophos in the urine accounted for 26 to 33% of the radioactive dose. On the basis of TLC and HPLC data, the principal urinary metabolites, N-methyl acetoacetamide (SD9112) and 3-hydroxy-N-methyl butyramide (SD11734), formed following cleavage of the phosphate-vinyl linkage, accounted for approximately 13-17% and 8% of the radioactive dose, respectively. Seventeen to 20% of the radioactive dose remained in the aqueous phase in the urine and could not be extracted using organic solvents. Faecal metabolites were not identified. On the basis of the metabolites identified, it was determined that the metabolism pathway in the rat is basically a detoxification route, involving the ester cleavage of monocrotophos, to first produce N-methyl acetoacetamide, which is then further degraded to produce 3-hydroxy-N-methyl butyramide. Given the excretion seen in expired air (approximately 6% of administered dose), the levels of radioactivity found in tissues were considered to be largely due to incorporation of carbon dioxide into the biological system.

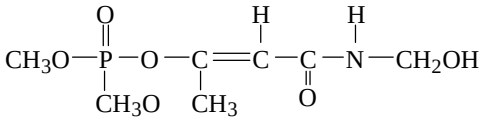
Metabolic Pathway for Monocrotophos



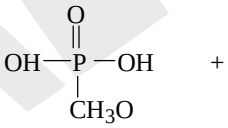
Monocrotophos



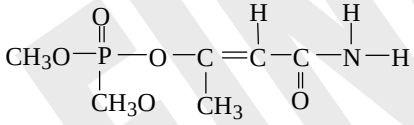
O-desmethyl monocrotophos



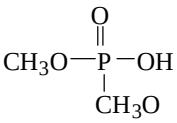
N-hydroxymethyl monocrotophos



Methyl Phosphate + Breakdown products



N-desmethyl monocrotophos



Dimethyl Phosphate + Breakdown products

Bull DL & Lindquist DA (1966) Metabolism of 3-hydroxy-N-methyl-cis-crotonamide dimethyl phosphate (AZODRIN) by insects and rats. J Agric Food Chem 14(2):105-109

The metabolism of monocrotophos by insects and rats was investigated by quantifying metabolites present in extracts of animals treated with ^{14}C- and ^{32}P-labelled monocrotophos and by incubating unlabelled monocrotophos with extracts from untreated animals. Metabolites found following this incubation included phosphoric acid, monomethyl phosphate, dimethyl phosphate, O-demethyl monocrotophos, monocrotophos acid and hydroxymethyl monocrotophos, however the relative proportions of metabolites formed were not quantified.

^{32}P-labelled monocrotophos was administered to Wistar rats (source not specified) at 5 mg/kg bw by IP injection. Urine was collected every 2 h for the first 12 h, then at intervals until 48 h after treatment. Faecal samples were also collected over this period. Monocrotophos was excreted rapidly in the urine, with 45% of the administered dose recovered in the first 6 h after treatment. Over the 48-h observation period, 61% of radioactivity was excreted in the urine, with 6% excreted in the faeces. In the first 2 h, the main compound excreted was unchanged monocrotophos (50% of radioactivity excreted), with approximately 30% of the excretion as dimethyl phosphate. At 24 h, dimethyl phosphate made up approximately 50% of the excreted material, while unchanged monocrotophos accounted for approximately 10%. Over 48 h, dimethyl phosphate made up 40% of urinary excreted metabolites, monocrotophos 28%, hydroxymethyl monocrotophos 19%, O-demethyl monocrotophos 10%, and phosphoric acid 3%.

2.2. Rat, goat and mouse.

Menzer RE & Casida JE (1965) Nature of toxic metabolites formed in mammals, insects and plants from 3(-dimethoxyphosphinyloxyl)-N, N-dimethyl-cis-crotonamide analog. J Agric Food Chem 13: 102-112.

Monocrotophos technical, and monocrotophos radiolabelled at a number of different sites were used to investigate the metabolism of monocrotophos in the rat and the goat. The radiolabelled compounds were ^{32}P -labelled monocrotophos, N-methyl ^{14}C labelled monocrotophos, and O-methyl ^{14}C labelled monocrotophos. These were administered both separately and in combination to a Saanen goat, and to white rats (supplied by Rolfsmeyer Farms, Wisconsin).

When a Saanen goat was given an unspecified dose of radiolabelled monocrotophos, there were no signs of toxicity. Milk, urine and faeces were collected at 'regular intervals' for 72 h. Radioactivity was determined by a liquid scintillation counter. Radioactivity in milk was 0.05 ppm 1 h after the dose, and decreased progressively from this point. The excreted material was analysed using a chromatograph to determine the metabolites, however the results were not reported.

Rats were treated with 1 mg/kg bw labelled ^{32}P-monocrotophos in aqueous solution by gavage, and faeces and urine were collected. Following the treatment, a number of metabolites were excreted. 13% of the administered dose was excreted as unchanged monocrotophos, while 50% of the administered dose was excreted as hydrolysis products (not further quantified).
N-hydroxymethyl amide and an amide product each were less than 2% of the administered dose. Overall, 63 - 71% of the dose given was excreted in 48 h, with 55% being excreted in the first 6 h.

2.3 Goat

Hall TDJ, Jameson CE & Shaffer SR (1987) Goat Metabolism Study of ^{14}C-DPX-Y2034. Lab: Analytical Bio-Chemistry Laboratories Inc. Sponsor: EI du Pont de Nemours & Company Inc. GLP:USEPA

^{14}C -labelled monocrotophos (radiochemical purity 98.5%, specific activity 21.3 µCi/mg; Shell Biological Science Research Centre) was administered to goats (Les Finding, Atlanta, Missouri) orally by gelatin capsules at 10 mg/d (approximately 0.2 mg/kg bw/d) for 3 days. Two goats were treated, and one maintained as a control. Goats were housed in individual metabolism cages; urine and faeces were collected daily, milk twice daily. Within 24 h of the final dose, all goats were killed. A gross necropsy examination was done, and samples of blood, omental fat, perirenal fat, muscle, kidneys, liver and GI tract/rumen contents were collected. The total radioactivity in all samples was quantified. For urine, faeces and milk, the metabolites were identified and quantified, where possible.

Feeding of monocrotophos at this dose did not produce any adverse clinical signs. By the end of the 3 d of collection, 66% of the administered radioactive dose was recovered from the urine, and approximately 13% recovered from the faeces. Overall, 79% of the administered dose was recovered from excreta. The study did not proceed with a sufficient observation period to enable a more complete excretion; additionally, the radioactivity expired as carbon dioxide was not measured. Less than 2% of the administered dose was recovered from the milk. The major metabolites identified were N-methyl acetoacetamide (SD9112) and 3-hydroxy-N-methyl butyramide (SD11734). The presence of 2 minor metabolites was also notified, however it was not possible to identify their structures. Tissue residue examinations were limited to quantifying the 'monocrotophos equivalent' based on radioactivity count. Levels in the kidney were approximately 0.17 ppm monocrotophos equivalents, while in the liver they were approximately 0.13 ppm.

Cattle Feeding Studies with SD-13311. Modesto Technical Report (undated) from Shell Chemical Technical Report Files

SD 13311 (3-dimethyl phosphate of 3-hydroxy-N-(glucosyloxymethyl)-*cis*-crotonamide), a metabolite of monocrotophos found in plants, was fed to two Guernsey cows to determine whether SD 13311 or its hydrolysis products (SD 12657 or SD 11319) would be present in the milk, meat or fat. Cows were milked twice daily. At each milking the cows received an initial 1 kg of grain; when this was consumed an additional 2 kg of grain was supplied. For 9 days, the first kg was treated with a water-alcohol solution to acclimatise the animals to treated grain. Following this, grain treated with water-alcohol solution containing 180 mg of SD 13311 was fed for 10 days. At the end of the 10-day period, the cows were slaughtered and samples of tissue taken for analysis.

Both cows were healthy and free of clinical signs throughout the test. Feeding SD 13311 did not appear to affect milk output. Milk samples were analysed for the presence of SD 12657, SD 11319 and SD 13311, as were tissue samples obtained at the end of the experiment. The results showed that residues were not detectable in this trial.

Potter JC (1965) Residues of AZODRIN insecticide in milk. Modesto, Shell Development Company. Tech Report M-24-65

The excretion pattern of monocrotophos in cattle was investigated by feeding ^{32}P -labelled monocrotophos twice daily in the feed to 2 lactating cows at doses of approximately 1 mg/kg bw/day (split into 2 doses of 0.5 mg/kg bw). Cattle were fed this dose for 14 days, with

collection of milk, urine and faeces. At the end of the feeding period, the cattle were slaughtered and tissue samples collected. Previous investigations had indicated that the main metabolites were likely to be a hydroxy-monocrotophos and N-hydroxymethyl-monocrotophos. Milk was also investigated specifically for the presence of a glucoside of N-hydroxymethyl, which had been previously found in plants.

Over the 14-day feeding period, the residues of monocrotophos in milk were on the order of 0.0061 - 0.022 ppm. The residues of hydroxy-monocrotophos were <0.0005 (LOD) to 0.0037 ppm, and the residues of N-hydroxy monocrotophos were <0.0005 to 0.002 ppm. In urine, the levels of monocrotophos excreted were 0.32 to 1.02 ppm, of hydroxy monocrotophos 0.01 to 0.06 ppm, and of N-hydroxy monocrotophos 0.083 to 0.63 ppm. Therefor there was significantly more excretion of monocrotophos and its metabolites in urine than in milk. Monocrotophos levels in skeletal muscle were 0.023 - 0.041 ppm, while liver levels were 0.11 - 0.13 ppm. Levels of metabolites in these tissues were not quantified.

3. ACUTE TOXICITY

3.1 Technical Grade Active Constituent

3.1.1 Median Lethal Dose Studies

The results obtained from acute toxicity studies conducted with monocrotophos are summarised in the following table.

Median Lethal Dose Studies

Species	Sex	Route	Vehicle	LD50(mg/kg bw) or LC50 (mg/m^3)	Reference
Mouse	M/F	PO	Peanut oil	15	Shellenberger & Newell (1963a)
Mouse	M/F	PO	Peanut oil	10	Shellenberger & Newell (1963b)
Mouse	M/F	PO	Peanut oil	11	Shellenberger & Newell (1963b)
Mouse (Swiss)	M/F	PO	Distilled water	11	Seshaiah (1955a)
Rat (CFE)	M/F	PO	?	8.4 - 8.7	Brown et al (1970)
Rat (Sprague Dawley)	M/F	PO	?	35 (M), 20 (F)	Newell & Dilley (1978)
Rat	M/F	PO	Peanut oil	23	Shellenberger & Newell (1963a)
Rat	M/F	PO	Peanut oil	13	Shellenberger & Newell (1963b)
Rat	M/F	PO	Peanut oil	21	Shellenberger & Newell (1963b)
Rat	M/F	PO	Peanut oil	15	Shellenberger & Newell (1964b)
Rat	M/F	PO	?	15	Shellenberger & Newell (1964d)
Rat	M/F	PO	?	17	Shellenberger & Newell (1964d)
Rat (Tif.RAI)	M/F	PO	Carboxymethyl cellulose	14	Sachsse & Bather (1975)
Rat (Wistar)	M/F	PO	Distilled water	9.6	Seshaiah (1995b)

Rat (Sprague Dawley)	M/F	Dermal	Undiluted	210(M) 206 (F)	Newell & Dilley (1978)
Rat (RAC)	M/F	Dermal	Carboxymethyl cellulose	330	Hurni & Sachsse (1969)
Rat (RifRAI)	M/F	Dermal	Distilled water	>2000 (M), approx 2000 (F)	Hartmann (1992)
Rat (Wistar)	M/F	Dermal	Undiluted	123	Deshmukh et al (1993a)
Rabbit	?	Dermal	Undiluted	354	Shellenberger & Newell (1963a)
Rabbit	?	Dermal	Undiluted	709	Shellenberger & Newell (1963b)
Rabbit	?	Dermal	Undiluted	354	Shellenberger & Newell (1963b)
Rabbit	?	Dermal	Water	420	Shellenberger & Newell (1964a)
Rabbit	?	Dermal	DMSO	223	Shellenberger & Newell (1964a)
Rabbit	?	Dermal	Xylene	149	Shellenberger & Newell (1964a)
Rabbit (NZW)	M/F	Dermal	Undiluted	347	Dushmukh et al (1993b)
Rat (CFE)	F	SC	Saline	7	Reift (1969)
Mice (MF-2)	M/F	IP	Carboxymethyl cellulose	11	Hurni & Sachsse (1970a)
Mice (MF-2)	M/F	IV	Carboxymethyl cellulose	11.5	Hurni & Sachsse (1970b)
Rat (Sprague Dawley)	M/F	IV	?	11.9(M) 9.2 (F)	Newell & Dilley (1978)
Rat	M/F	Inhalation		92 (1h), 80 (4h)	Sachsse et al (1973)
Rat (Tif RAI)	M/F	Inhalation	Distilled water	809 (4h)	Sachsse (1973)

3.1.1.1. Oral

Shellenberger TE & Newell GW (1963a) Report No 97, Ref Project B-1008., Stanford Research Institute, Menlo Park.

Monocrotophos technical (SD 9129, Code 1400, source, purity not specified) in peanut oil was administered orally to mice (10/group: source, strain, sex not specified) at doses of 10, 12.6, 15.9 or 20 mg/kg bw. It was not specified whether animals were fasted or non-fasted, nor was

the duration of the observation period stated. Clinical signs included diarrhoea, tremors, salivation and lacrimation, and began within 15 - 30 min of dosing. Survivors appeared normal after 24 h. The acute oral LD50 was determined to be 15 mg/kg bw.

Shellenberger TE & Newell GW (1963b) Report No 105, Ref Project B-1008. Stanford Research Institute, Menlo Park,.

Two compositions of monocrotophos were tested for acute oral toxicity. The first, SD9129 Code 4200 was 95% pure (source not specified) while the second, SD 9129 Code 3500 was approximately 90% pure (source not specified). SD 9129 code 4200 in peanut oil was administered to mice (source, strain, sex not specified) at 7.95, 10, 12.6 or 15.9 mg/kg bw (10/group). The LD50 was 10 mg/kg bw. SD 9129 code 3500 in peanut oil was administered to mice (source, strain sex not specified) at 7.95, 10, 12.6 or 15.9 mg/kg bw (10/group), and the LD50 was 11 mg/kg bw. Clinical signs observed included salivation, diarrhoea, tremors and clonic convulsions, and survivors appeared normal after 24 h.

Seshaiah A (1995a) Acute oral toxicity (LD50) study of monocrotophos technical to mice. Lab: Dept of Toxicology, Jai Research Foundation Sponsor: United Phosphorus Ltd.

Monocrotophos technical (purity 74.4%, batch no 307 supplier United Phosphorus Ltd) in distilled water was administered to Swiss albino mice (Animal House, Jai Research Foundation) in an initial range finding study and in the main study. Mice were housed in groups of 5, with *ad libitum* access to food and water (with the exception of required fasting periods).

In the range-finding study, monocrotophos was administered at 0, 8, 16 and 32 mg/kg bw (2/sex/group). Mice were observed for 5 days after treatment. All mice in the 16 and 32 mg/kg bw groups died. Toxic signs observed were lethargy, tremor, abdominal breathing and piloerection.

In the main study, monocrotophos was administered at 0, 8, 11 and 16 mg/kg bw (5/sex/group). Mice were observed for 5 h after dosing, then checked daily for 14 days. Body weights were recorded prior to administration, then on days 7 and 14. At the end of the study, survivors were euthanised and a gross post mortem examination performed.

Mortalities were observed in all treatment groups at the frequency 0/10, 3/10, 5/10 and 10/10. Clinical signs included gait changes, lacrimation, tremor and abdominal breathing. There were few abnormalities observed on gross post mortem examination, with lung congestion being the most notable. It was determined that the acute oral LD50 to mice was 11 mg/kg bw.

Brown VK, Dean B, Muir CMC, Pickering RG, & Reiff B (1970) Toxicity studies on AZODRIN; the effect of a single oral or subcutaneous dose on rats. Lab:, Shell Research Ltd Sittingbourne UK: TLTR.0005.68

The acute oral toxicity of monocrotophos technical (purity not given) and recrystallised monocrotophos (purity 99.8%) was investigated in Carworth Farm E rats (Tunstall Laboratories). The LD50 of technical grade monocrotophos was 8.4 mg/kg bw, that of the recrytallised monocrotophos was 8.7 mg/kg bw.

The effects of a single oral dose of monocrotophos technical on plasma, erythrocyte and brain ChE of female rats was investigated. A dose of 4 mg/kg bw produced significant (>20%) ChE inhibition plasma up to 24 h after dosing. Erythrocyte and brain ChE were inhibited for 15 days after dosing with 4 mg/kg bw.

The effects of concurrent administration of a single oral dose of monocrotophos and atropine sulphate were investigated. A dose of 6 mg/kg bw of either the pure or technical monocrotophos, with or without atropine sulphate, inhibited plasma ChE up to 24 h after dosing. Only pure monocrotophos produced inhibition of plasma ChE for 7 days after dosing. Erythrocyte ChE was inhibited until 10 days after dosing by monocrotophos pure or technical, with or without atropine. Technical monocrotophos, and monocrotophos with atropine produced significant inhibition of erythrocyte ChE at 20 days after dosing. Brain ChE was inhibited until 20 days after dosing by all treatments. Therefore, as expected, atropine sulphate did not appear to change the effect of monocrotophos on ChE inhibition.

Newell CW & Dilley JV (1978) Teratology and acute toxicology of selected chemical pesticides administered by inhalation. Springfield, US Dept of Commerce NTIS-PB-277.077

Monocrotophos technical (purity 61-64%, source: Battelle Memorial Institute Repository, Columbus Lab, Ohio) was administered by gavage to adult Sprague Dawley rats (Simonsen Laboratories, California) at unspecified doses (10/sex/group). Clinical signs included salivation, lacrimation, exophthalmos, defecation, urination and muscle fasciculations. The duration of the signs were dose dependent, with all survivors completely recovered by 10 - 14 days after treatment. The LD50 was determined to be 35 mg/kg bw for males and 20 mg/kg bw for females. Whole blood ChE inhibition was determined 6 h after treatment; in males at 25 mg/kg bw there was 82% inhibition, and in females at 15 mg/kg bw 89% inhibition.

Shellenberger TE & Newell GW (1963a) Report No 97, Ref Project B-1008. Stanford Research Institute Menlo Park.

Monocrotophos technical (SD 9129, code 1400, source, purity not specified) in peanut oil was administered orally by gavage to rats (source, strain, sex not specified) at doses of 16, 20, 25 or 32 mg/kg bw (10/group). It was not specified whether rats were fasted or non-fasted. Clinical signs included diarrhoea, tremors, salivation and lacrimation, and were seen within 15 to 30 min of administration. Survivors appeared normal 24 h after dosing. The LD50 was determined to be 23 mg/kg bw.

Shellenberger TE & Newell GW (1963b) Report No 105, Ref Project B-1008. Stanford Research Institute, Menlo Park.

Two compositions of monocrotophos were tested for acute oral toxicity. The first, SD9129 Code 4200 was 95% pure (source not specified) while the second, SD 9129 Code 3500 was approximately 90% pure (source not specified). SD9129 code 4200 in peanut oil was administered PO by gavage to rats (source, strain, sex not specified) at doses of 10, 12.6, 15.9 or 20 mg/kg bw (10/group). The LD50 was determined to be 13 mg/kg bw. SD9129 code 3500 in peanut oil was administered PO to rats (source, strain, sex not specified) at doses of 15.9, 20, 25.2 or 31.8 mg/kg bw (10/group). The LD50 was determined to be 21 mg/kg bw. Clinical signs included lacrimation, salivation, diarrhoea, tremors and clonic convulsions.

Shellenberger, TE & Newell GW (1964b) Report No 110, Ref Project B-1008. Stanford Research Institute, Menlo Park.

Monocrotophos technical (SD 9129, code 7300, source, purity not specified) in peanut oil was administered in peanut oil to rats (source, strain, sex not specified) at doses of 12.6, 15.9, 20 or 25.2 mg/kg bw. It was not specified whether rats were fasted. Clinical signs were tremors, salivation, lacrimation and diarrhoea. The LD50 was determined to be 15 mg/kg bw.

Shellenberger TE & Newell GW (1964d) Report No 114, Ref Project B-1008. Stanford Research Institute Menlo Park.

Monocrotophos (SD 9129 code 6200, code 9100 and code 9200, source and purity not specified) was administered PO to rats (source, sex, strain not specified) at a range of doses specific for each compound using 10 rats/group. SD 9129 code 6200 was administered at doses of 315, 398, 500 or 630 mg/kg bw; the LD50 was 420 mg/kg bw. SD 9129 code 9100 was administered at doses of 9.9, 12.5, 15.8 or 19.8 mg/kg bw; the LD50 was 15 mg/kg bw. SD 9129 code 9200 was administered at doses of 12.5, 15.8, 19.8 or 25 mg/kg bw; the LD50 was 17 mg/kg bw.

Sachsse K & Bathe R (1975) Acute oral LD50 of technical monocrotophos (C1414) in the Rat. Project No Siss 69 Ciba Geigy Limited, Basle, Switzerland

Technical monocrotophos (Batch OP 50/51, purity not given, source Ciba Geigy Ltd) in carboxymethyl cellulose was administered PO by gavage to fasted Tif.RAI SPF rats (Ciba Geigy Laboratories) at doses of 10, 12, 18 or 30 mg/kg bw (5 rats/sex/group). Rats were maintained in groups of 5 under standard conditions for 7 days. Clinical signs were seen in all treated rats, and included dyspnoea, chromodacryorrhoea, exophthalmus, salivation, hunched position, trismus, tonic-clonic muscle spasms and ruffled fur. Survivors recovered within 3 - 4 days of dosing. All survivors were euthanised after 7 days. Gross autopsy revealed no abnormal findings. The oral LD50 for both males and females was determined to be 14 mg/kg bw.

Seshaiah (1995b) Acute oral toxicity (LD50) study of monocrotophos technical to rat. Lab: Department of Toxicology Jai Research Foundation. Sponsor: United Phosphorus Ltd.

Monocrotophos technical (purity 74.4%, batch no 307, supplier: United Phosphorus Ltd) in distilled water was administered by gavage to Wistar rats (Animal House, Jai Research Foundation) in an initial dose range finding study, and the main trial. Rats were housed in groups of 5, with food and water available *ad libitum*, except during required pre-dosing fasts.

In the dose range-finding study, monocrotophos was administered at 0, 8, 16 or 32 mg/kg bw (2 rats/sex/group). Deaths occurred in the 16 mg/kg bw group. Clinical signs including tremors, abdominal breathing, chromodacryorrhea, exophthalmus and piloerection were observed for up to 5 days after dosing.

The main study used doses of 0, 12, 16, 20 and 25 mg/kg bw (5 rats/sex/group). Animals were observed hourly for 5 h after dosing, then daily for 14 days. Body weights were recorded prior to dosing and on days 7 and 14. A gross pathological examination was performed at the end of the study, and on animals dying during the study. Deaths occurred in all treatment groups (0/10, 8/10, 7/10, 9/10, 10/10). Tremor, lacrimation, exophthalmus and piloerection were seen in all treatment groups. On gross necropsy, there was congestion in the lungs, and patchy white discoloration of the liver. Organs were not preserved for histopathological examination. The acute oral LD50 of monocrotophos was determined to be 9.6 mg/kg bw.

Potrepka, RF (1994) Acute Oral Toxicity study of Monocrotophos Technical in Rats. Ciba Geigy Corporation, Laboratory Study No. F-00189 GLP:USEPA

Monocrotophos technical (C1414, Lot No. FL-940574, Batch No. OP 107001, purity 77.6%, source: Ciba-Geigy Crop Protection Division) was administered to Crl:CD(SD)BR VAF/Plus rats in a number of trials. Animals were housed individually during all trials, with food and water available *ad libitum*, except during pre-dosing fasting periods.

In the first trial, 3 mg/kg bw was administered by oral gavage to 5 rats/sex. Based on these observations, two additional groups were treated at 0.3 and 5 mg/kg bw. General physical examinations were done, and clinical signs recorded at 1, 2, 4 and 6 h after dosing, then daily for 7 days. Body weights were recorded prior to compound administration, and after 7 days. In the 2nd trial, monocrotophos in distilled water was administered by oral gavage at 3 mg/kg bw to 20 female rats, with controls receiving vehicle. The peak inhibition times for plasma, erythrocyte and brain ChE activity was determined by sacrificing 5 animals at 2, 4, 6 and 24 h after treatment. Animals were examined 1, 2, 4, 6 and 24 h after dosing (where applicable) for clinical signs. Body weights were recorded immediately prior to sacrifice In the 3rd trial monocrotophos in distilled water was administered by oral gavage at doses of 0, 0.01, 0.03, 0.1, 0.3 or 1 mg/kg bw with 5 rats/sex/group, with doses selected on the basis of results from the first 2 trials. General physical examinations were done prior to dosing, and approximately 1 and 2 h post-dose. ChE activities in plasma, erythrocytes and brain were determined. All animals in all trial were subject to a gross post mortem immediately after sacrifice.

In the first trial, there were no mortalities. There were no treatment related effects on body weight in either sex. Clinical signs of toxicity were seen at 3 and 5 mg/kg bw, and included muscle fasciculations, staining of the eyes, mouth and nose, diarrhoea, miosis, lacrimation and salivation. Signs were first seen at 1 h after dosing, peaked at 2 to 4 h, and were generally absent by 24 h although in some animals miosis persisted for the 7 d examination period. Gross necropsy revealed no treatment related findings. Incidental findings included a malformed/misshapen eye seen at 0.3 mg/kg bw, and a kidney cyst seen at 5 mg/kg bw; no histopathological examination was done.

In the second trial, there were no mortalities. There were decreases in body weight at 2 and 4 h post dosing, however this was not considered of biological significance. Clinical signs including flattened posture, muscle fasciculations, miosis, lacrimation, salivation and staining of the eyes, mouth and nose were seen from 1 to 2 h after dosing, Miosis was the only clinical sign still observed at 24 h after dosing. There were no significant findings on gross postmortem examination; histopathological examination was not done. ChE activities showed significant depression at all time periods examined. Plasma ChE activity was inhibited 80% at 2 h after administration and 32% at 24 h. Erythrocyte ChE was inhibited 72% at 2 h and 32% at 24 h. Brain ChE was inhibited 87% at 2 h and 27% at 24 h.

In the third trial, there were no significant change in bodyweights in the 2 h of observation. Clinical signs in males were muscle fasciculation and salivation at 1 mg/kg bw and miosis (>0.1 mg/kg bw). In females, the only observed abnormal clinical signs were miosis and staining of the nose. These did not show a clear dose relationship. Inhibition of ChE is presented below.

Mean percentage ChE inhibition

Dose (ppm)	Plasma ChE		Erythrocyte ChE		Brain ChE	
	Males	Females	Males	Females	Males	Females
0.01	10	4	0	2	1	3
0.03	21	0	6	7	0	7
0.1	34	0	13	7	17	15
0.3	56	29	44	65	44	26
1	83	57	66	75	72	88

There was significant inhibition of plasma ChE in males at 0.03 mg/kg bw at 2 h after dosing. In females, plasma ChE activity was significantly decreased at 0.3 mg/kg bw. Erythrocyte and brain ChE activity was significantly decreased in both sexes at 0.3 mg/kg bw/day. Based on the inhibition of plasma ChE, the NOEL for the study can be established at 0.01 mg/kg bw.

Hurni PH & Sachsse K (1970) Report on the determination of the acute oral LD50 to the rabbit of C-1414, Technical. Tierfarm AG Biomedical Research

Monocrotophos technical (source, purity, batch no not specified) was administered orally to rabbits (source, strain not specified) at doses of 16.7, 35.9, 46.4, 77.5 or 275 mg/kg bw using an unspecified number of animals/group. Rabbits were maintained for a 14-day observation period following dosing. Clinical signs in the lowest dose group included asynchronism of the extremities, spasm of the limb muscles and diarrhoea. These rabbits had recovered by 24 h after dosing. At the higher doses, signs included tachypnoea, clonic-tonic muscle spasms, hollow flanks, inhalation noises, salivation, lacrimation and diarrhoea. Survivors appeared normal after 48 h. Gross autopsy of animals dying during the study showed atalectases and haemorrhages of the lungs, and congested livers. No gross pathological changes were seen in animals euthanised at the end of the 14-day observation period. The oral LD50 was not determined in this study, and no information was presented on the number of mortalities.

3.1.1.2 Dermal

Newell CW & Dilley JV (1978) Teratology and acute toxicology of selected chemical pesticides administered by inhalation. Springrield, US Dept of Commerce NTIS-PB-277.077

Monocrotophos technical (purity 61-64%, source: Battelle Memorial Institute Repository, Columbus Ohio) was administered to the clipped skin of Sprague Dawley rats (Simonsen Laboratories, California) at unspecified doses, using 10 rats/sex/group. It was not specified how long the compound remained in contact with the skin, or whether the area was covered with an occlusive dressing. The LD50 for this trial were determined to be 210 mg/kg bw for males and 206 mg/kg bw for females.

Hurni H & Sachsse K (1969) Report on the determination of the acute dermal LD50 to the rat of monocrotophos technical. Toxicological Research Centre, Tierfarm Ag, Sisseln Switzerland

Monocrotophos technical (source, purity, batch no not specified) in carboxymethylcellulose was applied to the clipped dorsolumbar skin of RAC rats (Tierfarm AG, Switzerland) at doses of 60, 120, 240, 360, 600 or 1200 mg/kg bw and covered with an occlusive dressing for 24 h. At the end of this time, the dressing was removed, and the skin was washed with warm water. Rats were maintained for a 14-day observation period, with food and water available *ad libitum*. After 24 h, rats showed tachypnoea, clonic-tonic muscle spasms, prostration and lacrimation. The severity of the symptoms increased with increasing doses. The LD50 was determined to be 330 mg/kg bw.

Hartmann HR (1992) C 1414 technical Acute Dermal Toxicity in the Rat. Test No 911264 Ciba Geigy Ltd, Stein Switzerland.GLP - OECD/US EPA

Monocrotophos technical (batch no OP 107001, purity 77.6%, source: Ciba Geigy Ltd) in distilled water was applied to the shorn dorsolumbar skin of Tif RAI f (SPF) rats (source: Ciba Geigy Ltd Animal Production Switzerland) at doses of 100, 500 or 2000 mg/kg bw for males (5/group) and 500, 1000 or 2000 mg/kg bw for females (5/group). The application site was covered with an occlusive dressing for 24 h. All animals were maintained for at least 14 days

observation; females receiving 2000 mg/kg bw were monitored for 26 days. All animals were checked at least once daily for mortality and clinical signs. Body weight was determined weekly. A gross autopsy was done as soon as possible after death in the case of animals which died spontaneously. All animals were autopsied after scheduled sacrifice.

Clinical signs included piloerection, abnormal body positions, exophthalmus and dyspnoea. At 500 mg/kg bw and higher doses, tremor and decreased locomotor activity were seen. Ataxia was seen at 500 and 2000 mg/kg bw, while convulsions were seen only at 2000 mg/kg bw. Trismus was seen in males at 2000 mg/kg bw, but was not seen in females. Survivors did not show a decreased body weight at the end of the observation period. In the 1000 mg/kg bw group, 2/5 females died, while 2/5 females at 2000 mg/kg bw also died. There were no abnormalities seen on gross autopsy in any of the animals which died or were sacrificed. The LD50 for male rats was determined to be >2000 mg/kg bw, while the LD50 for females was determined to be approximately 2000 mg/kg bw. Overall, the LD50 for rats was determined to be >2000 mg/kg bw.

Deshmukh PB, Banerize RS & Patel SV (1993a) Acute dermal toxicity studies of monocrotophos technical in rats. Lag: Jai Research Foundation Sponsor: United Phosphorus Ltd.GLP: OECD

Monocrotophos technical (source, batch no, purity not specified) was administered dermally to Wistar:Haffkine strain rats (Jai Research Foundation) in a preliminary trial and main test. In both experiments, the material was applied to the clipped dorsolumbar region, and rats were observed for 15 days after dosing.

In the preliminary experiment, monocrotophos was applied at 100, 200 or 300 mg/kg bw (5/sex/group). It was not stated whether the area was covered with an occlusive dressing. The LD50 was determined to be between 100 and 200 mg/kg bw. For the main experiment, the doses used were 100, 125, 150 or 175 mg/kg bw, and the area was covered with an occlusive dressing for 24 h. It was not stated whether the area was washed following removal of the dressing. Clinical signs seen included lacrimation, salivation, exophthalmus, ataxia, dyspnoea and convulsions. The LD50 was determined to be 123 mg/kg bw.

Shellenberger TE & Newell GW (1963a) Report No 97, Ref Project B-1008. Stanford Research Institute, Menlo Park.

Monocrotophos technical (SD 9129, Code 1400, source, purity not specified) was applied to the skin of rabbits (source, strain not specified) at 125, 250, 500 or 1000 mg/kg bw using 3 rabbits/group. The length of exposure, whether the skin was covered or not, the degree of abrasion and the length of observation following exposure were not specified. Unspecified clinical signs described as 'typical of organophosphate toxicity' were seen within 1 h of application. Survivors were normal after 24 h. Mild erythema at the site of application was noted. The LD50 was determined to be 354 mg/kg bw.

Shellenberger TE & Newell GW (1963b) Report No 105, Ref Project B-1008. Stanford Research Institute, Menlo Park.

Two compositions of monocrotophos were tested for dermal toxicity. The first, SD9129, Code 4200 was 95% pure (source not specified) while the second, SD 9129, Code 3500 was approximately 90% pure (source not specified). The compounds were applied percutaneously to rabbits (source, strain, sex not specified). The application time was not specified, and it was not specified whether the area was covered. The skin was washed after exposure. SD 9129, Code 4200 was applied at doses of 250, 500, 1000 or 2000 mg/kg bw, and the LD50 was 709

mg/kg bw. SD 9129, Code 3500 was applied at doses of 125, 250, 500 or 1000 mg/kg bw, and the LD50 was 354 mg/kg bw. Clinical signs of tremors, diarrhoea, salivation and lacrimation were seen, and mild erythema was observed when the compound was removed.

Shellenberger TE & Newell GW(1964a) Report No 107 Ref Project B-1008. Stanford Research Institute, Menlo Park.

Monocrotophos technical (SD 9129, Code 4200, purity 95%, source not specified) was applied dermally to rabbits (source, strain, sex not specified) in water, DMSO or xylene using 3 rabbits/group. The compound was applied in water at doses of 125, 250, 500 or 1000 mg/kg bw; the LD50 was 420 mg/kg bw. The compound was applied in DMSO at doses of 62.5, 125, 250 or 500 mg/kg bw; the LD50 was 223 mg/kg bw. The compound was applied in xylene at doses of 62.5, 125, 250 or 500 mg/kg bw; the LD50 was 149 mg/kg bw. In all applications, clinical signs included tremors, salivation, diarrhoea and lacrimation; these signs increased in severity with dose, and were also more severe with xylene than with the other solvents.

Shellenberger, TE & Newell GW (1964c) Report No 111, Ref Project B-1008. Stanford Research Institute, Menlo Park.

Monocrotophos (SD 9129, Code 7347, source not specified, 40% in acetone) was applied dermally to rabbits (source, strain, sex not specified). It was not specified whether the hair was shorn, whether the area was occluded, and for how long the compound was applied. The signs observed were miosis, diarrhoea and dyspnoea. The LD50 was determined to be 342 mg/kg bw.

Dushmukh PB, Banerjee RS & Patel SV (1993b). Acute dermal toxicity studies of monocrotophos technical in rabbit. Lab: Jai Research Foundation Sponsor: United Phosphorus Ltd. GLP:OECD

Monocrotophos technical (batch no, source, purity not specified) was applied dermally to the shorn dorso-lumbar region of New Zealand rabbits (source: Jai Research Foundation) in a preliminary and a main trial. Rats were maintained in a specialised rabbit holder for 24 h after application before being returned to individual housing. The application area was not covered with an occlusive dressing.

In the preliminary trial monocrotophos was applied at 250, 500 or 750 mg/kg bw (2/sex/group). The LD50 was determined to lie between 250 and 500 mg/kg bw. In the main trial, monocrotophos was applied at 300, 325, 375 and 400 mg/kg bw (2/sex/group). Clinical signs were seen, including lacrimation, salivation, exophthalmus, ataxia, dyspnea and convulsions. The LD50 was determined to be 347 mg/kg bw.

3.1.1.3 Subcutaneous

Reift B (1969) Pharmacological studies into the toxic actions of cholinesterase inhibitors. Part 9. Shell Research Ltd, Sittingbourne UK TLGR.0008.69

Monocrotophos (analytical grade - 100% pure, source: WARC) in a saline vehicle was injected SC to female Carworth Farm rats (source not specified) at doses of 4.82, 5.79, 6.95, 8.34 or 10.0 mg/kg bw. Deaths were recorded at 24 h intervals over 7 days. The LD50 was determined to be 7 mg/kg bw.

Brown VK, Dean B, Muir CMC, Pickering RG, & Reiff B (1970) Toxicity studies on AZODRIN; the effect of a single oral or subcutaneous dose on rats. Lab: Shell Research Ltd, Sittingbourne UK. TLTR.0005.68

The effect of a single SC dose of 8.4 mg/kg bw monocrotophos on ChE inhibition was investigated in Carworth Farm E rats (Tunstall Laboratories). Plasma ChE was significantly inhibited 24 h after a single SC dose. Erythrocyte and brain ChE were inhibited for 7 days after a single SC dose. The inhibition had resolved by 14 days after dosing.

3.1.1.4 Intraperitoneal

Menzer RE & Casida JE (1965) Nature of toxic metabolites formed in mammals, insects and plants from 3(-dimethoxyphophinyloxyl)-N, N-dimethyl-cis-crotonamide analog. J Agric Food Chem 13: 102 - 112

The acute toxicity of monocrotophos and its metabolites was tested by IP administration to mice. The LD50 determined for monocrotophos was 8 mg/kg bw, while the LD50 for the N-hydroxymethyl metabolite was 12 mg/kg bw. The amide metabolite had an LD50 of 3 mg/kg bw, and thus was more toxic than the parent compound.

Hurni H & Sachsse K (1970) Report on the determination of the acute intraperitoneal LD50 to the mouse of C-1414, technical. Biomedical Research, Tierfarm AG, Switzerland

Monocrotophos technical (batch no, purity, source not specified) in carboxymethyl cellulose was administered by IP injection to MF-2 mice (Tierfarm AG, Switzerland) at doses of 4.64, 7.75, 10, 12.9 or 16.7 mg/kg bw using 5 mice/sex/group. Mice were housed in groups of 5 under controlled conditions, with food and water available *ad libitum* for an observation period of 14 days. At approximately 1 h after injection, mice showed slight tonic-clonic muscle spasms and tachypnoea, while mice at the 2 highest doses became recumbent. Gross autopsy of mice dying during the test generally revealed a pale liver, without other visible abnormalities. No abnormalities were found in mice autopsied at the end of the trial. The acute IP LD50 was determined to be 11 mg/kg bw.

3.1.1.5 Intravenous

Hurni H & Sachsse K (1970) Report on the determination of the Acute Intravenous LD50 to the Mouse of C1414 Technical. Tierfarm AG, Sisseln, Switzerland Ciba Geigy Ltd

Monocrotophos technical (source, purity not specified), diluted in sodium carboxymethyl cellulose was administered by IV injections to MF-2 mice (Tierfarm AG, Sisseln, Switzerland) at doses of 6, 10 , 12.9 or 16.7 mg/kg bw using 5 mice/sex/group. Mice were housed in groups of 5 under controlled conditions, with food and water available *ad libitum*. Animals were observed for 14 days following treatment. Clinical signs in the animals receiving 6 or 10 mg/kg bw included apathy, tachypnoea and lacrimation. In the animals at the higher doses, dyspnoea, clonic-tonic muscle spasms, and 'anxiety' were observed immediately after administration of the compound. Survivors were normal after 48 h. On gross autopsy, animals dying during the study had slightly congested livers. Animals surviving until the end of the observation period had no abnormal signs. The acute IV LD50 in mice was determined to be 11.5 mg/kg bw for males and females.

Newell CW & Dilley JV (1978) Teratology and acute toxicology of selected chemical pesticides administered by inhalation. Springrield, US Dept of Commerce NTIS-PB-277.077

Monocrotophos technical (source: Battelle Memorial Institute Repository, Columbus Ohio, purity 61 - 64%) was administered by IV injection to adult Sprague Dawley rats (Simonsen Laboratories, California) at unspecified doses using 10 rats/sex/group. Clinical signs included salivation, lacrimation, exophthalmus, defecation, urination and muscle fasciculations. Duration of clinical signs was dose related. The LD50 was determined to be 11.9 mg/kg bw for males

and 9.2 mg/kg bw for females. Whole blood ChE inhibition was measured 6 h after treatment. At 12 mg/kg bw in males, inhibition was 92%, while in females at 9 mg/kg bw, inhibition was 79%.

3.1.1.6 Inhalation

Newell GW & Shellenberger TE (1964) Letter Report No 2, Project B-4843. Stanford Research Institute, Menlo Park
and
Shellenberger TE (1965a) Letter Report No 5 Ref Project B-4843. Menlo Park, Stanford Research Institute

Monocrotophos technical (batch 4-5-0-0) was administered to 10 rats/sex (strain, source not given) in a vapor chamber for 1 h, with whole body exposure. The air flow was 3L/min saturated with the test solution. Rats were weighed before and after exposure, then weekly during the 14-d monitoring period. At the end of this time, rats were killed and examined grossly. The liver, kidney, spleen and heart of each animal was weighed, and these organs plus the lung, bronchial tube, testes/ovaries, prostate/uterus, lymph nodes, bone marrow, skeletal muscle, adrenals, thyroid and parathyroid were preserved for histopathology. There were no changes in body weight or organ weight relating to treatment. On gross necropsy, one treated male had a lung abscess, and one treated male had thyroid glands which were greatly increased in size. On histopathological examination, there was evidence of peribronchial infiltrate with lymphocytes associated occasionally with mild interstitial fibrosis or mild chronic bronchopneumonia. There were also signs of fibrosis in the thyroids. All effects were seen in both treated and control animals, and are not considered to be related to treatment.

Newell CW & Dilley JV (1978) Teratology and acute toxicology of selected chemical pesticides administered by inhalation. Springrield, US Dept of Commerce NTIS-PB-277.077

Monocrotophos technical (purity 61-64%, source: Battelle Memorial Institute Repository, Columbus Ohio) was administered by inhalation to adult Sprague Dawley rats (Simonsen Laboratories, California) at doses of 90, 97, 151, 162, 210, 308, 321 or 740 mg/m^3 with a particle size of 0.3 - 3 µm for an unspecified durations, using whole body exposure. Clinical signs included salivation, lacrimation, exophthalmus, defecation, urination and muscle fasciculations, and their duration was dose related. All survivors had were normal by 10 to 14 d after treatment. The LD50 was determined to be 162 mg/kg bw for males and 176 mg/kg bw for females. Whole blood ChE inhibition was measured 6 h after treatment. For males at 156 mg/kg bw, there was 74% inhibition, and for females at 192 mg/kg bw there was 69% inhibition of ChE.

Sachsse K, Ullmann L, Voss G & Hess R (1973) Measurement of inhalation toxicity of aerosols in small laboratory animals. in: Experimental model systems in toxicology and their significance to man. Proc. Eur. Soc. Study of Drug Toxicity Vol XV. Excerpta Medica Intern. Congress Series No 311, Zurich

Rats (strain not specified) were exposed to monocrotophos technical (source not specified) for either 1 or 4 h by nose-only inhalation. The doses used were not specified, and there was no description of clinical signs, or detailed presentation of mortality. The LD50 for a 1 h exposure was determined to be 94 mg/m^3, and for a 4 h exposure, 80 mg/m^3.

Sachsse K (1973) Acute inhalational toxicity of technical C-1414 (monocrotophos) in the rat. Project No Siss 2780, Ciba Geigy Ltd

Monocrotophos technical (source, batch no. and purity not specified) was administered as an aqueous dilution to Tif.RAI SPF rats (Ciba Geigy Laboratories) by inhalation, with nose-only exposure, for 4 h at doses of 38, 100 or 208 mg/m^3 with 9 rats/sex/group. Two control groups were maintained: one was exposed in to distilled water, the other control group was not exposed. Within 1 h of exposure commencing, rats showed dyspnoea, exophthalmus, trismus and tonic-clonic muscle spasms. Later rats also showed salivation. Rats were maintained under observation for 7 days. Survivors were normal by 48 h after exposure. At the end of the observation period, all rats were euthanised and a gross autopsy performed. Animals dying during the study showed lung and intestinal haemorrhages, and congested organs. There were no abnormal findings in animals surviving until the end of the study. The LC50 was determined to be 809 mg/m^3.

Deshmukh PB, Banerjee RS & Patel SV (1993c) Acute inhalation toxicity studies of monocrotophos technical in rat. Study no. NCTCF/R/006/91/01300. Report No. 366/JAIREF/TOXT/93 Lab: Jai Research Foundation. Sponsor United Phosphorus. GLP:OECD

Rats (source, strain unspecified) were exposed to monocrotophos technical (source, batch no, purity unspecified) for 4 h at 11 mg/L, with an air flow of 1.5 L/min. It was not specified whether the exposure was whole body or nose only. The concentration of monocrotophos maintained in the exposure chamber was not reported. Rats were observed every 15 min for 1 h, then hourly for 3 h. After exposure, rats were observed daily for 14 days. No mortality, abnormal clinical signs or weight loss were seen. Gross post mortem examination revealed no abnormalities.

3.1.2 Skin Irritation and Sensitisation Studies

Scibor G (1977a) Primary skin irritation test with AZODRIN insecticide, Code 288-55, 99.5% in albino rabbits. Industrial Biotest Laboratories Inc, Northbrook. Report No. 8530-10808

and

Sawin VL & Sommer KR (1981) Audit of Industrial Bio-Test Laboratories Study No. 8530-10808, "Eye irritation test and primary skin irritation with AZODRIN insecticide in albino rabbits". Shell Development Company, Houston. WRC RIR-92

Skin irritation tests were carried out with 6 New Zealand White rabbits (Pel Freeze Inc, 3/sex) using 99.5% pure monocrotophos (source:Shell Chemical Co, Code 288-55). The dorso-lumbar region was clipped, and one intact and one abraded area of skin was treated with monocrotophos (250 mg/site) and occluded for 24 h. The skin was washed with water, and the sites were examined and scored for irritation 1 h after removal of the dressing. The sites were assessed again 72 h after treatment. Monocrotophos produced a mild erythema and was classified as slightly irritant to rabbit skin.

Hagemann (1992a) Acute Dermal Irritation/Corrosion Study in the Rabbet. Test No 911265 C 1414 tech. Ciba Geigy Ltd Plant Protection. GLP: OECD/USEPA

Monocrotophos technical (Batch no. OP 107001, purity 77.6%, source not specified) was applied to the shaved right flank of 3 female NZW rabbits (Chemisch-Pharmazeutische Fabrik). The monocrotophos (0.5 g) was applied to a gauze patch previously moistened with 0.5% carboxymethylcellulose and aqueous polysorbate 80. The left flank was used as a control, and had a gauze patch moistened with solvents applied. The patches were covered with an occlusive bandage for 4 h. Skin reactions were assessed 1, 24, 48 and 72 h after removing skin

patches. Body weight was assessed pretest and on days 3 and 7. Animals were housed individually with food and water available *ad libitum*.

The mean skin reaction scores for erythema using the Draize scoring system for each rabbit over the 24-72 h period were 0.67, 0.67 and 1.67. No erythema was evident after 7 days. The mean skin reaction scores for oedema for each rabbit over the same period were 0, 0, and 1. No oedema was evident after 7 days. There was a slight weight loss in 2 rabbits after 3 days, however this had recovered by 7 days. Monocrotophos was classed as a mild skin irritant.

Seshaiah S (1995c) Acute dermal irritation study of monocrotophos technical to rabbit. Lab: Jai Research Foundation. Sponsor United Phosphorus GLP: OECD

Monocrotophos technical (purity 74.4%, batch no 307, source: United Phosphorus Ltd) was tested for skin irritation in New Zealand White rabbits (source: Jai Research Foundation). The dorso-lumbar region of each of 3 rabbits (sex not specified) was clipped at 2 sites, one treated with monocrotophos, and the other acting as control. Monocrotophos (100 mg) was applied to the treated site and covered with a gauze swab for 4 h. The area was then wiped with moist cotton prior to assessment of skin reaction. The skin reaction was assessed 1, 24, 48 and 72 h after the end of treatment. The Draize score for erythema and oedema was 1 in each rabbit at the end of 1 h. The scores were 0 for the rest of the test. Therefore monocrotophos technical was classes as a mild irritant to rabbit skin.

Deshmukh PB, Banerjee RS & Patel SV (1993c) Mucous membrane irritation studies of monocrotophos technical in rabbit. Lab: Jai Research Foundation. Sponsor United Phosphorus Ltd GLP:OECD

Monocrotophos technical (source, batch no, purity not specified) was applied to the vaginal mucous membranes of 6 female New Zealand White rabbits (Jai Research Foundation). The volume of monocrotophos applied was unspecified. Irritation was scored at 24 and 72 h. Very slight erythema was seen in the mucous membranes of 4/6 rabbits at 24 h; no oedema was seen. The mucous membranes were fully recovered at 72 h. Therefore monocrotophos was determined to cause very slight irritation to mucous membrane of rabbits.

Hurni H & Sachsse K (1970) Sensitizing effects on guinea pigs of C-1414, technical. Toxicological Research Centre, Tierfarm Switzerland

Monocrotophos technical (source, batch no, purity not specified) in carboxymethylcellulose was administered by intracutaneous injection to Pirbright White guinea pigs. The back and upper flanks of the guinea pigs were shorn, and a 1% solution administered, with the first injection being 0.05 mL, and the next 9 injections being 0.1 mL administered every 2nd day. Two weeks after the final injection, a challenge injection of 0.05 mL was administered.

Animals were examined 24 h after each injection to determine the reaction. Each of the intracutaneous injections produced a slight necroses, of about 1 mm diameter surrounded by slight erythuria. The reaction following the challenge injection was not more intense than that following each of the sensitising injections. Therefore, no sensitisation reactions were observed at the challenge sites. No raw data on individual animals was presented.

3.1.3 Eye Irritation Studies

Scibor G (1977b) Eye irritation test with AZODRIN insecticide in albino rabbits. Industrial Biotest Laboratories Inc, Northbrook. Report No. 8530-10808
and
Sawin VL & Sommer KR (1981) Audit of Industrial Bio-Test Laboratories Study No. 8530-10808, "Eye irritation test and primary skin irritation with AZODRIN insecticide in albino rabbits". Shell Development Company, Houston. WRC RIR-92
Monocrotophos (source: Shell Chemical Co, purity 99.5%, Code:288-55) was applied to the right conjuctival sac of 6 New Zealand White rabbits (Pel-Freez Inc, Arkansas), with the left eye serving at control. Seventy mg of the material was applied; the eyes were not washed. Within 30 min of application, the rabbits showed hyperpnea, hyperexcitability and miosis, with one rabbit showing salivation. All animals had recovered within 22 h of ocular exposure. Eye irritation was scored at 1, 24, 48, 72 and 96 h and 7 days after treatment, using the Draize scoring system. Irritation was present at 1 and 24 h, however no irritation was observed from 48 h. Monocrotophos was classed at mildly irritating to the rabbit eye.

Hagemann (1992) Acute Eye Irritation/Corrosion Study in the Rabbit. Text No 911266. C1414 tech. Ciba Geigy Ltd, Switzerland.
Monocrotophos technical (batch no. OP 107001, purity 77.6%, source not specified) was applied to the left conjunctival sac of 3 female New Zealand White rabbits (source: Chemisch Pharmazeutishe Fabrik), with 100 mg applied. The right eye was used as an untreated control. The ocular reactions were assessed 1, 24, 48 and 72 h after treatment. Miosis was seen from 10 min until 3 h after treatment in all treated eyes. Clinical signs included tremors, trismus, dyspnea, ataxia and diarrhoea, with some incidences of muscle twitching. Two animals showed weight loss on day 3. Mean scores for irritation using the Draize scoring system for each animal over the 24-72 h period were 0.33, 0 and 0 for the iris, for redness of the conjunctiva 1.67, 1,67 and 1.67 and for chemosis 0.33, 0.33 and 1 respectively. No effects to the cornea were reported and after 14 days no abnormal signs were detected. Monocrotophos was classed as a minimal irritant to the eye.

Seshaiah S (1995d) Acute eye irritation study of monocrotophos technical to rabbit. Lab: Jai Research Foundation. Sponsor United Phosphorus. GLP:OECD
Monocrotophos technical (purity 74.4%, batch no 307, source: United Phosphorus Ltd) in distilled water was applied in a volume of 0.1 mL (containing 10 mg) to one eye of each of 3 New Zealand White rabbits (source: Jai Research Foundation). Observations were made for reactions at 1, 24, 48 and 72 h after treatment. All rabbits showed conjunctival reactions at 1 h. No iris or cornea signs were seen at any stage, and the redness and discharge from the conjunctiva had resolved by 24 h. Based on this, monocrotophos was determined to be a minimal irritant to the rabbit eye.

3.1.4 Potentiation/Interaction studies

Shellenberger TE (1965e) Letter Report No 9, Ref Project No B-4843. Stanford Research Institute, Menlo Park.
Oral LD50 values in male Long Evans rats for monocrotophos (technical grade, 7-3-0-0) and 24 other cholinesterase-inhibiting pesticides were determined. The oral LD50 of monocrotophos was determined to be 10 mg/kg bw. Potentiation was determined by giving monocrotophos in combination with each of the pesticides. Initially, the LD10 of both pesticides was administered simultaneously. If mortality was less than 50%, the effect were considered to be merely

additive, with no potentiation, and no further investigation was done. If mortality was greater than 50%, both chemicals were administered at 1/2 the LD50. If mortality was greater than 50%, it was assumed that potentiation was occurring, and the LD50 of an equitoxic mixture of the chemical was determined.

Chemicals showing additive effects included dimethoate, malathion, parathion, azinphos and carbaryl. Potential potentiation was identified in chemicals including dicrotophos, crotoxyphos, diazinon, guthion, parathion-methyl, phosphamidon and fenchlorphos. Potentiation of lethality occurred only in the case of monocrotophos given with fenchlorphos.

3.2 Isomers, Metabolites and Impurities

3.2.1 Median Lethal Dose Studies

A summary of the median LD50s following administration of the *trans*-isomer of monocrotophos is presented in the table below.

Species	Sex	Route	Vehicle	LD50 (mg/kg bw) or LC50 (mg/m^3)	Reference
Mouse Tif:MAG	M/F	PO		118	Sachsse & Bathe (1976a)
Rat Tif:RAIf	M/F	PO	carboxymethyl cellulose	207	Sachsse & Bathe (1976b)
Rabbit Himalayan	M/F	PO		485	Sachsse & Ullman (1976a)
Rat Tif:RAIf	M/F	Dermal		>3170	Sachsse & Bathe (1976c)
Rat Tif:RAIf	M/F	Inhalation		805	Sachsse & Ullman (1976b)
Rat Tif:RAIf	M/F	IP		202	Sachsse & Bathe 1976d)

3.2.1.1 Oral

Sachsse K & Bathe R (1976a) Acute oral LD50 in the mouse of monocrotophos, trans isomeres. Project No: Siss 5559 Ciba Geigy Ltd

Monocrotophos (trans isomer - source, batch no, purity not specified) was administered PO by gavage to fasted Tif MAG(SPF) mice (source: Ciba Geigy Ltd) at doses of 46.4, 77.5, 100, 129, 147 or 167 mg/kg bw using 5 mice/sex/group. Mice were housed in groups of 5 under controlled conditions with food and water available *ad libitum*. Signs included sedation, dyspnoea, chromodacryorrhea, exophthalmus, trismus, clonic-tonic muscle spasms and ruffled fur. Survivors had returned to normal within 9 days. There were no abnormalities found on gross post-mortem examination, either of animals dying during the study or animals examined at terminal sacrifice. The LD50 was determined to be 118 mg/kg bw.

Shellenberger TE (1966) Subacute toxicity and cholinesterase study of Shell Compound SD 13311 - Rat. SRI Project SS05908. Stanford Research Institute, Menlo Park

The oral LD50 of the beta-D-glycosyl conjugate of hydroxymethyl monocrotophos, a monocrotophos metabolite produced in mammals, was determined in non-fasted Long-Evans rats (source not specified). The compound was administered at doses of 126, 159, 200 or 252 mg/kg bw PO by gavage. Clinical signs included tremors, salivation, diarrhoea and tonic and clonic convulsions. The LD50 was determined to be 168 mg/kg bw.

Sachsse K & Bathe R (1976b) Acute oral LD50 in the rat of monocrotophos, trans isomeres. Project No: Siss 5559. Ciba Geigy Ltd

Monocrotophos, trans-isomer (purity, batch no, source not specified) diluted in carboxymethyl cellulose was administered PO by gavage to fasted Tif:RAIf rats (source: Ciba Geigy Ltd) at doses of 167, 180, 200, 205, 215 or 230 mg/kg bw (5/sex/group). Rats were housed in groups of 5 under controlled conditions, with food and water supplied *ad libitum*. Within 2 h of treatment, clinical signs included sedation, dyspnoea, chromodacryorrhoea, exophthalmus, curved or ventral body positions, tonic-clonic muscle spasms, trismus and ruffled fur. Survivors had recovered within 9 - 12 days. On post mortem examination, no gross abnormalities could be seen either in animals dying during the study, or those killed at the termination of the study. The LD50 was determined to be 207 mg/kg bw.

Sachsse K & Ullman L (1976a) Acute oral LD50 in the rabbit of monocrotophos, trans-isomeres. Project No Siss 5559. Ciba Geigy Ltd

Monocrotophos (trans isomer - source, batch no, purity not specified) in carboxy-methylcellulose was administered PO by gavage to fasted Himalayan rabbits (source Ciba Geigy Ltd) at doses of 100, 215, 359, 464 or 600 mg/kg bw using 2 rabbits/sex/group. Rabbits were maintained under controlled conditions, and food and water were available *ad libitum*. Clinical signs were seen within 1 h of treatment at doses of 215 mg/kg bw and greater, and included tonic-clonic muscle spasms, ataxy, tremor, lateral or ventral position and sedation. Survivors had recovered within 2 or 3 days. No gross post mortem signs were seen in any animals, either those dying during the study, or those euthanised at the end of the study. The LD50 was determined to be 485 mg/kg bw.

3.2.1.2 Dermal

Sachsse K & Bathe R (1976c) Acute dermal LD50 in the rat of monocrotophos, trans-isomeres. Project No Siss 5559. Ciba Geigy Limited.

Monocrotophos (trans-isomer - source, batch no, purity not specified) was applied to the shorn dorso-lumbar skin of Tif: RAIf (SPF) rats (3/sex/group) at doses of 1000, 2150, 2780 or 3170 mg/kg bw and covered with an occlusive dressing for 24 h. After 24 h, the dressing was removed and the skin cleaned with lukewarm water. Rats were maintained under controlled conditions with food and water available *ad libitum*.

Within 24 h, rats in all dosage groups showed sedation, dyspnoea, chromodacryorrhoea, exophthalmus, curved position, trismus, tonic-clonic muscle spasms and ruffled fur. One female each at 2150 and 2780 mg/kg bw died within 48 h; all other rats survived. No local skin irritations were observed. Survivors had recovered from all clinical signs within 10 to 13 days. Gross autopsies revealed no abnormalities in any animals. The LD50 was determined to be in excess of 3170 mg/kg bw.

3.2.1.3 Inhalation

Sachsse K & Ullman L (1976b) Acute Inhalation Toxicity in the rat of monocrotophos, trans-isomeres. Project No Siss 5559. Ciba Geigy Ltd.

Monocrotophos (trans isomer - batch no, source, purity not given) was administered by nose-only inhalation exposure to Tif RAIf (SPF) rats (source: Ciba Geigy Ltd) at 360, 530 or 710 mg/m^3 for 4 h using 9 rats/sex/group. The concentration and particle size of the administered material was monitored at 1 h intervals throughout the exposure. Following the 4 h exposure, rats were returned to their cages and monitored for 14 d. Rats were housed in groups of 9, and food and water were available *ad libitum*.

Within 2 h of the start of the exposure, rats in all concentrations showed dyspnoea, exophthalmus, tremor, tonic-clonic muscle spasms, curved positions and ruffled fur. The symptoms increased in severity with dose. Surviving animals recoveredwere normal within 3 to 6 days. Gross autopsies on animals dying during the study showed haemorrhages in the lungs and intestines, while there were no abnormalities seen in the animals surviving until the end of the study. The LD50 was determined to be 805 mg/m^3.

3.2.1.4 Intraperitoneal

Sachsse K & Bathe R (1976d) Acute Intraperitoneal LD50 in the rat of monocrotophos, trans-isomer. Project No Siss 5559. Ciba Geigy Ltd, Switzerland

Monocrotophos (trans isomer - batch no, source, purity not specified) in carboxy-methylcellulose was administered by IP injection to Tif RAI f (SPF) rats (source - Ciba Geigy Ltd) at doses of 77.5, 147, 167, 215, 278, 359 or 600 mg/kg bw using 5 rats/sex/group. Rats were housed in groups of 5 under controlled conditions, with *ad libitum* access to food and water and were maintained for a 14-d observation period after injection.

Signs of toxicity were seen within 2 h after treatment, and were dyspnoea, trismus, chromodacryorrhea, exophthalmus, tonic-clonic muscle spasms and ruffled fur. Survivors were normal in 8 - 12 days. On gross examination of animals dying during the study and those sacrificed at the end of the study, no abnormalities were found. The LD50 was determined to be 202 mg/kg bw.

3.2.2 Skin irritation

Sachsse K & Ullman L (1976c) Skin irritation in the rabbit after single application of monocrotophos, trans isomeres. Project No Siss 5559. Ciba-Geigy Ltd.

Monocrotophos (trans isomer - batch no., source, purity not given) was applied to the skin of Himalayan rabbits (source not specified). Rabbits were housed individually under controlled conditions. The back and flank of the rabbits was clipped 2 days prior to the test. Immediately before application, the left side of each rabbit (3/sex) was scarified. Gauze patches with 0.5 mL of test material was applied and covered with an occlusive dressing for 24 h. Skin irritation was scored immediately following removal of the gauze patch, and 48 h later (ie. 24 and 72 h after treatment).

On the intact skin after 24 h, one rabbit had well defined erythema. No other signs of irritation were seen at any time on intact skin.

On the scarified skin at 24 h, all rabbits showed either slight (5/6) or well defined (1/6) erythema. Rabbits also showed very slight (2/6), slight (2/6) or moderate (2/6) oedema. At 72

h, 4/6 showed very slight erythema, with no other signs seen. Therefore the test material caused a mild skin irritation to rabbits.

Sachsse K & Ullman L (1976a) Repetitive skin irritation test in rabbits of monocrotophos, trans isomeres. Project No. Siss 5559 Ciba Geigy Ltd

Monocrotophos (trans isomer - batch no, purity, source not specified) was applied to the shorn dorsolumbar skin of Himalayan rabbits (source not specified) once daily for 5 d (3/sex). Five mL of the material was applied to a gauze patch, placed on the shorn skin area and covered with an occlusive bandage for 24 h. At the end of the 24-h period, the dressing was removed, and the skin reaction evaluated using the Draize index before another gauze patch and occlusive dressing was applied. Rabbits were maintained for observation following the 5-d treatment period in individual housing under controlled conditions, with free access to food and water.

Within 48 h of commencing treatment, rabbits showed salivation, trismus, convulsions, ataxia, diarrhoea and sedation. Signs became more severe over time, and all animals died between days 5 and 7. The body weight of all animals decreased over the test period. The skin reaction after the first 24 h was scored at 0.83. The mean reaction score for days 1 to 5 was 1.9, and the final reaction score for day 5 was 2.5. Based on these scores, monocrotophos trans-isomer was determined to be a mild irritant to rabbit skin following repeated exposure.

3.2.3 Eye irritation

Sachsse K & Ullman L (1976d) Eye irritation in the rabbit of monocrotophos, trans-isomeres. Project No. Siss 5559. Ciba Geigy Ltd.

Monocrotophos (trans isomer - source, batch no, purity not specified) was applied (0.1 mL) to the left conjunctival sac of 3 male and 3 female Himalayan rabbits (source not specified), with the right eye an untreated control. In 3 rabbits, the test solution was rinsed out after 30 seconds. Rabbits were housed individually under controlled conditions, with *ad libitum* food and water. Eye irritation was assessed on days 1, 2, 3, 4 and 7. There was no evidence of any irritation to the cornea, conjunctiva or iris at any assessment time, in either the rinsed or unrinsed eyes. Therefore the trans isomer of monocrotophos was non-irritating to the eye.

Sachsse K & Ullman L (1976b) Repetitive eye irritation test in rabbits of monocrotophos, trans isomeres. Project No. Siss 5559 Ciba Geigy Ltd

Monocrotophos (trans isomer - source, batch no, purity not specified) was tested for repetitive irritation in Himalayan rabbits. 0.1 mL of monocrotophos trans isomer was placed in the left eye of 6 rabbits (3/sex). The right was maintained as an untreated control. In 3 animals, the treated eye was rinsed 30 seconds after application. The treatments were repeated once daily for 5 days. The reaction was assessed 24 h after each application and scored using the Draize system, and also assessed 8, 9 and 10 days after commencement of treatment. Animals were housed individually under controlled conditions with free access to food and water.

No adverse clinical signs were reported during the trial, and there was no loss in body weight in any of the treated animals. The primary reaction score after 24 h was 1, the mean reaction score over days 1 to 5 was 0.4, and the score on day 5 was 0. Monocrotophos, trans isomer, was therefore considered to be a minimal irritant to the rabbit eye.

3.2.4 Skin sensitisation

Sachsse K & Ullman L (1976e) Skin sensitizing (Contact allergenic) effect in guinea pigs of monocrotophos, trans isomeres. Project No. Siss 5559. Ciba Geigy Ltd

Monocrotophos (trans isomer - source, purity, batch no not specified) was administered by intracutaneous injection to Pirbright white guinea pigs (source: Ciba Geigy Ltd) using 10 animals/sex/group. A 0.1% dilution in saline was administered in 0.1 mL injections, with saline used as negative control and dinitrochlorobenzene (DNCB) used as positive control. On the first d, animals received an injection in the right flank and in the back; every second d they received an injection in the back, for a total of 10 injections. Fourteen d after the final induction injection, animals received a challenge injections of 0.1 mL of the test solution in the left flank. Animals were housed individually under controlled conditions with *ad libitum* food and water.

Reactions were scored based on the diameter and skin thickness of the reaction seen. Each injection was scored 24 h after administration. The injections administered during the first week of induction were used to establish a standard of reactivity for each animal. Any reaction greater than one standard deviation above this reaction in this animal was considered to be positive, and to be an allergic reaction. No allergic reactions were seen in the negative control, while all animals administered DNCB showed positive reactions. In the monocrotophos (trans isomer) test animals, 9/20 showed positive reactions. Many of these animals showed no skin reaction at all during induction, and the magnitude of the reaction was considerably less than that seen with the positive control. Therefore monocrotophos, trans isomer, was determined to induce allergic responses.

3.3 Monocrotophos formulations

3.3.1 Median Lethal Dose Studies

A summary of the median lethal dose studies with different monocrotophos formulations are presented in the table below.

Formulation	Species	Sex	Route	LD50 (mg active/ kg bw) or LC50 (mg/m^3)	Reference
60%:acetone	Rat	M/F	PO	5.8	Brown et al (1968)
60%; acetone	Rat	M/F	PO	10	Muir (1970a)
24%; acetone and isopropyl alcohol	Rat	M/F	PO	11.9	Brown et al (1968)
24%; isopropyl alcohol	Rat	M/F	PO	7.7	Brown et al (1968)
24%; acetone	Rat	M/F	PO	7.7	Brown et al (1968)
10%; hexylene glycol	Rat	M/F	PO	4.5	Brown et al (1970)
24%; isopropanol and acetone	Rat	M/F	PO	12	Brown et al (1970)

24%; hexylene glycol	Rat	M/F	PO	3.6	Carter (1976)
15%; acetone	Rat	M/F	PO	7.8	Cassidy (1978)
40%; hexylene glycol	Rat	M/F	PO	7.1	Muir (1970a)
60%; acetone	Rat	M/F	PO	6.1	Muir (1970a)
10%; hexylene glycol	Rat	M/F	PO	4.1	Muir & Brown (1968)
15%; hexylene glycol	Rat	M/F	PO	4.5	Muir & Brown (1968)
10%; acetone	Rat	M/F	PO	7.2	Muir & Brown (1968)
15%; acetone	Rat	M/F	PO	7.7	Muir & Brown (1968)
40%; acetone	Rat	M	PO	8.4	Newell (1965)
20%: oxitol acetate	Rat	M/F	PO	m: 10.4, f: 1.08	Price (1982b)
20%; hexylene glycol	Rat	M/F	PO	m: 9.8, f: 8	Price (1982b)
5%; oil	Rat	M/F	PO	5.5	Simpson & Carter (1975)
60%; acetone	Rat	M/F	Dermal	80-100	Brown et al (1968)
24%; acetone, isopropyl alcohol	Rat	M/F	Dermal	<80	Brown et al (1968)
24%; isopropyl alcohol	Rat	M/F	Dermal	>125	Brown et al (1968)
24%; acetone	Rat	M/F	Dermal	<80	Brown et al (1968)
24%: hexylene glycol	Rat	M/F	Dermal	m: 114, f: 143	Carter (1976)
15%; acetone	Rat	M/F	Dermal	75 - 78	Cassidy (1978)
57%; hexylene glycol	Rat	M/F	Dermal	m: 86-114, f: 57-86	Muir (1968)
40%; hexylene glycol	Rat	M/F	Dermal	114	Muir (1970a)
60%; acetone	Rat	M/F	Dermal	78.6	Muir (1970a)
5%, granules	Rat	M/F	Dermal	>500	Muir (1970b)
25%; dioxitol	Rat	M/F	Dermal	m: 197, f: 227	Price (1982b)
15%; hexylene glycol and Shellsol AB	Rat	M/F	Dermal	11-17	Price (1982b)
20%; oxitol acetate	Rat	M/F	Dermal	m: 135, f: 132	Price (1982b)
20%; hexylene glycol	Rat	M/F	Dermal	m: 189, f: 145	Price (1982b)

40%; excipient not specified	Rat	M/F	Dermal	155	Hurni & Sachsse (1969)
60%; acetone	Rabbit	M/F	Dermal	>120	Lazzara & Paa (1975) Sawin (1980)
40%; hexylene glycol	Rabbit	M/F	Dermal	210	Coombs (1975)
40%; acetone	Rabbit	M/F	Dermal	137	Newell & Shellenberger (1964)
40%; acetone	Rabbit	M/F	Dermal	338	Shellenberger (1965a)

3.3.1.1 Oral

Brown VK, Muir CMC & Barrett J (1968) The acute oral and percutaneous toxicities of four AZODRIN formulations. Lab: Shell Research Ltd, Sittingbourne UK. TLTR.0005.68.
Monocrotophos in one of 4 formulations was administered to fasted Carworth Farm E rats (Tunstall Laboratories) in a single gavage dose using 4 rats/sex/group. The formulations were Azodrin 5 (60% monocrotophos in acetone), EF 2629 (24% monocrotophos; Azodrin 5 in isopropyl alcohol), EF 2448 (24% monocrotophos in isopropyl alcohol) and EF 2672 (24% monocrotophos in acetone) and were administered at 5, 10 or 20 mg monocrotophos/kg bw. Water was available *ad libitum*, and food was available after dosing. No detail of the clinical signs observed were given. The LD50s, based on active ingredient, were 5.8, 11.9, 7.7 and 7.7 mg/kg bw respectively. The LD50s based on administration of formulation were 10.2, 42.3, 26.6 and 28.6 mg/kg bw.

Brown VK, Dean B, Muir CMC, Pickering RG, & Reiff B (1970) Toxicity studies on AZODRIN; the effect of a single oral or subcutaneous dose on rats. Lab: Shell Research Ltd Sittingbourne UK. TLTR.0005.68
Monocrotophos formulations were administered to fasted Carworth Farm E rats (Tunstall Laboratory) by gavage. Rats were observed for at least 10 days after dosing; observation time was continued where rats showed any sign of poisoning at the end of 10 days. No details on the clinical signs observed were presented. The LD50s ranged from 4.5 mg active/kg bw for a 10% solution of monocrotophos in hexylene glycol, to 12 mg active/kg bw for a 24% solution of monocrotophos in isopropanol and acetone.

Carter BI (1976) The acute toxicity of AZODRIN 24% in hexylene glycol (FX 1363). Lab: Shell Research Ltd, Sittingbourne UK. TLTR.0015.76
Monocrotophos (24% in hexylene glycol) was administered as a single oral dose to fasted Wistar or CFE rats (Shell Toxicology Laboratory, Tunstall) at doses of 10, 20, 30, 40 or 50 mg formulation/kg bw using 5 rats/sex/group. All doses resulted in clinical signs including piloerection, fibrillation, fasciculations, salivation and chromolacrymation. The acute oral LD50 was 15 mg formulation/kg bw, equivalent to 3.6 mg active/kg bw.

Cassidy SL (1978) Toxicology of insecticides: Acute toxicity of a 15% AZODRIN in acetone formulation to rats. Lab: Shell Research Ltd, Sittingbourne UK. TLTR.003.78
Monocrotophos technical (78.1% purity, source: Shell Toxicology Laboratory) was mixed with acetone to give a 15% w/v formulation, which was administered to fasted SPF Wistar rats (Tunstall Breeding Unit) at doses of 40, 50, 63, 80 or 100 mg formulation/kg bw using

6 rats/sex/group. Rats were maintained for 14 days with free access to food and water. A gross post mortem examination was done, however the results were not reported. Rats were described as showing signs typical of cholinesterase inhibition for the first day after dosing, however the signs were not specified. The LD50 was determined as either 52 or 53 mg formulation/kg bw, depending on the method of determination, equivalent to 7.8 mg active/kg bw.

Muir CMC (1970a) The acute oral and percutaneous toxicities to rats of an AZODRIN 40% WSC (EF 2820) in comparison with AZODRIN 5. Shell Research Lrd, Sittingbourne UK. TLGR.0066.70

Monocrotophos, either as a 40% solution in hexylene glycol, or a 60% solution in acetone was administered by gavage to fasted Carworth Farm E rats (Tunstall Breeding Unit). The doses used were not specified; 5 rats/sex/dose were used. Rats were then observed for 10 days following treatment. The oral LD50 for the 40% formulation was 17.7 mg formulation/kg bw (equivalent to 7.1 mg active/kg bw), and the oral LD50 for the 60% formulation was 10.2 mg formulation/kg bw (equivalent to 6.1 mg active/kg bw).

Muir CMC & Brown VKH (1968) The acute oral toxicity of some AZODRIN formulations. Shell Research Ltd, Sittingbourne UK. TLTR.0012.68

Four formulations of monocrotophos, all supplied by the Physical Chemistry Division of Woodstock Agricultural Research Centre (location not specified) were used. The formulations were a 10% and 15% solution in hexylene glycol, and a 10% and 15% solution in acetone. The formulations were administered to fasted Carworth Farm E rats (Tunstall Breeding Unit) at doses not specified, using 4 rats/sex/group. Rats were initially maintained for 10 d; at the end of this period some rats showing weight loss were maintained for a continued period (unspecified). Clinical signs observed included trembling, salivation and chromodacryhorrhea, however the doses at which these signs were seen was not specified. The LD50 determined in this trial were: 15% monocrotophos in hexylene glycol - 30 mg formulation/kg bw (4.5 mg active/kg bw); 10% monocrotophos in hexylene glycol - 41 mg formulation/kg bw (4.1 mg active/kg bw); 15% monocrotophos in acetone - 51 mg formulation/kg bw (7.65 mg active/kg bw) and 10% monocrotophos in acetone - 72 mg formulation/kg bw (7.2 mg active/kg bw).

Newell GW (1965) Letter Report No 3 Project B-4843. Stanford Research Institute, Menlo Park

Monocrotophos (40% in acetone, source not specified) further diluted in peanut oil was administered by gavage to young male non-fasted Long Evans rats (source not specified) at doses of 15.2, 20, 25.5 or 31.8 mg formulation/kg bw. At the higher doses, signs of toxicity, including lacrimation, salivation, diarrhoea, tremors and clonic convulsions were observed. Deaths occurred between 25 min and 20 h after dosing, with the survivors appearing normal after 24 h. The LD50 was determined to be 21 mg formulation/kg bw (8.4 mg active/kg bw).

Price JB (1982b) Toxicology of AZODRIN formulations; The acute percutaneous toxicity of EF 5801 and EF 5803 and the acute oral and percutaneous toxicity of EF 5811 and EF 5843. Shell Research Ltd, Sittingbourne SBGR.81.112

In two studies, monocrotophos formulations, 200 g/L in oxitol acetate (EF 5811), and 200 g/L in hexylene glycol (EF 5843), were administered to fasted Wistar rats (Tunstall Breeding Unit) by gavage. The first trial was a dose-ranging study, and the rats received doses between 2.5 and 80 mg formulation/kg bw. The second study was a definitive study to determine the LD50. EF 5811 was administered at doses between 30 and 95 mg formulation/kg bw, and EF 5843 was administered at doses between 25 and 80 mg formulation/kg bw. Rats were observed for 14 d

after dosing. Clinical signs included salivation, lacrimation and muscle fasciculations. The LD50 for EF 5811 was 52 mg formulation/kg bw (10.4 mg active/kg bw) for males and 54 mg formulation/kg bw (10.8 mg active/kg bw) for females. The LD50 for EF 5843 was 48 mg formulation/kg bw (9.8 mg active/kg bw) for males and 40 mg formulation/kg bw (8 mg active/kg bw) for females.

Shellenberger TE (1965a) Letter Report No 5 Ref Project B-4843. , Stanford Research Institute, Menlo Park.

Monocrotophos (40% in acetone, source: Shell Chemical Co, Code 8-10-4-13) was diluted in water and administered by gavage to fasted rats (source, strain not specified). The doses for males were 15.8, 19.9, 25 or 31.5 mg formulation/kg bw, and the doses for females were 12.5, 15.8, 19.9 or 25 mg formulation/kg bw, using 10 rats/sex/group. Signs of salivation, lacrimation and tremors were observed. Survivors were normal 24 h after treatment. The acute oral LD50 for males was 23 mg formulation/kg bw (9.2 mg active/kg bw), and the acute oral LD50 for females was 18 mg formulation/kg bw (7.6 mg active/kg bw).

Simpson BJ & Carter BI (1975) Acute oral toxicity of AZODRIN, Formulation EF 3668 to rats. Shell Research Ltd, Sittingbourne UK. TLTR.0023.75

Monocrotophos (50 g/L in an unspecified oil) was administered orally by gavage to fasted rats (strain not specified, source: Tunstall Laboratories) at doses of 20, 40, 80, 121, 141 or 161 mg formulation/kg bw. Rats were observed for 9 d, during which period food and water were available *ad libitum*. Signs of toxicity associated with organophosphorous poisoning (not specified) were seen at doses of 40 mg formulation/kg bw and above. The LD50 was determined to be 111 mg formulation/kg bw (equivalent to 5.5 mg active/kg bw).

3.3.1.2 Dermal

Brown VK, Muir CMC & Barrett J (1968) The acute oral and percutaneous toxicities of four AZODRIN formulations. Lab: Shell Research Ltd, Sittingbourne UK. TLTR.0005.68.

Monocrotophos in one of 4 formulations was administered to Carworth Farm E rats (Tunstall Laboratories) by percutaneous application (4/sex/group). The formulations were Azodrin 5 (60% monocrotophos in acetone), EF 2629 (24% monocrotophos: as Azodrin 5 in isopropyl alcohol), EF 2448 (24% monocrotophos in isopropyl alcohol) and EG 2672 (24% monocrotophos in acetone). Undiluted formulation was applied to the shaved dorso-lumbar area at doses of 60, 80, 100, 125 or 150 mg monocrotophos/kg bw, and a waterproof occlusive dressing applied for 24 h. After 24 h, the dressing was removed and the skin washed with a dilute detergent solution. Water was available *ad libitum*, however food was removed during the exposure period. The LD50s were: Azodrin 5 - 140 - 170 mg formulation/kg bw (80 - 100 mg active/kg bw), EF 2629 - <257 mg formulation/kg bw (<80 mg active/kg bw), EF 2448 - >415 mg formulation/kg bw (>125 mg active/kg bw) and EF 2672 - <268 mg formulation/kg bw (<80 mg active/kg bw).

Carter BI (1976) The acute toxicity of AZODRIN 24% in hexylene glycol (FX 1363). Lab: Shell Research Ltd, Sittingbourne UK. TLTR.0015.76

Monocrotophos (24% in hexylene glycol) was applied to the shorn dorso-lumbar area of Wistar or Carworth Farm E rats at doses of 208, 313, 417, 625, 834, 1042 or 1251 mg formulation/kg bw (4 /sex/group). The area was covered with an occlusive dressing for 24 h, after which the dressing was removed and the skin was washed. Rats were observed for 12 d after exposure. All animals had slightly splayed back legs on the day of dosing. Animals dosed at 417 mg formulation/kg bw and higher showed signs typical of organophosphorus poisoning.

An unspecified number of animals lost weight in the days after exposure. The LD50 was 475 mg formulation/kg bw for males and 596 mg formulation/kg bw for females (equivalent to 114 mg active/kg bw and 143 mg active/kg bw respectively).

Cassidy SL (1978) Toxicology of insecticides: Acute toxicity of a 15% AZODRIN in acetone formulation to rats. Lab: Shell Research Ltd, Sittingbourne.. TLTR.003.78

Monocrotophos (78.1% purity, source: Shell Toxicological Laboratory) was formulated as a 15% solution in acetone, and applied to the shorn dorso-lumbar skin of SPF Wistar rats (Tunstall Breeding Unit) under an occlusive dressing for 24 h at doses of 320, 400, 500, 630, 790 or 1000 mg formulation/kg bw (4/sex/group). At 24 h the dressing was removed and the skin washed. Rats were observed for 14 days following treatment, and it was noted that they showed signs of cholinesterase inhibition for 24 h after dosing, and lost weight from then until the end of the trial. The LD50 was determined to be 498, 521 or 523 mg formulation/kg bw (equivalent to 75 or 78 mg active/kg bw)

Muir CMC (1968) Azodrin FX 1364 WMC. Shell Research Ltd, Sittingbourne: Bioassay Card dated 5/12/68.

Monocrotophos (57% in hexylene glycol) was applied to the skin of CFE rats (source not specified) for an unstated time. It was not indicated whether this was an occluded or a non occluded test. Clinical signs included convulsions and considerable weight loss (not quantified). The LD50 was determined to be 150 - 200 mg formulation/kg bw in males and 100 - 150 mg formulation/kg bw in females (equivalent to 86 - 114 mg active/kg bw in males and 57 to 86 mg active/kg bw in females).

Muir CMC (1970a) The acute oral and percutaneous toxicities to rats of an AZODRIN 40% WSC (EF 2820) in comparison with AZODRIN 5. Shell Research Ltd, Sittingbourne. TLGR.0066.70

Monocrotophos, as either a 40% solution in hexylene glycol, or a 60% solution in acetone was applied to the shorn dorso-lumbar skin of Carworth Farm E rats (Tunstall Breeding Unit) and covered with an occlusive dressing for 24 h. The dressing was then removed and the skin washed. The doses applied were not stated, however 4 rats/sex/group were used. The animals were observed for 10 days, and the LD50 of the 40% solution was determined to be 285 mg formulation/kg bw (equivalent to 114 mg active/kg bw), and the 60% solution to be 132 mg formulation/kg bw (equivalent to 78.6 mg active/kg bw).

Muir CMC (1970b) The acute percutaneous toxicity of AZODRIN 5% Granules (FX 1551) to rats. Shell Research Ltd, Sittingbourne. TLGR.0010.70

Monocrotophos (FX1551, 5% granules) was applied to the shorn dorso-lumbar skin of Carworth Farm E rats (source not specified) at doses of 2500, 5000, 7500 or 10 000 mg formulation/kg bw (8/sex/group), and covered with an occlusive dressing for 24 h. The skin was then washed, and animals were observed for 24 h. The LD50 was determined to be greater than 10 000 mg formulation/kg bw (>500 mg active/kg bw).

Muir CMC (1971) Toxicity studies on Azodrin. The effect of time of exposure on the acute percutaneous toxicity to rats of a 40% w/v WSC (EF 2820) and dilutions of this concentrate in Shellsol A and water. Shell Research Ltd, Sittingbourne TLGR.0020.71

Three formulations of monocrotophos were used to determine the effect of dermal exposure time on toxicity. The formulations were a 40% solution in hexylene glycol, a 40% solution in hexylene glycol diluted to 20% in Shellsol A, and a 40% solution in hexylene glycol diluted to 20% in water. The 24-h occluded LD50 was determined by applying the formulation to the

shaved dorso-lumbar skin of Carworth Farm E rats (Tunstall Breeding Unit), and covering the area with an occlusive dressing for 24 h; the skin was then washed. Animals were observed for 10 days. The doses used were not specified. There were 4 rats/sex/group. The LD50 determined were 107 mg active/kg bw for the 40% in hexylene glycol (268 mg formulation/kg bw), 17 mg active/kg bw (85 mg formulation/kg bw) for the Shellsol A dilution, and 113 mg active/kg bw (565 mg formulation/kg bw) for the water dilution. The effect of time of exposure on toxicity was investigated by applying the formulation to shorn skin for periods of time ranging between 1 min and 4 h, then washing the skin and observing the animals for 10 d. The results are set out in the table below.

Effect of time of exposure on toxicity

Exposure time	Percutaneous LD50 value (mg active/kg bw)		
	40% in hexylene glycol	Shellsol A dilution	Aqueous dilution
1 min	>1000	821	>500
10 min	796	130	>500
30 min	846	-	-
1 h	868	-	-
4 h	467	-	-

It can be seen that the toxicity of the compounds increases significantly with the length of time in contact with the skin, and rapid washing is important in order to reduce toxicity. The LD50 determined for each of the formulations indicated that the formulation with Shellsol A had the highest toxicity, while the aqueous formulation had the lowest toxicity.

Price JB (1982b) Toxicology of AZODRIN formulations; The acute percutaneous toxicity of EF 5801 and EF 5803 and the acute oral and percutaneous toxicity of EF 5811 and EF 5843. Shell Research Ltd, Sittingbourne. SBGR.81.112

Monocrotophos formulations were assessed for percutaneous toxicity. The formulations used were 250 g/L in dioxitol (EF 5801), 150 g/L in hexylene glycol and Shellsol AB (EF 5803), 200 g/L in oxitol acetate (EF 5811) and 200 g/L in hexylene glycol (EF 5843). The formulations were applied to the shorn dorso-lumbar skin of Wistar rats (Tunstall Breeding Unit) over a range of doses and covered with an occlusive dressing for 24 h. The skin was then washed with a detergent solution, and the animals were observed for 14 d. The doses used are set out in the following table:

Doses, expressed as active, applied to shorn dorso-lumbar skin

Formulation	Range finding study - 1 rat/sex/group	Definitive study 6 rats/sex/group
EF 5801	32.5 - 527 mg/kg bw	39.5 - 395 mg/kg bw
EF 5803	3.75 - 30 mg/kg bw	2.85 - 28.5 mg/kg bw
EF 5811	25.8 - 412 mg/kg bw	76.2 - 247 mg/kg bw
EF 5843	25 – 401 mg/kg bw	74.2 - 240 mg/kg bw

The clinical signs observed during the trial included fasciculations, tremors, increased lacrimation and salivation, chromodaccryorrhea and signs of ocular damage. Signs with EF 5803 were of shorter duration than those observed with other compounds. The LD50s determined are set out below.

Dermal LD50 for formulations, expressed as active.

Formulation	Males	Females
EF 5801	196.5 mg/kg bw	227 mg/kg bw
EF 5803	11 - 18 mg/kg bw	11 - 18 mg/kg bw
EF 5811	136 mg/kg bw	152 mg/kg bw
EF 5843	189 mg/kg bw	195 mg/kg bw

Hurni H & Sachsse K (1969) Report on the determination of the Acute Dermal LD50 to the rat of NUVACRON 40. Tierfarm AG, Sisseln, Switzerland.
Monocrotophos (as Nuvacron 40; no other formulation details, source or batch no. specified) was applied to the shorn dorso-lumbar skin of RAC rats (source: Tierfarm AG, Switzerland) at doses of 100, 200, 300, 500 or 800 mg formulation/kg bw (3/sex/group). The lowest dose was diluted in sodium carboxymethylcellulose; the other doses were applied undiluted. The test area was shorn 6 h prior to application. After application, the area was covered with an occlusive bandage for 24 h. The bandage was then removed and the skin washed with warm water. Animals were housed individually for the 14-d observation period, with free access to food and water.

Clinical signs were seen during and after the application period, and included dyspnoea, exophthalmus, lacrimation and prostration. At 800 mg/kg bw muscle spasms, erection of the tail, and salivation were also seen. Gross pathological examination of animals dying during the study revealed bloating in the gastrointestinal tract and 'stained' livers. In animals euthanised at the end of the study, gross pathological findings included stained livers, inflamed intestines with haemorrhagic contents, and pale kidneys. The LD50 was determined to be 388 mg formulation/kg bw (approximately 155 mg active/kg bw).

Hurni H & Sachsse K (1969) Report on the determination of the Acute Dermal LD50 to the rat of NUVACRON EC 40. Tierfarm AG, Sisseln, Switzerland.
Monocrotophos (as Nuvacron 40EC; formulation details, batch no, and source not specified) was applied to the shorn dorso-lumbar skin of RAC rats (source:Tierfarm AG, Switzerland) at doses of 100, 200, 400, 500 or 1000 mL formulation/kg bw (3/sex/group). As the specific gravity of the formulation was not supplied, a dose in mg/kg bw could not be calculated. The application area was covered with an occlusive dressing for 24 h, after which the dressing was removed and the skin washed with warm water. Rats were housed individually with *ad libitum* food and water for the 14-d observation period.

Clinical signs were seen after 24 h, and included clonic-tonic muscle spasms, prostration, exophthalmus, lacrimation, dyspnoea and salivation. The severity of the symptoms increased with increasing dosage. Survivors were normal within 7 to 10 days. Gross pathological examination of animals dying during the study revealed haemorrhages in the small intestine and colon, atalectases in lungs, fatty degeneration in the liver and congested organs. No

abnormalities were seen in the animals euthanised at the end of the trial. The LD50 was determined to be 350 mL formulation/kg bw.

Lazzara K & Paa H (1975) Acute dermal toxicity study with AZODRIN 5 water miscible insecticide in male albino rabbits. Lab: Industrial Bio-Test, Report No 601-07485
and
Sawin VL (1980) Audit of Industrial Bio-Test Laboratories Study No 601-07485, "Acute dermal toxicity study in rabbits" Shell Development Company, Houston WRC RIR-13
Monocrotophos (60% in acetone, Code AC 14150, batch 2-TCL-23) was applied to the shaved dorso-lumbar skin of 6 male albino rabbits (source, strain not specified) at a dose of 200 mg/kg bw. The application site was covered with impervious plastic sheeting for 24 h, after which the skin was washed. Test sites were examined for local skin reactions, and the animals were observed for 14 d for mortality, local skin reactions, and behavioural abnormalities. Initial, 7- and 14-d body weights were recorded, and necropsies were done on all animals at the end of the observation period.

No animals died during the study, and there was no significant weight loss during the 14-d observation period. The material was slightly irritating to unabraded skin, producing pale red erythema at 24 h. No skin reactions were seen at 7 or 14 d. No gross pathologic abnormalities were found. The LD50 was determined to be >200 mg/kg bw. This IBT study was independently validated.

Coombs AD (1975) Acute percutaneous toxicity of AZODRIN formulation EF 2820 in the rabbit (non occluded). Shell Research Ltd, Sittingbourne. TLTR.0025.75
Monocrotophos (400 g/L in hexylene glycol) was applied to the shorn dorso-lumbar area of New Zealand White rabbits (Ranch Rabbits, Crawley, Sussex) at doses of 0.322, 0.386, 0.463, 0.556 or 0.667 mL/kg bw using 2 rabbits/sex/group. The skin was left non-occluded during the experiment. Food and water were available *ad libitum* after dosing; the rabbits were observed over a 9-d period. At the end of 9 d, rabbits that had lost weight were kept until their weight had returned to normal. Typical signs of poisoning were seen at doses of 0.556 mL/kg bw and above. Diarrhoea and weight loss were also observed, with the majority of affected animals not regaining normal weight for 2 -4 weeks after dosing. The LD50 was calculated at 0.49 mL/kg bw, equivalent to 527 mg formulation/kg (210 mg active/kg bw).

Newell GW & Shellenberger (1964) Letter Report No 2, Project B-4843. Stanford Research Institute, Menlo Park.
Monocrotophos (40% in acetone, batch no 7-3-4-7) was applied to the closely clipped dorso lumbar skin of New Zealand White rabbits (source not specified) at 96, 192, 384, and 784 mg/kg bw (3/group). The application was not occluded, and skin was washed with water after 6-h exposure. Symptoms included diarrhoea, miosis and dyspnoea, with the severity related to dose. The dermal LD50 for this 6 h nonoccluded trial was 342 mg formulation/kg bw (137 mg active/kg bw).

Shellenberger TE (1965a) Letter Report No 5 Ref Project B-4843. Stanford Research Institute, Menlo Park
Monocrotophos (40% in acetone, source: Shell Chemical Co, Code 7-3-4-7) was applied to the closely clipped skin of New Zealand White rabbits at doses of 237, 474, 948 or 1895 mg formulation/kg bw (3/group). The application area was not covered, and the material was removed by washing after 6-h exposure. Diarrhoea, miosis and dyspnoea were observed. The LD50 was determined to be 845 mg formulation/kg bw (338 mg active/kg bw).

3.3.1.3 Inhalation

Blair D & Wilson AB (1972) Toxicity studies on insecticide AZODRIN (SD 9129): Acute inhalation exposure of rats to aqueous mist (median droplet size less than 10 µm) Lab: Shell Research Ltd, Sittingbourne. TLTR.0002.72.

Azodrin soluble concentrate (40% monocrotophos in hexylene glycol) (source: Woodstock Agricultural Research Centre, Sittingbourne) was administered as an aerosol to Carworth Farm E strain rats (Tunstall Laboratories). Rats (2/sex/group) were restrained with their heads in an exposure chamber and exposed for 4 h. The estimated concentrations of monocrotophos in the atmosphere were 2, 4, 7, 10, 17, 18 or 36 µg/L.

All exposed animals exhibited tremors, excess salivation and dyspnoea. At 4 µg/L, the animals were exposed for 90 min before tremor was observed. At concentrations of 17 µg/L and above, all rats died, while all rats exposed to 7 µg/L or less survived. One female exposed at 10 µg/L died. No NOEL could be established for this study, as signs were seen at the lowest dose tested. There was no calculation of the LD50.

Wilson AB (1970) Toxicity studies on the insecticide AZODRIN (SD 9129): Acute inhalational exposure of rats to a 40% w/v soluble concentrate in an aqueous spray. Shell Research Ltd, Sittingbourne. TLGR.0078.70

Monocrotophos (40% solution in hexylene glycol) was administered by inhalation as an aqueous spray to Carworth Farm E rats (Tunstall Laboratories) at concentrations of 0.4% and 0.75% for 4 h, using 4 rats/sex/group. One group of rats at each dose were not protected from spray deposition on their body; an additional group at 0.4% received exposure only to their heads, with the rest of the body protected. Rats were fitted with collars to prevent grooming during exposure. Immediately following exposure, rats were washed, dried and returned to the cage for 10 d observation.

During exposure, treated rats showed tremors, dyspnoea and lethargy, and several died. The mortality in the unshielded group at 0.4% was 1/3 males and 1/3 females. One rat escaped, and another removed its collar and commenced washing. These animals were not included in the final result. In the 0.4% head only exposure group, 1/4 males died during the experiment. In the 0.75% group, 1/4 males and 4/4 females died. Therefore there appeared to be lower mortality when head-only exposure was used. No LC50 was determined.

3.3.2 Skin irritation Studies

Cagen SZ (1981a) Primary skin irritation of AZODRIN-5. Shell Development Company, Houston. WRC RIR-171

Azodrin 5 (60% monocrotophos in acetone; source: Shell Biological Sciences Research Centre) was applied at 0.25 mL/rabbit to shaved abraded and non abraded skin of New Zealand White rabbits (Ray Nicholls Rabbitry, Lumberton, Texas) under an occlusive dressing for 24 h (6/sex/group). Sites were assessed shortly after removal of dressing and at 72 h after application. The formulation was determined to be slightly irritating, with erythema and oedema the only signs. No clinical signs were reported for this investigation.

Shellenberger TE (1965a) Letter Report No 5 Ref Project B-4843. Stanford Research Institute, Menlo Park

Monocrotophos (40% solution in acetone, Shell Chemical Co, Code 8-10-4-13) was applied to the closely clipped skin of 6 rabbits (source, strain not specified). The material was applied to

two spots, each area receiving 0.5 mL. Following application, the area was covered (type of covering not specified) for 24 h. The area was then washed with warm tap water, and sites graded for erythema and edema development. The average erythema at 24 h was 0.8, and at 72 h was 1.0. The average edema score was 0 at 24 h, and mild edema was seen at 2 sites at 72 h. Thus, the compound was deemed to be mildly irritating.

3.3.3 Eye irritation

Cagen SZ (1981b) Eye irritation of AZODRIN-5. Shell Development Company, Houston. WRC RIR-173

A 60% formulation of monocrotophos in acetone (Azodrin 5; undiluted, source, batch no not specified) was applied in a volume of 0.1 mL to the right eyes of 5 New Zealand White Rabbits (Roy Nicholls Rabbitry, Lumberton, Texas). Three males also had 0.1 mL applied, with the eye washed with tap water 30 seconds after application. Based on assessment of irritation at 1, 24, 48 and 72 h and 7 and 14 d after application, Azodrin 5 was found to be severely irritating to rabbit eyes. Washing the eye 30 seconds after application did not diminish the effect. The irritation was considered to be due to the acetone solvent.

Newell GW & Shellenberger TE (1964) Letter Report No 2, Project B-4843. Stanford Research Institute, Menlo Park.

Monocrotophos (40% in acetone, batch 8-10-21-1, source not specified) was instilled into the left eye of 9 rabbits at a dose of 0.1 mL/eye. The solution was washed out of the eyes after 30 sec, after 5 min or was not washed out (3/group). Control animals had the left eye treated with acetone. Eyes were graded for ocular lesions over the next 24 h, and on 3 occasions during the 14-d observation period. Monocrotophos induced transitory ocular effects, with corneal opacity, miosis and chemosis resolving within 24 h. Monocrotophos was classed at mildly irritating, given the transitory nature of the signs.

3.4 Monocrotophos mixtures with other pesticides

Cassidy SL (1979) Toxicology of insecticides: Acute toxicity of AZODRIN/DDT ULV formulation EF 5485 to rats. Shell Research Ltd, Sittingbourne. TLTR.79.010

A ULV formulation of monocrotophos (in cyclohexanone and ethyl dioxitol, percentage of monocrotophos not stated) (EF 5485) was administered to fasted SPF Wistar rats (Tunstall Breeding Unit) by gavage at doses of 44, 55, 69, 77, 110. 137. 158 or 217 mg formulation/kg bw using 6 rats/sex/group. Additionally, the formulation was applied to the shorn dorso-lumbar areas of similar rats with an occlusive dressing for 24 h at doses of 200, 250, 320, 400, 500, 630 or 790 mg formulation/kg bw using 6 rats/sex/group.

In the oral gavage study, all rats showed tremors and lacrimation within 5 h of dosing, with survivors recovering on day 2. In the dermal study, these signs were seen at doses of 320 mg formulation/kg bw and above, and resolved on day 3. At day 7, a dose related bodyweight loss (amount of loss not specified) was observed; this was resolving by day 14. The oral LD50 was calculated to be 123 mg formulation/kg bw, and the dermal LD50 was calculated to be 358 mg formulation/kg bw. It was not possible to determine the LD50 based on quantity of active.

Cassidy SL (1980a) Toxicology of insecticides; Acute oral and percutaneous toxicity of a RIPCORD/AZODRIN ULV formulation, EF5254, to rats. Shell Research Ltd, Sittingbourne. TLGR.79.182

Monocrotophos (100 g/L in hexylene glycol and Shellsol AB, with RIPCORD 25 g/L (not otherwise identified)) was administered by gavage to fasted SPF Wistar rats (Tunstall Breeding Units) at 48, 60, 77, 96, 120, 151, or 189 mg formulation/kg bw. Additionally, the formulation was applied to the shaved dorso-lumbar region of rats (6/sex/group) at doses of 125, 160, 200 or 250 mg formulation/kg bw and covered with an occlusive dressing for 24 h.

At oral dose levels of 60 mg formulation/kg bw and above, salivation, tremors and lacrimation were observed within 3 h of dosing. Survivors showed a decreased body weight over the first 7 d, followed by recovery by day 14. In the dermal study, salivation, tremors and lacrimation were observed within 90 min of dosing in all rats. Survivors showed a decreased body weight over the first 7 d, with the majority recovering by day 14. The oral LD50 was calculated to be 89 mg formulation/kg bw (8.9 mg active/kg bw), while the dermal LD50 was calculated to be 186 mg formulation/kg bw (18.6 mg active/kg bw).

Cassidy SL (1980b) Toxicology of insecticides: Acute oral and percutaneous toxicity of a RIPCORD/AZODRIN ULV formulation, EF 4830, to rats. Shell Research Ltd, Sittingbourne. TLGR.80.007.

Monocrotophos (20% formulation in Shellsol AB) was administered to fasted SPF Wistar rats (Tunstall Breeding Unit) by gavage at doses of 20, 40 or 80 mg formulation/kg, using 2 rats/sex/group. Additionally, the formulation was applied to the shaved dorso-lumbar skin of similar rats under an occlusive dressing for 24 h at 125, 250, 500 or 1000 mg formulation/kg bw, using 2 rats/sex/group. The acute oral LD50 was estimated to be between 20 and 40 mg formulation/kg bw (between 4 and 8 mg monocrotophos/kg bw), while the acute dermal LD50 was estimated to be between 125- 250 mg formulation/kg bw (between 25 and 50 mg monocrotophos/kg bw).

Cassidy SL (1980c) Toxicology of insecticides: Acute oral and percutaneous toxicity of a RIPCORD/AZODRIN ULV formulation, EF 4831, to rats. Shell Research Ltd. Sittingbourne. TLGR.80.009

Monocrotophos (20% formulation in isopropyl alcohol, diluted in hexylene glycol) was administered to fasted SPF Wistar rats (Tunstall Breeding Unit) by gavage at doses of 20, 40 or 80 mg formulation/kg bw, using 2 rats/sex/group. Additionally the formulation was applied to the shaved dorso-lumbar skin of similar rats at doses of 125, 250, 500, 1000 or 2000 mg formulation/kg bw under an occlusive dressing for 24 h, using 2 rats/sex/group. The estimated acute oral LD50 was between 20 and 40 mg total formulation/kg bw (between 4 and 8 mg monocrotophos/kg bw), while the acute percutaneous LD50 was estimated to be 500 mg formulation/kg bw (100 mg monocrotophos/kg bw).

Cassidy SL (1980d) Toxicology of insecticides: Acute oral and percutaneous toxicity of a RIPCORD/AZODRIN EC formulation, EF 5312, to rats. Shell Research Ltd, Sittingbourne TLGR.80.006

Monocrotophos (25% in mixed petroleum/xylene: diluted to 2.5% in water) was administered to fasted SPF Wistar rats (Tunstall Breeding Unit) by gavage at doses of 6.25, 12.5, 25 or 50 mg total formulation/kg bw, using 2 rats/sex/group. Additionally, the formulation was applied to the shaved dorso-lumbar skin of similar rats at doses of 30, 60, 130, 250 or 500 mg formulation/kg bw, using 2 rats/sex/group.

Rats in the oral study showed lethargy, piloerection and chromodacryorrhoea at all dose levels. Deaths occurred approximately 5 h after dosing. Rats in the dermal study showed tremors, lachrymation and lethargy, with death occurring approximately 1 h after dosing. The acute oral

LD50 was estimated to be approximately 25 mg total formulation/kg bw (6 mg monocrotophos/ kg bw), while the acute percutaneous LD50 was estimated to be between 60 and 130 mg formulation/kg (between 15 and 33 mg monocrotophos/kg bw).

Cassidy SL (1980e) Toxicology of insecticides: Acute oral toxicity of a RIPCORD/AZODRIN EC formulation, EF 4832, to rats. Shell Research Ltd, Sittingbourne. TLGR.80.008

Monocrotophos (24% in mixed petroleum/xylene diluted in water) was administered to fasted SPF Wistar rats (Tunstall Breeding Unit) at doses of 20, 40 or 80 mg formulation/kg bw, using 2 rats/sex/group. The estimated acute oral LD50 was estimated to be between 20 and 40 mg formulation/kg bw (between 5 and 10 mg monocrotophos/kg bw).

Dewar AJ (1981a) Toxicology of RIPCORD/AZODRIN formulations: The acute percutaneous toxicity of the ULV formulations EF 5832 and EF 5833. Shell Research Ltd, Sittingbourne. SBGR.81.032

Monocrotophos (150 g/L ULV formulation with (EF 5832) or without (EF 5833) hexylene glycol, source: Physical Chemistry Division, Sittingbourne Research Centre) was applied to the shaved dorso-lumbar area of Wistar rats (Tunstall Breeding Unit).

In an initial dose ranging study, EF 5832 was applied at doses of 100, 200, 300, 500, 750 or 1000 mg total formulation/kg bw and covered with an occlusive dressing for 24 h. Based on the results of this study, the doses for a definitive LD50 determination were 314, 396, 500, 630, 793 and 1000 mg total formulation/ kg bw. Clinical signs in the dose-ranging study included salivation, lacrimation, fasciculation, tremor, lethargy and in some cases ataxia. A marked loss in body weight was also seen. In a number of animals, the eyes became swollen, discoloured, bloody and opaque towards the end of the first week of dosing. Clinical signs persisted in survivors into the second week of testing. The LD50 for EF 5832 was determined to be 811 mg formulation/kg bw (122 mg active/kg bw) in males and 619 mg formulation/kg bw (93 mg active/kg bw) in females.

A dose-ranging study for EF 5833 was carried out at the same doses as for EF 5832. A definitive study was carried out at doses of 314, 396, 500, 630, 793 or 1000 mg formulation/kg bw, in a similar manner to the above study. Clinical signs were similar to those seen for EF 5832, including ocular effects. The LD50 for EF 5833 was determined to be 682 mg formulation/kg bw (102 mg active/kg bw) in males and 690 mg formulation/kg bw (104 mg active/kg bw) in females.

Dewar AJ (1981b) Toxicology of AZODRIN/DDT formulations: The acute percutaneous toxicities of the ULV formulations SEF 0001/81 and SEF 0002/81. Shell Research Ltd, Sittingbourne. SBGR.81.143

The formulations SEF 0001/81 and 0001/82 both contain 125 g/L monocrotophos and 300 g/L DDT. SEF 0001/81 is based on Shellsol AB solvent, while SEF 0002/81 is based on Dioxitol solvent. The acute percutaneous toxicities of both of these mixtures was investigated in Wistar rats (Tunstall Breeding Unit), housed under controlled conditions. The pesticides were applied to intact shaved dorsal skin. The area of application was covered by an occlusive dressing for 24 h after application. At this time, the dressing was removed and the area washed. Animals were observed for 14 days for signs of toxicity.

SEF 0001/81 was applied at doses of 9, 15, 23, 30, 38 or 60 mg formulation/kg bw, using 6 rats/sex/group. Clinical signs observed included lethargy, bleeding from the nose, and

chromodacryorrhoea, however the occurrence of these were sporadic, and all animals had recovered by day 3. Following this, SEF 0001/81 was applied at doses of 30, 48, 76, 121, 192 or 305 mg formulation/kg bw. At doses of 76 mg/kg bw and above, clinical signs including muscle fasciculations, salivation and lacrimation were seen. Survivors had generally recovered by day 3, however in one individual tremor persisted until day 7. The LD50 was determined to be 159 mg formulation/kg bw.

SEF 0002/81 was applied at doses of 94, 150, 238, 300, 378, or 600 mg formulation/kg bw. Some animals in the top two dose groups showed signs of intoxication, including salivation, fasciculation and ataxia. Signs appeared either on or after day 2, and recovery had occurred by day 8. The LD50 was determined to be 539 mg formulation/kg bw.

Price JB (1982a). Toxicology of AZODRIN mixtures: The acute percutaneous toxicities of AZODRIN/DDT (EF 5837), AZODRIN/BELMARK (EF 5741) and AZODRIN/RIPCORD (EF 5798). Shell Research Ltd, Sittingbourne. SBGR.81.111.

The acute percutaneous toxicity of Azodrin/DDT (90/200 g/L), Azodrin/Belmark (100/25 g/L) and Azodrin/Ripcord (100/50 g/L) was investigated using Wistar rats (Tunstall Breeding Unit). A dose-ranging study for each formulation was carried out, using one rat/sex/group, prior to a determination of the LD50 using 6 rats/sex/group. For each trial, the dose was applied to the shaved dorso-lumbar skin and covered with an occlusive dressing for 24 h. At the end of this time, the dressing was removed and the skin washed with detergent solution. The animals were observed for 14 days after dosing. Where rats had a lower body weight at 14 days than at the start of the trial, they were maintained until they had returned to at least 95% of initial weight. Clinical signs seen in all three trial included tremors, increased salivation and lacrimation, muscle fasciculation and chromodacryorrhea. Additionally, a number of animals in each study showed corneal opacity suggestive of ocular damage. In one animal this resolved within 7 days; the remainder of the animals showing this sign were euthanised on humane grounds due to significant weight loss.

In the Azodrin/DDT trial, the doses for the dose ranging trial were 602, 1183, 1828, 2365, 3010 or 3548 mg/kg bw. In the definitive trial the doses used were 1183, 1505, 1935, 2365, 3010 or 3768 mg/kg bw, and the LD50 for males was 1729 mg/kg bw and for females was 1398 mg/kg bw.

In the Azodrin/Belmark trial, the doses for the dose-ranging trial were 511, 1022, 1544, 2044, 2555 or 3066 mg/kg bw/day, and for the definitive trial were 1022, 1329, 1635, 2044, 2555 or 3270 mg/kg bw. The LD50 for males was 1533 mg/kg bw and for females was 1329 mg/kg bw.

In the Azodrin/Ripcord trial, the doses for the dose-ranging trial were 257, 515, 772, 1029, 1286 or 1544 mg/kg bw, and for the definitive trial were 123, 206, 319, 515, 823 or 1338 mg/kg bw. The LD50 for males was 823 mg/kg bw. The LD50 for females could not be calculated, however was between 319 and 515 mg/kg bw.

Rose GP (1980) Toxicology of RIPCORD/AZODRIN formulations: The acute oral and percutaneous toxicity of EF 5632 and EF 5644. Shell Research Ltd, Sittingbourne. TLTR.80.003

The acute oral and percutaneous toxicity of two formulations of monocrotophos were tested using Wistar rats (Shell Toxicology Lab Breeding Unit). EF 5632, containing monocrotophos at 125 g/L and Ripcord at 30 g/L in dioxitol, and EF 5644, containing monocrotophos at 125 g/L and Ripcord at 30 g/L in hexylene glycol and Shellsol A were used. The formulation

were administration orally, initially at 2% in corn oil, and then in 4% in corn oil, by gavage, to fasted rats using 6 rats/sex/group. The formulations were also applied to the shorn dorso-lumbar skin of rats (6/sex/group), and covered with an occlusive dressing for 24 h. All rats were observed for 14 d following treatment.

EF5632 was initially administered orally at doses between 10 and 160 mg formulation/kg bw. The oral LD50 was estimated to be approximately 160 mg formulation/kg bw. Clinical signs of lacrimation, salivation and muscle fasciculations were seen at doses above 64 mg/kg bw. A second study was done, using doses ranging from 80 to 252 mg formulation/kg bw, and the LD50 was determined to be 168 mg/kg bw in males and 140 mg/kg bw in females.

EF 5644 was initially administered orally at doses between 9 and 149 mg formulation/kg bw. The oral LD50 was estimated to be approximately 149 mg/kg bw. A second study using doses ranging between 93 and 372 mg/kg bw was done, and the oral LD50 for males was determined to be 171 mg/kg bw, and for females 145 mg/kg bw.

EF 5643 was applied dermally at doses from 50 to 500 mg formulation/kg bw. The LD50 was estimated to be >500 mg/kg bw. A second trial was done, using doses from 500 to 2000 mg/kg bw. The LD50 was estimated to be >2000 mg formulation/kg bw. There were no clinical signs seen in rats at any dose.

EF 5644 was administered at doses between 47 and 239 mg formulation/kg bw, and the LD50 was determined to be >239 mg/kg bw. Typical clinical signs were seen at all doses. A second trial using doses between 149 and 735 mg formulation/kg bw was performed. Tremors and ataxia were seen at doses of 288 mg/kg bw and greater. The LD50 was determined to be 288 mg formulation/kg bw.

Rost GP (1984) Toxicity of pyrethroids plus AZODRIN: The acute oral and percutaneous toxicity of AZODRIN ULV. Shell Research Ltd, Sittingbourne SBGR.83.368

A ULV formulation containing 100 g/L monocrotophos and 16 g/L Ripcord in trioxitol G was evaluated for acute oral and percutaneous toxicity using STCF Wistar rats (Tunstall Breeding Unit). In the oral trial, the formulation was administered by gavage to fasted rats (6/sex/group) at doses of 82, 104, 125, 166, 208 or 160 mg/kg bw. Animals were observed for 14 d after dosing. Deaths occurred in the first 24 h after dosing. Clinical signs included muscle fasciculations, ataxia, tremor, splayed hind-leg gait, salivation, lacrimation, lethargy, piloerection and chromodaccryorrhea. The acute oral LD50 was determined to be 198 mg/kg bw for males and 125 mg/kg bw for females.

In the percutaneous trial, the formulation was applied to the shaved dorso-lumbar skin of the rats at doses of 655, 822, 1040, 1310, 1664 or 2080 mg/kg bw, and covered with an occlusive dressing for 24 h. At the end of this time, the skin was washed with a detergent solution, and the animals were observed for 14 d. Clinical signs were similar to those seen in the oral study. The LD50 was determined to be >2080 mg/kg bw for males, and 1706 mg/kg bw for females. Deaths occurred mainly in the first 3 d after dosing, however delayed deaths occurred until 14 d after dosing.

3.5 Antidote Studies

Reift B (1969) Pharmacological studies into the toxic actions of cholinesterase inhibitors. Part 9. Shell Research Ltd, Sittingbourne. TLGR.0008.69

Monocrotophos (analytical grade, purity 100%, source WARC) was administered in a saline vehicle by SC injection to female CF rats. This was followed by injections of atropine methonitrate (18.02 mg/kg bw), atropine sulphate (17.4 mg/kg bw), P-2-S (50 mg/kg bw), Toxogonin (dichloride bis[4-hydroxylminomethyl pyridinium-(1)-methyl]) (90 mg/kg bw), atropine sulphate and P-2-S (dose as previously) or atropine sulphate and Toxogonin (dose as previously) administered when signs of toxicity were observed. Deaths were recorded at 24 h intervals for 7 days. Additionally, monocrotophos was administered SC at doses of 10 mg/kg bw either with or without antidotal treatment. Immediately after death, or after 2 h, the brains were perfused, removed and the ChE activity determined.

The LD50 of monocrotophos in this strain of rats had previously been determined at 7 mg/kg bw. All antidotes were effective in lowering the toxicity; results are presented below.

SC LD50 of monocrotophos following antidote administration

Chemical	LD50 (mg/kg bw)
Monocrotophos	7
Monocrotophos + atropine methonitrate	21.1
Monocrotophos + atropine sulphate	81.5
Monocrotophos + P-2-S	18.3
Monocrotophos + atropine sulphate + P-2-S	198.7
Monocrotophos + Toxogonin	32.6
Monocrotophos + atropine sulphate + Toxogonin	163

In rats given 10 mg/kg bw monocrotophos SC, signs were seen after 9 min, with death following at approximately 23 min after dosing. The brain ChE activity was 63% inhibited in comparison to controls. When antidotes were administered, rats survived to the 2 h scheduled sacrifice. The brain ChE activity following atropine sulphate treatment was 58% inhibited, following atropine sulphate and P-2-S was 60% inhibited, and following atropine sulphate and Toxogonin administration was 61% inhibited. It appears that the brain ChE inhibition it not altered significantly following antidote administration, although the LD50 is substantially changed.

Mehani, S, El-Habashi A & Soliman S (1978) Evaluation of certain oximes and atropine in the treatment of rats intoxicated with organophosphorus insecticides. Ain Shams Med J 29(5/6): 383-389

Monocrotophos (as Azodrin and Nuvacron, source, purity not specified) was administered orally to Wistar rats (source not specified) by gavage at doses of 10 mg/kg bw for Azodrin, or 20 mg/kg bw for Nuvacron using 5 rats/sex/group. This was immediately followed by an IP injection of an antidote, or combination of antidotes. The antidote was repeated by SC injection after 4 h. The antidotes used were 2-PAM, dichloride bis[4-hydroxylminomethyl pyridinium-(1)-methyl](Toxogonin), parlidoxime hydroxyiminomethyl-2 methyl-1 pyridinium (Contrathion) and atropine sulphate. 2-PAM was administered at 100 mg/kg bw, Toxogonin at 5 mg/kg bw, Contrathion at 4 mg/kg bw and atropine sulphate at 20 mg/kg bw. Additionally, atropine was administered in combination with 2-PAM, with Toxogonin and with Contrathion.

Azodrin administered with no antidote resulted in 5/10 rats dying within 24 h. Following atropine, PAM, atropine and PAM, or atropine and Contrathion in combination, all rats survived this dose of Azodrin. Atropine and Toxogonin in combination resulted in 1/10 rats dying, with both Toxogonin or Contrathion alone, 2/10 rats died. Following Nuvacron administration with no antidote 9/10 rats died within 2 days. The only fully effective antidotes was atropine in combination with either Toxogonin or Contrathion. Atropine, 2-PAM or Toxogonin alone resulted in 2/10 rats dying, while a combination of atropine and PAM resulted in mortality in 3/10 rats. Contrathion alone resulted in 5/10 rats dying.

Therefore atropine and 2-PAM either alone or in combination effectively reduce the mortality following an administered dose of monocrotophos.

Gough BJ & Shellenberger TE (1977-78) In vivo inhibition of rabbit whole blood cholinesterase with organophosphate inhibitors and reactivation with oximes. Drug Chem Toxicol 1(1): 25-43

Monocrotophos technical (source: Shell Development, purity not given) in saline solution was administered by IV infusion to adult male New Zealand White rabbits (3/group) at 0.106 mg/min for 60 minutes, followed by treatment with one of five oxime reactivators, 2-PAM, P-2-S, 1,1'-trimethylene-bis-[4-(hydroxyiminomethyl)-pyridinium bromide] (TMB4), isonitrosoacetone (MINA) and diacetyl monoxime (DAM). Whole blood ChE activity was measured prior to, during and after the chemical infusion.

Monocrotophos produced significant inhibition of ChE activity by 20 minutes after the commencement of infusion. Levels decreased to approximately 40% of preadministration levels (estimated from graph) by the end of the infusion, and recovered gradually from this point. The administration of 50 mg/kg bw 2-PAM did not change the ChE activity from that which would be expected with spontaneous recovery. In a second test comparing the 5 oxime reactivators, MINA and DAM at high doses did not affect ChE activity recovery. 2-PAM and TMB-4 administration at 50 mg/kg bw resulted in a return to normal values at 60 minutes after the administration of the reactivator. P-2-S administration at 50 mg/kg bw also produced an increase in ChE activity over 60 minutes, however the levels had not returned to normal. When the rabbits were not dosed with the oxime reactivator, ChE activity did not return to normal over the period of examination.

Brown AK (1964) The efficacy of atropine and oxime therapy as an antidote to poisoning by SD9129 in guinea-pigs. Shell Research Ltd, Sittingbourne. Tech Memo Tox 20/64

A series of 2 experiments was conducted using monocrotophos (batch no FC 1342, source not given). The first involved establishing the LD50 by the SC route in guinea-pigs of the 'P' strain (source not given), and the second was to determine the effectiveness of atropine alone or in combination with P-2-S.

In the first test, a 2% aqueous solution of monocrotophos was injected SC into the flank of guineapigs (1/sex/group) at doses of 37.5, 40, 45 or 60 mg/kg bw. The LD50 was determined to be 45 mg/kg bw. For the antidote test either 5 times the LD50 was administered to controls, and either 5 or 10 times the LD50 was administered to animals treated with atropine alone (17.4 mg/kg bw SC) or atropine and P-2-S (17.4 mg/kg bw and 50 mg/kg bw respectively). At 5 times the LD50, all control animals died, while no deaths were seen in either of the antidote groups. Ten times the LD50 was not administered to controls, as complete mortality was assumed. With atropine treatment, mortality following this dose was 3/5 males and 5/5 females. With atropine and P-2-S, mortalities were 1/5 males and 2/4 females. The mortalities seen in

these groups were delayed by several hours in comparison to deaths seen in control animals. Therefore, it appears that atropine plus P-2S is an effective antidote, and has a greater affect than atropine alone at high doses.

Shellenberger (1980) Organophosphate pesticide inhibition of cholinesterase in laboratory animals and man and effects of oxime reactivators. J environ Sci health B 15(6): 795-822

Monocrotophos (source, purity not given) was administered to New Zealand White rabbits by IV infusion via the lateral ear vein at the rate of 0.106 mg/min. The oxime reactivators 2-PAM, P-2-S and TMP4 were administered at 25 or 50 mg/kg bw, and the level of cholinesterase inhibition determined. 2-PAM at 50 mg/kg bw resulted in minimal ChE inhibition 1 h after commencement of treatment, as did 50 mg/kg bw of TMP4. P-2-S was not as effective at decreasing inhibition. At the lower dose of 25 mg/kg bw, none of the antidotes were effective at preventing ChE inhibition.

An absorption study was also done. Monocrotophos technical, and combined with water, acetone, DMSO or xylene was administered percutaneously to the clipped skin of New Zealand White rabbits, and exposed for a period of time. The dose applied and the period of exposure were not specified. The absorption was estimated using ChE inhibition. Water did not increase the penetrance of monocrotophos. Including acetone or DMSO increased absorption to approximately twice that seen with monocrotophos alone. The use of xylene as a solvent dramatically increased the absorption of monocrotophos, estimated to increase it by approximately 40 times.

4 SHORT-TERM REPEAT DOSE STUDIES

4.1 Oral Administration in mice for five weeks

Hend RW & Gellatly JBM (1979) Toxicity studies on the insecticide AZODRIN: a five week feeding study in mice. Shell Research Ltd, Sittingbourne. TLGR.79.163

Monocrotophos (batch no 8-26-0-0, supplier: BSRC, Modesto, USA, purity not given) was fed to SPF CD mice (Tunstall Breeding Laboratories) at doses of 0. 0.1, 0.5, 1, 10, or 100 ppm (equivalent to 0, 0.015, 0.075, 0.15, 1.5 or 15 mg/kg bw/day), using 8 mice/sex/group (16/sex/group in controls) for five weeks. Food and water were available *ad libitum*. Mice were observed daily for clinical signs and physical appearance. Body weights and food intakes were determined weekly.

At the end of exposure, blood was taken for haematology and clinical chemistry. Haematological examinations included total and differential leucocyte count, erythrocyte count, Hb, MCV, MCH and MCHC. Clinical chemistry examination included measurement of protein, urea, AP, ALT and AST. Plasma, erythrocyte, and brain ChE were also determined. Full gross necropsy was done on all animals. Histopathological examination of the liver, Harderian gland, adrenals, kidneys, alimentary tract, ovaries, uterus, prostate, spleen, lymph nodes, lungs, thyroid, urinary bladder, pancreas and brain of all animals in the control, 10 and 100 ppm treatment groups was performed.

General health and behaviour were unchanged. Body weight was significantly reduced in 100 ppm males from the first week. In females, the only significant decrease was seen in the 100 ppm treatment groups in weeks 1 and 2. As the decreases in these groups were greater than 10%, they are considered of biological significance. While body weights were decreased in other treatment groups, the decreases were not great enough to be considered significant. Food intake was decreased in high-dose males throughout the study, and in high-dose females in weeks 1 to 3.

There were no alterations in haematology related to treatment. In males on 100 ppm, there was a significant increase in AP levels. There was also an increase in the ratio of liver weight to body weight in this group, however there were no histopathological abnormalities. There were no significant findings on macroscopic examination. At the end of the study, reduced pigment levels seen in the Harderian glands of mice fed 100 ppm were thought to reflect subclinical corneal irritation, although no clinical or histological evidence of irritation was observed.

Changes in ChE activity are presented below.

Mean percentage ChE inhibition

Dose (ppm)	Plasma ChE		Erythrocyte ChE		Brain ChE	
	Males	Females	Males	Females	Males	Females
0.1	35	0	26	0	0	0
0.5	25	16	21	0	11	0
1	22	32	21	14	14	13
10	81	74	65	42	74	60
100	99	97	70	65	90	91

It can be seen that there was significant (>20%) plasma ChE inhibition in males at all doses, and in females from 1 ppm. Erythrocyte ChE activity was also inhibited in males at all doses, while females showed inhibition at 10 ppm. Brain ChE was inhibited in both sexes at 10 ppm. Based on the effects seen in males in both plasma and erythrocyte ChE, no NOEL can be established for this study. A NOEL for brain ChE could be established at 1 ppm in the diet. The LOEL for the study is 0.1 ppm (equivalent to 0.015 mg/kg bw/day), based on effects on plasma and erythrocyte ChE in males.

4.2 Oral Administration in Rats for 5 Days.

Brown, VKH & Muir CMC (1970) Toxicity studies on AZODRIN: The effect of repeated oral doses on the rat. Shell Research Ltd, Sittingbourne. TLGR.0027.70

Monocrotophos (purity, source not given) was administered by gavage to Carworth Farm E (CFE) strain rats (Tunstall Laboratories) at doses of 0, 1.68, 3.36 or 6.72 mg/kg bw/day for 5 days (5/sex/group). The doses were calculated to be 0%, 20%, 40% or 80% of the LD50 in this strain. Food and water were available *ad libitum* throughout the study, and survivors were maintained for 10 days after the final dose.

At the lowest dose tested, there were slight signs following the first dose, which persisted until 4 d after the final dose. There were no mortalities seen in this group. In the mid-dose group, there were strong cholinergic symptoms from the first dose; these persisted in survivors until 5 d after the final dose. No mortalities were seen in the females, while 3/5 males died during treatment. Males were generally more affected than females. In the high-dose group, all rats were severely affected after the first dose and died on day 2 of the study.

Therefore, no NOEL could be established, given the clinical signs seen at 1.68 mg/kg bw/day, the lowest dose tested.

4.3 Intraperitoneal administration in Mice for six weeks

Gupta M, Bagchi G, Bandyopadhyay S, Sasmal D, Chatterjee T & Dey SN (1982) Haematological changes produced in mice by NUVACRON or FURADAN. Toxicology 25:255 - 260.

Monocrotophos (source, purity not given) in normal saline was administered to male albino (Swiss) mice (20/group) at 0.8 mg/kg bw by IP injection, once weekly for six weeks. Mice were killed 18 h after the final dose. Erythrocyte and total and differential leucocyte counts were done. Clotting time, Hb, platelet count, Hct, MCV, MCH and MCHC were measured. Bone marrow and splenic measurements were done, including an estimation of the number of nucleated cells per spleen.

Following monocrotophos administration, clotting times increased by approximately 3-fold. It was proposed that this may be related to a liver abnormality, however no attempt was made to quantify this. Erythrocyte count was decreased by a similar factor, as was the platelet count. There were no other significant abnormalities detected on examination of the blood. There was mild depression of the bone marrow, with erythrocyte precursors decreased. The weight of the spleen was unchanged following monocrotophos administration, however the number of splenic cells had increased by a factor of 4. This was considered to be a reactive erythropoiesis, in response to the depression of the bone marrow.

Gupta M, Bagchi GK, Gupta SD, Sasmal D, Chatterjee T & Dey SN (1984) Changes of acetylcholine, catecholamines and amino acid in mice brain following treatment with Nuvacron and Furadan. Toxicology 30: 171 - 175.

Monocrotophos (source, purity not given) was administered by IP injection to Swiss male albino mice at 0.8 mg/kg bw (75 mice/group) once weekly for 6 weeks. Animals were killed by immersion in liquid air 30 min after the last dose. The brain was dissected out and kept frozen. Brains from 30 mice were used to assess brain acetylcholine and ChE. Another group was used for the spectrofluorometric determination of 5-hydroxy tryptamine and catecholamines, including adrenaline, noradrenaline and dopamine, while the remainder were used for a determination of GABA, using paper chromatography.

Acetylcholine concentration in the brain was increased, while ChE activity was decreased in mice treated with monocrotophos ($p<0.01$). The concentration of adrenaline, noradrenaline and dopamine were significantly increased, while the concentration of GABA was unchanged.

4.4 Oral Administration in Rats for up to 13 Weeks

Hend RW & Brown VKH (1981) A reversibility study on cholinesterase activity in rats fed AZODRIN for 8 weeks. Shell Research Ltd, Sittingbourne. TLGR.79.154

Monocrotophos (batch no 8-28-0-0, supplier: BSRC Modesto USA, purity 78.7%) was fed to SPF Wistar rats (Shell Toxicology Laboratory Breeding Unit) at doses of 0, 0.1, 0.25, 0.5, 2.0 or 8.0 ppm in the diet (equivalent to 0. 0.005, 0.0125, 0.025, 0.1 or 0.4 mg/kg bw/day), using 10 rats/sex/group (except controls with 20 rats/sex/group). Three experiments were performed. In the first, rats were fed monocrotophos in the diet for 8 weeks prior to euthanasia; in the second, rats were fed monocrotophos for 13 weeks, and in the third, rats were fed monocrotophos for 8 weeks, then maintained on control diets for 5 weeks.

Rats were observed daily for clinical signs. Body weight and food consumption were determined weekly for the first 8 weeks, then body weight was determined in remaining animals in weeks 9, 11, 12 and food consumption in weeks 9 and 12. Rats dying during the study underwent gross postmortem examination, but no blood samples were taken. At the end of each of the studies, surviving animals were euthanised, and blood samples taken for ChE activity. Brains were also removed for ChE determination.

There were no treatment-related mortalities or clinical signs observed during the trial. Body weights of male rats in the high-dose group were decreased in the first weeks of feeding in each experiment; although the decreases were statistically significant ($p<0.05$), they were less than 10% in comparison to controls, and were considered not to be of biological significance. Data on the inhibition of ChE activity is presented below.

Mean ChE inhibition in rats fed monocrotophos for 8 weeks

Dose (ppm)	Plasma ChE		Erythrocyte ChE		Brain ChE	
	Males	Females	Males	Females	Males	Females
0.1	5	8	4	13	4	4
0.25	5	14	14	19	8	10
0.5	5	28	28	19	18	18
2.0	21	43	59	61	46	43
8.0	51	79	83	86	72	75

Mean ChE inhibition in rats fed monocrotophos for 13 weeks

Dose (ppm)	Plasma ChE		Erythrocyte ChE		Brain ChE	
	Males	Females	Males	Females	Males	Females
0.1	0	22	1	13	4	3
0.25	6	21	18	17	7	10
0.5	11	26	31	37	17	15
2.0	28	45	61	65	43	42
8.0	51	77	80	83	72	70

Mean ChE inhibition in rats fed monocrotophos for 8 weeks, followed by 5 weeks recovery

Dose (ppm)	Plasma ChE		Erythrocyte ChE		Brain ChE	
	Males	Females	Males	Females	Males	Females
0.1	0	0	7	0	4	2
0.25	0	0	19	19	4	1
0.5	0	0	11	11	5	3
2.0	0	0	22	17	11	10
8.0	0	0	31	26	17	15

It can be seen that significant inhibition of plasma ChE was produced in males fed at 2.0 ppm in the diet after either 8 or 13 weeks exposure, and in treated females fed at 0.1 ppm after 13 weeks exposure. Plasma ChE activity had recovered in all animals after 5 weeks of consumption of untreated food. Erythrocyte ChE inhibition after 8-weeks dosing was significant in males at 0.5 ppm, and in females at 2.0 ppm, while after 13 weeks the inhibition was significant in both males and females at 0.5 ppm. After 5-weeks recovery, erythrocyte ChE remained inhibited from 2.0 ppm in males and 8.0 ppm in females. Brain ChE activity was inhibited at 2.0 ppm after either 8- or 13-weeks exposure in both males and females; all groups had recovered after 5 weeks on control diet. The NOEL for erythrocyte ChE was 0.25 ppm, while the NOEL for brain ChE was 0.5 ppm. Plasma and brain ChE activity showed a more rapid return to normal than did erythrocyte ChE activity. No NOEL for plasma ChE inhibition can be set in this trial, as females at the lowest dose (0.1 ppm) showed significant inhibition of plasma ChE.

4.5 Oral Administration in Rats for 5 Weeks

McAusland, HE & Gellatly JBM (1979) A five week feeding study of AZODRIN in rats. Shell Research Ltd, Sittingbourne. TLGR.79.162

Monocrotophos technical (spurce: BSRC, batch no 8-26-0-0) was fed in the diet to Wistar rats (Shell Toxicology Laboratory Breeding Unit) at doses of 0, 0.1, 0.5, 1.0, 10 or 100 ppm (equivalent to 0, 0.005, 0.025, 0.05, 0.5 or 5 mg/kg bw/d) for five weeks, using 8/sex/group (16 rats/sex in control). Food and water were available *ad libitum*.

Rats were observed daily for general health and physical appearance. Body weight and food consumption was measured weekly throughout the experiment. Three days before the end of the study, blood was taken for estimation of glucose concentration. At the end of the trial, blood was taken for clinical chemistry and haematological examination. Clinical chemistry parameters examined were protein, BUN, AP, glucose, chloride, AST, ALT, sodium and

potassium. Haematological evaluation included total and differential leucocyte count, erythrocyte count, Hb, Hct, MCV, MCH, MCHC and prothrombin time. Plasma, erythrocyte and brain ChE activity was determined. A gross pathological examination of all animals was performed. The brain, heart, testes, kidneys, liver and spleen were weighed. In the 0, 10 and 100 ppm groups the following organs were examined histologically: adrenals, brain, eyes with Harderian glands, testes or ovaries/fallopian tubes, heart, small intestine, large intestine, kidneys, liver, lungs, lymph nodes (both mesenteric and submaxillary), pancreas, pituitary, prostate, salivary gland, spleen, stomach, trachea and oesophagus, thyroids and parathyroids, thymus, urinary bladder, and any abnormalities identified on gross necropsy. The following tissues were stored: bone with marrow, caecum, skin with mammary gland, skeletal muscle, nasal cavity, sciatic nerve, seminal vesicles, vertebrae/spinal cord, tongue and vas deferens.

No rats died during the study. Treatment-related clinical signs were seen in the 100 ppm group, including slight tremors, nervous and unsteady condition and a 'congested chatter'. These animals were in poor condition, and a number had unspecified damage to the eyes. Body weight was significantly reduced in both males and females on 100 ppm from week 1, with the decrease ranging between 40 and 46% in comparison to controls in males, and 32% for females. While the body weight of the 10 ppm group was statistically significantly decreased ($p<0.05$), the decreases were only around 6% in comparison to controls, and were not considered of biological significance. The food intake was decreased in both of these groups. There were a number of changes noted on clinical chemistry examination, including decreased plasma protein and increase AP, AST and ALT in the high dose animals. These were associated with a decrease in the relative liver weight, also seen at 10 ppm, and a decrease in periportal parenchymal vacuolations. These effects were suggested to be due to the decreased food intake, rather than a direct compound-related effect.

The ChE inhibition data are presented below.

Mean percentage ChE inhibition

Dose (ppm)	Plasma ChE		Erythrocyte ChE		Brain ChE	
	Males	Females	Males	Females	Males	Females
0.1	3	13	7	16	4	12
0.5	5	24	19	39	14	17
1	18	30	43	43	20	25
10	56	78	100	100	74	77
100	82	93	100	100	91	91

It can be seen that significant plasma ChE inhibition occurred in females from 0.5 ppm, and in males from 10 ppm. Erythrocyte ChE inhibition occurred in females from 0.5 ppm, and in males from 1 ppm. Brain ChE inhibition occurred in both sexes from 1 ppm. Based on the effects on erythrocyte and plasma ChE in females, the NOEL for ChE is 0.1 ppm, equivalent to 0.005 mg/kg bw/d.

On macroscopic examination, an increase in corneal opacity was noted in animals at 100 ppm (0/32, 0/16, 0/16, 0/16, 0/16, 5/16). There was also a similar incidence of pale retro-orbital tissues. On histological examination, these animals were found to have subacute keratitis, with local pannus formation and corneal oedema. In some cases this had progressed to include formation of an anterior synechiae. There was also pigment depletion in the Harderian gland found in 8/32 controls, 4/16 at 10 ppm and 15/16 at 100 ppm. It was suggested that this may

be related to hypersecretion. The NOEL can be set at 10 ppm, based on the ocular effects seen at 100 ppm.

Overall, the NOEL for the study can be set at 0.1 ppm in the diet, equivalent to 0.005 mg/kg bw/d, based on the inhibition of plasma and erythrocyte ChE seen in females at 0.5 ppm.

4.6 Dermal Administration in Rats

Hageman (1992) 28 Day Repeated Dose Dermal Toxicity Study in the Rat. Test No. 911267 C1414 tech. Final Report. Ciba Geigy Ltd. GLP: OECD/USEPA

Monocrotophos technical (batch no OP 107001, purity 77.6% source: Ciba Geigy Ltd) in distilled water was applied to the shorn skin of Tif RAIf (hybrids of RII/1 x RII/2) rats once daily for 28 d at doses of 0, 0.2, 1, 10 or 100 mg/kg bw/d (5/sex/group). The hair was clipped prior to the first application and then as required. The applied dose was placed on a gauze swab, which was then applied to the shorn area and secured with adhesive tape. The gauze was left in place for 6 h, then carefully removed. It was not covered with an occlusive bandage.

Rats were housed individually with free access to food and water. Clinical signs, mortality and skin irritation were assessed daily. Body weight and food consumption was assessed once weekly. At the end of the trial, blood was taken for haematological and clinical chemistry examination, and ChE activity in plasma, erythrocytes and brain was determined. The haematological parameters examined were erythrocyte count, Hb, Hct, MCV, MCH, MCHC, total and differential white cell count, prothrombin time and methaemoglobin. The clinical chemistry parameters examined were glucose, BUN, creatinine, total protein, albumin, globulins, sodium, potassium, calcium, chloride, inorganic phosphorus, AST, ALT and AP. Gross postmortem was done on all animals, and the brain, heart, liver, kidneys, adrenals, thymus, ovaries/testes and spleen weighed. Histopathological examinations were done on the skin application site, a remote skin site, liver, kidney, thymus, spleen and thyroid with parathyroid gland. Other tissues were preserved in formalin, but were not examined at this time.

There were no mortalities during the trial. Piloerection, hunched posture and dyspnoea were seen in rats at 10 and 100 mg/kg bw/d. Tremor, trismus, clonic-tonic muscle spasms, ventral recumbency and exophthalmus were seen at 100 mg/kg bw/d. Skin was assessed 23 h after each application; no skin irritation was seen during the trial. There was no significant change in body weight in any of the treatment groups during the trial, and haematological examinations at the end of the trial were normal. ALP levels were significantly decreased in male rats at the high dose; this is of questionable biological significance.

The decreases in ChE activity are detailed below.

Mean percentage ChE inhibition

Dose (ppm)	Plasma ChE		Erythrocyte ChE		Brain ChE	
	Males	Females	Males	Females	Males	Females
0.2	7	21	6	12	3	0
1	5	17	15	7	2	0
10	34	56	48	17	25	25
100	76	93	65	21	61	63

It can be seen that there was significant inhibition of plasma ChE in females at the lowest dose; however, this was not consistent, as the inhibition seen at the next dose was not significant. In both males and females, plasma ChE was significantly inhibited at 10 mg/kg bw/d. Males showed significant inhibition of erythrocyte ChE from 10 mg/kg bw/d, while females showed inhibition at 100 mg/kg bw/d. In both males and females, brain ChE was significantly inhibited from 10 mg/kg bw/d. Based on the inhibition of erythrocyte ChE in males, the inhibition of brain ChE in both sexes and the inhibition of plasma ChE at 10 mg/kg bw/d, the NOEL can be established at 1 mg/kg bw/d.

There were no abnormalities found on gross pathological examination, and no significant change in organ weights were detected. On histopathological examination, findings included inflammatory cell infiltration in the skin, liver and kidneys. This was not dose related, and was not considered to be related to treatment.

Overall, the NOEL for the study can be established at 1 mg/kg bw/day, based on the ChE inhibition seen at 10 mg/kg bw/d.

4.7 Dermal studies in rabbits

Newell GW & Shellenberger TE (1964) Letter report No 2, Project B4843. Stanford Research Institute, Menlo Park
and
Newell GW (1965) Letter Report No 3, Project B-4843. Stanford Research Institute, Menlo Park.

Monocrotophos (40% in acetone, source not specified) was applied in aqueous solution to intact or abraded skin of New Zealand White rabbits (5/sex/group) at doses 0, 42 or 84 mg formulation/kg bw (equivalent to 0, 17 or 34 mg active/kg bw) once daily for 6 h, 5 d/week for 3 weeks. Skin abrasions were 2-3 cm apart, and were deep enough to penetrate the dermis without drawing blood. The skin was not covered during application, and was washed with water at the end of each day's application period. Animals were housed individually.

In the-low dose group, some animals showed occasional laboured breathing and diarrhoea. In the 84 mg formulation/kg bw/d group, tremors were pronounced, and diarrhoea was more frequent, particularly in the animals with abraded skin. Body weight was decreased in animals on the highest dose, to an extent considered biologically significant. The animals with abraded skin showed a greater decrease in body weight. Mortalities were increased in animals on the higher dose, with 5 treated animals dying during the study. Two control and one low-dose animal died.

Histopathological examination revealed no significant treatment related findings. All groups showed an increased frequency of nonspecific interstitial pneumonitis, which is common in laboratory rabbits. The increased frequency in this trial was considered to be due to the physical trauma of repeated skin applications. There was also an increase in focal aseptic fat necrosis in all groups, which was not related to treatment. There were no changes to the skin noted on histopathological examination.

Based on the clinical signs seen at the lowest dose, no NOEL could be established for this experiment.

Doyle RL & Elsea JR (1965) Repeated applications of technical BIDRIN insecticide and AZODRIN to the skin of rabbits. Hill Top Research Inc. Miamiville. Report No P-44 Sponsor: Shell

Monocrotophos (60% solution, lot no. 389-21-10, supplier: Shell Chemical Co; no other formulation details supplied) in distilled water was applied to albino rabbits (H&M Breeders, Kentucky). A dose of 36 mg formulation/kg bw/day was applied to 11 males and 9 females, and a dose of 72 mg formulation/kg bw/day was applied to 8 males and 12 females. Ten rabbits/sex served as controls; distilled water was applied to these animals. The abdominal and lateral skin area were clipped. In one-half of the animals in each group, the abdominal skin was abraded initially and at the start of the 2nd and 3rd experimental weeks, by a series of longitudinal minor epidermal incisions spaced 1 - 2 cm apart. The abrasions penetrated the epidermis, but did not induce bleeding.

The control and test material were applied once daily, 5 days/week to an area approximately equivalent to 10% of the total body surface. The dose was applied under a binder of rubber dental dam secured around the trunk, and was covered with an outer layer of gauze secured with adhesive tape to ensure the test material remained in contact with the skin. At the end of the 6-h exposure period, the covering materials were removed, and the area sponged with a moistened towel to remove any unabsorbed material.

Body weights were determined initially, once weekly during the study, and at termination. Initially and on day 13, haematological determinations, including Hb and Hct determinations, and total and differential white cell counts, were done on all abraded skin animals. Rabbits were observed at least once daily for signs of dermal irritation and clinical signs. Food and water consumption were estimated from the quantity remaining from the previous day's allocation.

At the end of the trial, a complete gross autopsy was done on each rabbit. The weight of the heart, liver, kidney, adrenals, spleen and gonads were recorded. An histopathological examination was made of the abraded skin animals in the control and high dose groups and the skin, heart, liver, kidney, adrenal, spleen, stomach, small intestine, gonad and sternal bone marrow were examined. All tissues were preserved for future reference.

In general, rabbits showed no evidence of systemic toxicological or pharmacological effects. A small number of animals had diarrhoea and wheezing, however this was not related to treatment. Mild skin irritation was seen in a number of animals, particularly those with abraded skin. This was generally erythema and atony, with atony more common in animals in the high dose group. There were no treatment-related effects on body weight. On haematological examination, the only differences noted were an elevation in the white cell count of treated rabbits at week 3. While the levels seen were almost 50% greater than controls, they are considered to be within the normal range for rabbits of this strain and age. On gross autopsy, kidney congestion, either generalised or limited to the cortico-medullary junction was seen at 0/20, 2/20 and 4/20 animals in the control, low-dose and high-dose groups. In 2 rats at the high dose, the spleen was enlarged. The mean spleen weight in comparison to body weight in males was increased relative to controls (0.045%, 0.046% and 0.081%). No abnormalities were found on histopathology.

Given the increase in atonia, the increase in spleen weight, and the increased incidence of congestion of the kidney at the high dose, the NOEL can be set at 36 mg formulation/kg bw (equivalent to 22 mg monocrotophos/kg bw).

Coombs AD (1977) AZODRIN toxicity: cholinesterase inhibition in rabbit blood following the percutaneous administration of Azodrin and Azodrin containing 5% w/v chloromonocrotophos for five days. Shell Research Ltd, Sittingbourne TLTR.0001.77

Monocrotophos (SD 9121, 20% w/v dilution in acetone) and monocrotophos technical, containing 5% w/v chloromonocrotophos (SD 10791: 20% w/v dilution in acetone) (sources not given) were administered separately to New Zealand White rabbits (Goreside, Northchurch, Berhamsted, Herts). A dose of 3 mg monocrotophos/kg bw/d was applied percutaneously to the shorn neck region for 5 d (4/sex/group), the dosage being determined from preliminary studies using 3 mg/kg bw/d or 30 mg/kg bw/d. Control animals were treated with acetone. Food and water were available *ad libitum*. Animals were observed for any abnormal clinical signs. Pre-exposure blood samples were taken 7 and 4 days before exposure, and also on the morning of exposure. Blood was taken on days 7, 10, 14, 17, 21, 38 and 63 after the first dosing day.

No abnormal clinical signs were reported. Plasma ChE was significantly inhibited in both males and females by both monocrotophos and technical monocrotophos up to 7 d after the first d of administration. Both males and females on monocrotophos had significant plasma ChE inhibition on day 10. Erythrocyte ChE showed inhibition over a longer period, with significant inhibition in seen until day 14 in both sexes for the technical monocrotophos, and inhibition seen until day 17 (males) or day 21 (females) for the monocrotophos alone. A similar degree of ChE inhibition was seen with both preparations, and therefore there were few differences between monocrotophos alone and monocrotophos with 5% chloromonocrotophos.

5 SUBCHRONIC TOXICITY

5.1 Rat

Shellenberger TE & Newell GW (1964e) Subacute toxicity and cholinesterase study of Shell Compound SD 9129 - Rat and dog. Techn. Report Part 1. Stanford Research Institute, Menlo Park.

Monocrotophos technical (SD9129, source not specified) in corn oil was fed in the diet to weanling Long Evans rats (source not specified) at doses of 0, 0.5, 1.5, 15, 45 or 135 ppm (equal to 0, 0.03, 0.1, 1.0, 3.3 or 11 mg/kg bw/d for males and 0, 0.04. 0.1, 1.3, 4.0 or 13.3 mg/kg bw/d for females) for 12 weeks. In the 0 and 15 ppm groups, there were 42 rats/sex, in the 0.5 and 1.5 ppm groups there were 30 rats/sex, and in the 45 and 135 ppm groups, 12 rats/sex. Food and water were available *ad libitum*. Body weight and food consumption were determined weekly, and records of physiological response, behaviour and mortality were kept. Hb, Hct, erythrocyte and total and differential white cell counts were determined on 4 rats/sex in the 0, 15, 45 and 135 ppm groups at the beginning, and every 4 weeks throughout the study. After 12 weeks, 12 rats/sex in the 0, 15 and 45 ppm groups, and 12 males and 9 females at 135 ppm were euthanised and examined. The liver, kidney, spleen, heart and testes/ovaries were weighed. Sections of these organs and of the lung, adrenal, pancreas, stomach, small intestine, prostate/uterus, skeletal muscle, femur, brain, pituitary, submaxillary and sublingual glands, lymph node, thyroid, parathyroid, Harderian gland, lacrimal gland and thymus were preserved for examination. Blood and brain ChE activities were determined at weeks 2, 4, 8 and 12 on 5 rats/sex from the 0, 0.5, 1.5 and 15 ppm groups. These groups were maintained on a control diet for 4 weeks following the end of the trial, and blood and brain ChE activities were determined after 2 and 4-weeks recovery.

Toxic signs at 135 ppm included tremors in all rats. Bodyweight gains were reduced at 135 ppm in both males and females, with weights decreased between 17 and 40% in comparison to controls. Three female rats in the high dose group died by the third week. Food intake was normal at 135 ppm. The average weights of liver and kidneys relative to body weight measured at the end of the study were significantly increased at 135 ppm. Haematology values were normal at all doses and there were no macro- or microscopic pathological changes. Incidental histopathological changes included a leiomyosarcoma in the uterus of one high-dose female, and bone marrow hypoplasia with associated haematopoiesis in the spleen were seen in another high-dose female. Individual animal pathology results were not reported.

Changes in whole blood and brain ChE activities are presented below.

Mean percentage inhibition of ChE activity.

Dose (ppm)	Plasma ChE		Erythrocyte ChE		Brain ChE	
	Males	Females	Males	Females	Males	Females
0.5	8	7	7	10		
1.5	49	50	36	36		
15	79	82	76	80		

It can be seen that there was significant ChE inhibition (both blood and brain from) 1.5 ppm in both males and females. The NOEL for ChE inhibition can therefore be set at 0.5 ppm (equal to 0.03 mg/kg bw/day).

Shellenberger TE (1966) Subacute toxicity and cholinesterase study of Shell Compound SD 13311 - Rat. SRI Project SS05908. Stanford Research Institute, Menlo Park.
and
Newell GW (1966) Letter Report No 1 Project B5908. Stanford Research Institute, Menlo Park.

The beta-D-glycosyl conjugate of N-hydroxymethylmonocrotophos (SD 13311) was fed to weanling Long-Evans rats (source not specified: 42 rats/sex/dose; except 90 ppm at 22 rats/sex) at doses of 0, 1 and 18, 3, 9 or 90 ppm (equivalent to 0, 0.1 and 1.8, 0.3, 0.9 or 9 mg/kg bw/d). The 1 and 18 ppm level involved feeding at 1 ppm for the first 7 weeks and at 18 ppm for the last 5 weeks. Rats were then maintained on control diets for 4 weeks to determine recovery. The LD50 for SD 13311 had previously been determined as 168 mg/kg bw.

Food and water were available *ad libitum*. Body weight and food consumption were determined weekly throughout the study. Blood was taken for haematological examination, including Hct, Hb determination, erythrocyte count,and total and differential leucocyte count from 4 rats/group at the beginning of the trial and at the end of week 4, 8 and 12. Whole blood ChE activity was determined at the end of week 1. After weeks 2, 4, 8 and 12, 5 rats/sex of the control, 1-18, 3 and 9 ppm groups were sacrificed and the whole blood and brain ChE activity was determined. After 2 and 4 weeks recovery, 5 rats/sex/group were sacrificed for determination of whole blood and brain ChE activity.

At the end of the 12-week feeding period, 12 rats/sex/group were euthanised and examined for any gross pathological changes. The kidney, liver, spleen and testes/ovaries were weighed. Whole blood and brain ChE activity were determined. The kidney, liver, spleen, testes/ovaries, skeletal muscle, femur, brain, stomach, small and large intestine, lung, prostate/uterus, lymph node, adrenal, pituitary, pancreas, thyroid, parathyroid, submaxillary, sublingual, Harderian and extraorbital lacrimal glands were preserved for histopathological examination. Tissues from the control and 90 ppm groups were examined.

Bodyweights were reduced in the 90 ppm group from week 7 until the end of the study (males 11-18%, females 10-14%). In females, bodyweight returned to normal by week 12. There were no abnormal haematological findings. No significant changes in relative organ weight were seen. Data on the inhibition of whole blood and brain ChE activities are presented below.

Mean percentage inhibition in ChE activity

Dose (ppm)	Plasma ChE		Erythrocyte ChE		Brain ChE	
	Males	Females	Males	Females	Males	Females
1	1.5	4	4	0		
3	8	7	7	5		
9	24	26	13	9		
18	56	62	36	39		
90	98	95	74	62		

There was significant inhibition of whole blood ChE activity at 9 ppm, and significant brain ChE activity inhibition at 18 ppm. Therefore, the NOEL for ChE inhibition can be set at 3 ppm (equivalent to 0.3 mg/kg bw/d). The blood ChE activity of all groups had recovered after a 4 week recovery period. Brain ChE of animals at 90 ppm was still inhibited after 4 weeks, although lower doses had returned to normal.

There were no abnormal finding on gross pathological examination. On histopathological examination, there were a number of instances of non specific pneumonitis, reactive hyperplasia in the lymph nodes, and myeloid hyperplasia. These findings were seen in both the control and 90 ppm treatment groups, and there were no abnormalities related to treatment. Therefore, the NOEL for histopathological effects was 90 ppm (equivalent to 9 mg/kg bw/d).

Overall, based on the ChE inhibition seen at 9 ppm, the NOEL for the study can be set at 3 ppm, equivalent to 0.3 mg/kg bw/d.

5.2 Dog

Shellenberger TE & Newell GW (1964e) Subacute toxicity and cholinesterase study of Shell Compound SD 9129 - Rat and dog. Techn. Report Part 1. Stanford Research Institute, Menlo Park..

Monocrotophos technical (SD 9129, source not specified) in corn oil was fed to dogs (source not specified) in the diet in two trials. The first, a dose ranging experiment, used doses of 0, 0.17, 0.5, 1.5, 4.5, 15, 45, 135 or 400 ppm in the diet (equivalent to 0, 0.004, 0.013, 0.038, 0.11, 0.38, 1.1, 3.4 or 10 mg/kg bw/d) using 2 dogs/group for 2 weeks. Dogs of mixed breeds were used for this trial. Body weights were obtained weekly. Plasma and erythrocyte ChE levels were determined pretest. After the 2-week trial, the dogs were euthanised and plasma, erythrocyte and brain ChE activities determined.

In the second trial monocrotophos was fed in the diet at doses of 0, 0.5, 1.5, 15, 45 or 135 ppm (equivalent to 0, 0.013, 0.038, 0.38, 1.1 or 3.4 mg/kg bw/d) to Beagle dogs (source not specified) using 4/sex/group, except in the 45 and 135 ppm groups in which 2 dogs/sex/group were used. After 8 weeks, the 135 ppm dose was increased to 270 ppm for weeks 9 and 10, to 540 ppm for weeks 11 and 12 and to 1080 ppm for week 13. As each dose increment was fed for a short time, and there was a very small number of animals, the significance of any changes observed in these high-dose animals was difficult to determine.

Hb, Hct, erythrocyte and total and differential white cell count were determined pretest and after weeks 4, 8 and 12. Plasma and erythrocyte ChE were determined pretest and after weeks 2, 4, 8, and 12 of feeding and weeks 1, 2 and 4 of the recovery period. Brain ChE activity was determined at the end of week 12 and at the end of a 4-week recovery period. AP and BUN were determined pretest and at weeks 4 and 10.

In the 2-week dose range-finding study, dogs receiving up to 45 ppm monocrotophos gained weight in a similar fashion to control animals. At 135 ppm, the animals failed to gain weight, while at 400 ppm the dogs lost an average of 800 g. There was no indication of the initial or final weights of the dogs, however the statement was made that they were extremely variable. No individual results were reported, however it was indicated that there was no plasma or erythrocyte ChE inhibition at 0.17 or 0.5 ppm, marginal inhibition at 1.5 ppm and marked inhibition at 4.5 ppm.

In the 12-week study, the 135 ppm group showed mild tremors on handling after 3 weeks. There was no decrease in body weight noted in the treated groups in the first 8 weeks of the study; following an increase in dose from 135 to 270 ppm there was some decreased weight gain, which continued as doses were increased. No alterations were observed in haematology, AP or BUN. Variations in spleen size were not treatment-related and reductions in average liver weights in males and females and in ovary weights in females at 135-1080 ppm were

thought to reflect decreased food intake. Histopathology was normal except for some isolated instances of gonadal changes, including Sertoli cell proliferation with occasional multinucleated cells in one high dose male, testicular atrophy in two males (one at 0.5 ppm and one at 45 ppm), and immature ovarian follicles, suggestive of maturation arrest, in 2 females. None of these effects were dose-related, and the findings were considered to be incidental.

ChE inhibition is presented below.

Mean percentage ChE inhibition

Dose (ppm)	Plasma ChE		Erythrocyte ChE		Brain ChE	
	Males	Females	Males	Females	Males	Females
0.5	7	5	3	10	0	4
1.5	14	19	18	20	19	20
15	36	33	44	49	43	56
45	51	54	60	65		
135	64	70	77	78		

There was significant inhibition of plasma, erythrocyte and brain ChE activity from 15 ppm, and the NOEL is therefore 1.5 ppm (equivalent to 0.038 mg/kg bw/day). All activities had recovered following 4 weeks on control diet. The overall NOEL for the study was 1.5 ppm, equivalent to 0.038 mg/kg bw/d, based on ChE inhibition.

Shellenberger TE (1965d) Subacute toxicity study of Shell Compound SD 9129 - Dog. Addendum to Tech. Report/Part 1. Stanford Research Institute, Menlo Park.
Monocrotophos technical (source not specified, Code 7-3-0-0) was fed in the diet to Beagle dogs at doses of 0, 0.5, 45 or 135 ppm (equivalent to 0, 0.01, 1.1 or 3.4 mg/kg bw/d) for 12 weeks. Dogs were offered 225 g of food daily; generally the entire amount was consumed. Dogs were weighed weekly, and their responses, behaviour and mortality was recorded. After 12 weeks, all dogs were euthanised, and a gross postmortem done. The liver, spleen, stomach, large and small intestine, kidney, pancreas, lymph node, adrenal gland, bone marrow and either testes and prostate or ovaries and uterus were preserved for histopathological examination.

The report stated that there were no significant changes in body weight during the study, however raw data was not supplied and this conclusion could not be verified. Tremors were seen in some animals on the high dose after 3 to 4 weeks; no abnormal clinical signs were seen in other treatment groups. One dog in the low-dose group died in the first 2 weeks of the study; the cause of death was determined to be acute pneumonitis.

Gross pathological examination revealed no abnormalities. On histopathological examination, one low-dose female showed signs of granulomatous arteritis in both kidneys, and a number of animals showed pyelitis or pyelonephritis. These signs were not considered to be related to treatment. Most males showed normal testicular development with normal spermatogenesis and no evidence of Sertoli cell hyperplasia. In most females there was normal follicular development, however no evidence of corpora lutea. As the animals were 11 - 13 months old at the time of death, it would be normal for ovulation to have occurred. However, as the finding was seen in both control and treated animals, it is not considered to be related to treatment.

6 CHRONIC TOXICITY

6.1 Mice

Brown VK (1982) A two year oncogenicity study in mice fed AZODRIN. Project No 194/82. Sponsor SICC/CSAA. Lab: Sittingbourne Research Centre, UK.

Monocrotophos (purity 78.7%, batch no 8-28-0-0, source: Shell Chem Co, Denver Colorado) was administered in the diet to CD mice (source: Shell Toxicology Laboratory Breeding Unit) at 0, 1, 2, 5 or 10 ppm (equivalent to 0.15, 0.3, 0.75 or 1.5 mg/kg bw/d) for 2 years, using 77 mice/sex/group. Fresh diets were prepared twice weekly. Interim kills were performed at 55 and 78 weeks, with the terminal kill at 104 weeks. Mice were housed individually under controlled conditions and food and water were available *ad libitum*.

Clinical signs were monitored daily, with any abnormalities recorded. Body weight and food intake were determined weekly for the first 13 weeks. From week 14, body weight were determined every 2 weeks and food intake determined monthly. Every 3 months, groups of mice from 0 and 10 ppm groups had an ophthalmoscopic examination.

Blood samples were obtained from animals scheduled for interim slaughter (at 55 and 78 weeks) and from all animals at the end of the study. Haematological examinations, including erythrocyte count, total and differential white cell count, Hb, Hct, MCV, MCH and MCHC were conducted, and plasma and erythrocyte ChE activity was determined. Gross necropsies were performed on all animals dying during the study, and on all scheduled sacrifices, and the brain, heart, liver, kidney and testes were weighed. The following tissues were preserved for histopathological examination: adrenals, brain, eyes, gall bladder, head (selected cases), heart, intestine, kidneys, lacrimal gland, larynx, liver, lung, lymph nodes, mammary gland, muscle, oesophagus, ovaries or testes and epididymis, pancreas, pituitary, salivary gland, sciatic nerves, seminal vesicles, spinal cord, spleen, stifle joint, stomach, thymus (if present), thyroids, tongue, trachea, urinary bladder and uterus or prostate. Additionally, any abnormalities seen on macroscopic examination were preserved for examination.

Clinical signs seen in all groups included poor condition, distended abdomen, skin sores and fur loss. These signs were not treatment related. A dose-related increase in the number of mice having stress-related convulsions was seen. Spontaneous convulsions had previously been noted in this strain of mice in this laboratory, and this effect was not investigated further. There was no treatment-related increase in mortality or changes in bodyweight gains or food intake. Haematology was normal and organ weights were not altered. Ophthalmology examinations did not reveal any treatment related abnormalities. Retinopathies present in both treated and control animals were suggested to be related to the light intensity the mice were exposed to, given that the frequency of occurrence could be related to housing position.

Mean percentage ChE inhibition is detailed below:

ChE inhibition (mean percentage)

Dose (ppm)	Plasma ChE		Erythrocyte ChE		Brain ChE	
	Males	Females	Males	Females	Males	Females
1	31	26	15	24	23	18
2	38	39	31	18	35	31
5	59	63	43	48	53	49
10	74	72	69	58	68	60

It can be seen that there was significant plasma ChE inhibition in both males and females from 1 ppm, the lowest dose tested. Erythrocyte ChE inhibition was seen in males from 2 ppm and in females from 1 ppm. Inhibition of brain ChE was seen in males from 1 ppm and in females from 2 ppm. Therefore it was not possible to set an NOEL for ChE inhibition, based on the effects seen in plasma in both sexes and in brain in males at 1 ppm (equivalent to 0.15 mg/kg bw/d).

Gross and histopathological examination did not reveal any significant treatment related findings. Although there was a higher incidence of pulmonary tumours relative to controls in males fed monocrotophos for 78 weeks (1/30, 4/15, 3/15, 2/15, 5/15), this increase was not seen at the terminal sacrifice (34/40, 15/22, 12/20, 9/19, 11/19), and was not seen in females at any examination time. The apparent increase in the incidence of pulmonary tumours at the 78 week sacrifice would appear to be due to the low incidence in control animals, and was therefore not considered significant. Overall the incidence of non-neoplastic lesions and tumours in control and treated groups was similar.

Overall, based on the effects seen in plasma and brain ChE at the lowest dose tested, no NOEL could be established for this study. The LOEL, based on plasma and brain ChE was established at 2 ppm (equivalent to 0.3 mg/kg bw/d).

6.2 Rat

Johnston CD (1966) AZODRIN. Safety evaluation by chronic feeding study in the rat and the dog for two years. Interim report: 52 weeks. Lab: Woodard Research Corporation. Sponsor: Shell Development Company
and
Johnston CD, Howard DH & Donoso J (1967b) AZODRIN safety evaluation by a chronic feeding study in the rat for two years. Final Report. Lab: Woodard Research Corporation. Sponsor: Shell Development Company.
Monocrotophos (purity, source not given; batch 7-3-4-16) was administered to Charles River rats (Charles River Breeding Laboratories) in the diet at doses of 0, 1, 10 or 100 ppm (nominally equivalent to 0, 0.05, 0.5 or 5 mg/kg bw/d) for 104 weeks, using 25 rats/sex/group in treated groups (40 rats/sex in control). Fresh diets were prepared weekly. Analysis of diets at 15 and 40 weeks showed degradation of monocrotophos of up to 60%, therefore actual concentrations were much lower than nominal concentrations. Animals were housed individually and food and water were available *ad libitum*.

Rats were observed daily for any change in general condition or behaviour, and were examined and weighed weekly. Food consumption was also determined at the weekly examination. Haematological examination, with Hb, Hct and total and differential leucocyte counts were done at weeks 6, 13, 19, 26, 39, 52, 78, 91 and 104 for 5/sex in control and high dose group. Plasma and erythrocyte ChE were determined at weeks 6, 13, 26, 52, 78 and 104 for 5/sex in each treatment group and 10/sex in control.

Toxic signs, including tremors and diarrhoea, were observed at 100 ppm. Bodyweight gain was reduced at 100 ppm throughout the study in both sexes and food intake was reduced in males only. Absolute weights of liver, gonads, thyroid and pituitary glands were reduced in females at 100 ppm. Haematology was normal. There were no macroscopic or microscopic pathological changes due to treatment, except for decreased frequency of hepatic vacuolation at 100 ppm (22/48, 19/26, 11/20, 4/18) and an increase in the occurrence of degenerative and/or chronic

inflammatory changes in one or both eyes at 100 ppm (0/80, 0/50, 1/50, 4/50). The incidence of neoplasms was similar in control and treated groups.

The mean inhibition of ChE throughout the study is presented below.

Mean percentage ChE inhibition

Dose (ppm)	Plasma ChE		Erythrocyte ChE		Brain ChE	
	Males	Females	Males	Females	Males	Females
1	25	18	18	18	32	34
10	68	68	69	76	50	36
100	84	93	96	96	82	75

It can be seen that plasma ChE was significantly inhibited in males from 1 ppm, and in females from 10 ppm. Erythrocyte ChE was significantly inhibited in both sexes from 10 ppm, while brain ChE was significantly inhibited from 1 ppm. Based on the effects seen on brain ChE activity and on plasma ChE in males at the lowest dose tested, no NOEL can be established for this study. The LOEL is 1 ppm (equivalent to a nominal concentration 0.05 mg/kg bw/d), based on plasma and brain ChE effects.

Brown, VK (1983) A long-term feeding study with AZODRIN in rats to investigate chronic toxicity and oncogenicity (6, 12 and 24 month necropsies) Lab: Shell Research Ltd, Sittingbourne. SBGR.82.062

Monocrotophos (purity 78.7%, batch no 8-28-0-0, source: Shell Chemical Co, Denver) was fed at 0,0.01, 0.03 0.1, 1 or 10 ppm (equivalent to 0, 0.0005, 0.0015, 0.005, 0.05 or 0.5 mg/kg bw/d) in the diet to Wistar rats (Shell Toxicology Laboratory Breeding Unit) for 2 years, using 85 rats/sex/group (170 rats/sex in the control group). Dose levels were determined from previous studies (not specified). Rats were housed individually under controlled conditions, with food and water available *ad libitum*.

Clinical observations were made on all animals twice daily during the week, and once daily on weekends and public holidays. Bodyweight and food intake was determined weekly for the first 14 weeks, then fortnightly for the rest of the study. Interim necropsies were done at 6, 12 and 18 months. Urine samples were taken from rats scheduled for interim autopsy approximately 3 weeks prior to scheduled necropsy. Blood samples were taken 2 weeks prior to scheduled sacrifice, and protein, BUN, AP, glucose, chloride, calcium, potassium, sodium, LDH, AST, ALT, cholesterol and bilirubin levels determined. Additionally, haematological examination including Hb, erythrocyte and leucocyte (total and differential) counts, Hct, MCH, MCV, reticulocytes and prothrombin time were performed. ChE activity in plasma, erythrocyte and brain were also determined.

Detailed necropsies were performed on all animals. Major organs (not specified) were weighed and a range of tissues were examined histologically from all animals at scheduled necropsies and from those which died or were killed prior to the scheduled dates.

Clinical signs observed included piloerection, poor condition, blood around the nose, sore hocks and abnormal gaits, but these signs were seen in all groups and thus were not related to treatment. Abnormal gaits were noted in one control and one high dose animal after 210 days. In the latter parts of the study between 1 and 4 animals per group were affected, with onset generally occurring after 600 days of treatment. Bodyweight gains were statistically

significantly reduced in males at 10 ppm, however as the difference in comparison to controls was less than 10% these were not considered biologically significant. Mortalities were slightly increased in the high-dose group, particularly in females, however this was not statistically significant. Haematology, clinical chemistry (with the exception of ChE inhibition) and urinalysis revealed no consistent treatment-related changes. Ophthalmoscopic examinations showed no abnormalities. Therefore the NOEL based on body weight, clinical chemistry, and haematological examination was 10 ppm (equivalent to 0.5 mg/kg bw/day).

ChE inhibition is presented in the table below:

ChE inhibition (mean percentage)

Dose (ppm)	Plasma ChE		Erythrocyte ChE		Brain ChE	
	Males	Females	Males	Females	Males	Females
0.01	1	0	2	4	1	1
0.03	2	1	10	5	1	2
0.1	2	3	5	8	3	4
1.0	22	28	41	34	28	26
10	62	76	78	83	75	74

It can be seen that significant ChE inhibition (plasma, erythrocyte and brain) occurred at 1.0 ppm, thus the NOEL for ChE inhibition was 0.1 ppm, equivalent to 0.005 mg/kg bw/d.

There were no treatment-related macroscopic changes observed at any of the scheduled necropsies. On histopathological examination, there was a high incidence of pituitary adenomas, with no dose relationship seen. There was a slight increase in the incidence of malignant gliomas in male rats (2/100, 1/50, 1/50, 0/50, 3/50 and 2/50), however the numbers were small and there was no clear dose relationship.

The incidence of neurological findings is presented in the table below.

Percentage incidence of neurological findings in animals surviving to study completion

Observed signs	0 ppm	0.01 ppm	0.03 ppm	0.1 ppm	1 ppm	10 ppm
Spinal cord fibre degeneration - males	12%	21%	17%	26%	20%	29%
Spinal cord fibre degeneration - females	14%	44%	25%	9%	15%	31%
Peripheral nerve fibre degeneration - males	92%	71%	83%	86%	80%	85%
Peripheral nerve fibre degeneration - females	82%	89%	96%	77%	85%	94%

There was no clear dose-relationship for either of these findings, although the incidence of spinal cord fibre degeneration in all treated males was higher than that in controls. Observations earlier in the trial, at 6, 12 and 18 months on sciatic nerves indicated that there was no dose related findings at these times, and there was no evidence of acceleration of degeneration based on dosing with monocrotophos. Given that the observed findings in the

central and peripheral nerves were not linked with any abnormal clinical findings, there is no evidence clearly linking the finding with monocrotophos treatment.

Overall, the NOEL for the study was 0.1 ppm (equivalent to 0.005 mg/kg bw/d) based on ChE inhibition seen at 1.0 ppm.

6.3 Dog

Johnston CD (1966) AZODRIN. Safety evaluation by chronic feeding study in the rat and the dog for two years. Interim report: 52 weeks. Lab: Woodard Research Corporation. Sponsor: Shell Development Company
and
Johnston CD, Thompson WM & Donoso J(1967b) AZODRIN safety evaluation by a chronic feeding study in the dog for two years. Final Report. Lab: Woodard Research Corporation. Sponsor: Shell Development Company.

Monocrotophos technical was fed to purebred Beagle dogs (Richard E Saunders Corporation, Virginia) at doses of 0, 0.16, 1.6 or 16 ppm (nominally equivalent to 0, 0.004, 0.04 or 0.4 mg/kg bw/d) for 104 weeks, 3/sex/group in treated groups, and 4/sex as controls.. After 52 weeks, another group of 2/sex commenced receiving 100 ppm (equivalent to 2.5 mg/kg bw/d) for 54 weeks. Diets were prepared freshly once a week; from week 47 fortified diets were stored at 2°C. Analysis of diets at 15 and 40 weeks showed deviations of up to 60% in the concentration of monocrotophos.

Dogs were examined daily for clinical signs and behaviour. Body weight and condition were determined weekly. Electrocardiograms, heart rates, blood pressure and ophthalmic examinations were done at 0, 6, 13, 26, 39, 52, 84, 85 and 104 weeks; the group which commenced treatment at 52 weeks had these examination at the same time as the original groups of dogs. Haemograms, including determination of Hb, Hct, sedimentation rate, thrombocyte counts and total and differential leucocyte counts were done at weeks 0, 6, 14, 18, 26, 40. 53, 78, 93 and 104, with the dogs commencing treatment at week 52 having examination at the same time as dogs already on the study. Clinical chemistry examinations were also done at this time, with levels of BUN, blood glucose, AP, AST and ALT being determined. Additionally plasma and erythrocyte ChE levels were determined, and qualitative urinalyses was performed. At the end of the study, a gross autopsy was done on all dogs, and the heart, liver, kidneys, spleen, lungs, brain, gonads, adrenals, thyroid, pituitary, prostate/uterus, bone marrow, pancreas, urinary bladder, trachea, salivary gland, stomach, mammary gland, peripheral nerve, oesophagus, thymus, small intestine, large intestine, spinal cord, skin, gall bladder, aorta, lymph node, skeletal muscle and eye preserved from controls, the 100 ppm (54 week) group, and the 16 ppm (full term) group. Selected organs were preserved from the low- and mid-dose groups.

Clinical signs were seen in the dogs on 100 ppm in the first 8 weeks of treatment. These animals showed tremors, salivation and constricted pupils. A number of these animals also had soft stools or diarrhoea during this time. There were no treatment-related effects on growth, mortality, haematology, clinical chemistry parameters (except ChE), urinalysis parameters or physiological measurements including ophthalmoscopy. Macroscopic and microscopic pathology was normal.

Cholinesterase inhibition data are presented below. It should be noted that the brain ChE assay was performed twice; on the first occasion there were technical flaws in the assay, and these results were not considered in determining the mean percentage ChE inhibition.

Mean percentage ChE inhibition

Dose (ppm)	Plasma ChE		Erythrocyte ChE		Brain ChE	
	Males	Females	Males	Females	Males	Females
0.16	0	7	6	0	0	12
1.6	0	9	5	6	0	36
16	39	28	39	35	22	45
100	28	46	93	94	48	40

It can be seen that plasma and erythrocyte ChE was inhibited at 16 ppm. Brain ChE was inhibited in females, but not males, at 1.6 ppm. Therefore the NOEL based on plasma and erythrocyte ChE inhibition can be established at 1.6 ppm (equivalent to 0.04 mg/kg bw/d). Overall, the NOEL for the study is 0.16 ppm (equivalent to 0.004 mg/kg bw/day), based on inhibition of brain ChE in female dogs. The exceptionally poor stability of monocrotophos in the diet of the 2 year study thus giving only nominal dietary concentrations further complicates the setting of a NOEL for this end point.

7 REPRODUCTION STUDIES

7.1 Mouse

7.1.1 Mouse Sperm Morphology Assay

Vijaya Kumar D & Janardhan A (1988) Mutagenicity of monocrotophos in mice. Bull Environ Contam Toxicol 41: 189-194

Technical monocrotophos (purity 98%; batch number not reported; source: National Organic Chemical Industries Ltd, Bombay) was administered to male Swiss albino mice (10/group) by gavage at 0, 0.9, 1.8 or 3.6 mg/kg bw. Doses used were claimed to be 1/20, 1/10 and 1/5 that of the LD50 and all were reduced by 4/5 (ie 0, 0.18, 0.36 or 0.72 mg/kg bw/day) for subsequent daily administration over the next 5 days. Mice were sacrificed 35 days after the first dose and caudae epididymides sperm examined for abnormal morphology. Histopathology was limited to the examination of one testis from each mouse.

Apart from the absence of any histopathological changes accompanying a dose-related increase in the number of abnormal sperm, no other signs were reported. The percentage of abnormal forms increased from 2.1% in controls to 2.1%, 3.6% and 5.4% for the low-, mid- and high-dose treatments. Statistical significance ($p<0.01$) was achieved at the mid and high dose. The investigators asserted that the presence of abnormal forms of sperm indicated DNA damage, however, no evidence was provided to demonstrate such a linkage.

7.2 Rat

7.2.1 One-Generation Oral Gavage Study

Adilaxmamma K, Janardha A & Reddy KS (1994) Monocrotophos: Reproductive toxicity in rats. Ind J Pharmacol 26: 126-129

Technical monocrotophos, synthesized and supplied by the Indian Institute of Chemical Technology, Hyderabad (80% purity, batch not stated), was administered by gavage to groups of 10 female Wistar rats (140-170 g; National Institute of Nutrition, Hyderabad) at 0, 0.3, 0.6 or 1.2 mg/kg bw/d for 2 weeks prior to mating and continued throughout gestation, parturition and lactation. Dose selection was based on 1/40, 1/20 and 1/10 of the oral LD50 (12 mg/kg bw) dose. Three female rats were mated with an untreated male (usually overnight) until evidence of vaginal spermatozoa was observed (day 0).

Rats were observed at regular intervals throughout the study for deaths, clinical signs, bodyweight, and food consumption. Maternal reproductive parameters determined were fertility (no. pregnant/no. mated) and parturition (no. pups delivered/no. pregnant) indices. Offspring were assessed for live to dead pup ratio, viability (live pups at day 4/total delivered), birth weight, litter size, crown-rump (CR) length, and survival (live pups at day 21/live pups at day 4). Gross pathological examinations were performed on all rats that died during treatment and after weaning. Only ovaries were examined histopathologically.

One pregnant rat from the highest dose group died on day 17 of gestation of an unspecified cause. Necropsy revealed 9 normal fetuses, 3 resorptions and fibrotic ovaries with attritic follicles and marked interfollicular fibrosis. Apart from 1 dam at 0.3 mg/kg bw/day and another at 0.6 mg/kg bw/day with a haemorrhagic vaginal discharge at day 14 of gestation, no other

clinical signs were reported. Necropsy of these 2 dams revealed resorption of fetuses (though completeness not specified).

Dams apparently had a dose-related decline in bodyweight throughout treatment (data not shown) and although fertility and parturition indices were slightly, though not significantly reduced (~10%) among all treated dams, pup birthweight, crown-rump length, viability, and survival throughout lactation were more markedly reduced. At the highest dose of 1.2 mg/kg bw/day, birth weight and size (CR length) were significantly ($p<0.05$) reduced (12% and 6% respectively) relative to control, the viability index was reduced to 3% on day 4 post partum, and no pups survived to day 21. At 0.3 and 0.6 mg/kg bw/day the viability index (at day 4) was reduced to 72% and 65% respectively relative to a control value of 90%. Survival during lactation was reduced from 84% in controls to 80% and 38% at 0.3 and 0.6 mg/kg bw/day respectively, suggesting monocrotophos and/or metabolite excretion in milk. One pup (1/70) at 0.3 mg/kg bw/day had anophthalmia, 2 dams at 0.3 mg/kg bw/day had enlarged haemorrhagic ovaries but ovaries from high-dose rats (1.2 mg/kg bw/day) were small.

This study is not suitable for regulatory purposes because it does not provide sufficient detail for maternal bodyweight or litter data.

7.2.2 Two-Generation Rat Study

Dix KM (1981) Reproduction study in rats fed AZODRIN. Sittingbourne, Shell Research Ltd SBGR.81.143

Technical grade monocrotophos (78.7% purity, batch no 8-28-0-0, source: Shell Chemical Co, Denver Colorado) was fed in the diet at 0, 0.1, 1, 3 or 10 ppm (equivalent to 0, 0.005, 0.05, 0.15 or 0.5 mg/kg bw/d) to SPF Wistar rats (Shell Toxicology Laboratory Breeding Unit) using 13 males and 26 females per group. Fresh diet was given twice a week, and food and water were available *ad libitum*. Reproductive effects were studied in two consecutive generations (F0, F1). Both generations were bred to produce one litter.

Rats were examined daily, and any abnormal clinical signs recorded. Body weights were determined for F0 animals aged 5, 8, 12, 16 and 20 weeks, and for F1 animals aged 3, 4, 8, 12, 16 and 19 weeks. At 20 weeks of age, following 15 weeks of treatment, males were housed with 2 treated females from the same treatment group. The day of detection of coitus (a vaginal smear positive for sperm) was taken as day 0 of gestation. If there was no evidence by day 7, the male was replaced with a proven breeder from the same treatment group for an additional 7 days. Male rats were sent for necropsy in the week following pairing. Females presumed unmated were kept for up to 5 weeks. Litter observations included gestation length, number of pups born alive, number and sex of pups born dead, number and sex of pups dying during lactation, number and sex of pups weaned, individual litter weights at days 1, 4, 7, 14 and 21, individual pup weights at days 4,7, 14 and 21 and the general condition of pups. Gross and histopathological examination of 5 pups/sex/group was done at 21 days. At this time, 13 males and 26 females were randomly selected from each treatment group to form the F1 population. These animals were exposed to monocrotophos in the diet for 18 weeks prior to mating. At weaning of the F2, 5 rats/sex/group were selected for gross and histopathological examination, and the rest were discarded.

Gross and histopathological examination of the following tissues from F1 adults and F1 and F2 weanlings was conducted: brain, pituitary, eyes, nasal cavity, salivary gland, lymph nodes (mesenteric and submaxillary) thyroids and parathyroids, larynx, trachea, oesophagus, thymus,

mammary glands, heart, lungs, liver, spleen, pancreas, stomach, small and large intestine, adrenals, kidneys, tongue, urinary bladder, prostate, testes, ovaries, uterus, fallopian tubes, epididymides, seminal vesicles, skin, skeletal muscle, bone marrow, spinal cord, peripheral nerve and any grossly abnormal tissues. Gross examination of the above organs was done on F0 adults; any grossly abnormal tissue was examined histopathologically.

Statistically significant decreases in body weights were seen at 10 ppm, during weeks 3, 7 and 15 in F0 males and from weeks 8 to 19 in the F1 males. F1 females aged 4 weeks on 10 ppm also showed a statistically significant decrease in body weight in comparison to controls. None of the decreases noted were greater than 10%, and therefore they are considered to be of limited biological significance. Clinical signs seen in all treatment groups included fur loss, tail damage and skin sores. In the F0 generation, 2 females at 10 ppm showed poor teat development; in the F1 generation, the frequency of poor teat development increased (0/26, 0/26, 0/26, 1/26, 8/26). Small dark faecal pellets were observed in 11/13 males and 23/26 females in the F0 group.

There were no changes in sperm head counts in F0 or F1 parent males. At 10 ppm, gestation length was statistically significantly increased in F0 and F1 females. In F0 females, approximately 75% had gestation lengths of 23 or 24 days, in comparison to control females where more than 80% had a gestation length of 22 days. In F1 females, 90% of controls had a gestation length of 22 days while approximately 70% of high dose females had a gestation length of 23 days. At 10 ppm the mean litter size of F1 pups at birth and the F1 male mating index were both statistically significantly smaller, with the mean litter size of the high dose group being 7.5 pups, whereas control size was 10.9 pups. Pre-weaning losses were higher for the F1 and F2 pups at 10 ppm with 37% losses in the F1 and 48% losses in the F2 between birth and weaning. These losses may be due to poor mammary development and lactation in dams in the higher dose groups. Mean litterweights were reduced at 10 ppm.

There were no treatment-related structural abnormalities in dead or weanling pups, or in F1 adults. There were no significant gross histopathological findings, apart from the poor mammary development in a few treated dams and unilateral keratitis and corneal ulceration in the eyes of one 3 ppm and one 10 ppm male. No adverse effects on reproduction were observed in F0 generation rats exposed to 3, 1 or 0.1 ppm monocrotophos or in F1 rats exposed to 1 or 0.1 ppm.

Therefore the NOEL for reproductive and maternal effects was 1 ppm (equivalent to 0.05 mg/kg bw/d), based on the effects on teat development in females, and on decreased pup survival seen at 3 ppm.

7.2.3 Three-Generation Study in Rat

Eisenlord G & Loquvam GS (1965) Results of short route reproduction study of rats fed diets containing SD 9129 insecticide over three generations. Lab: Hine Laboratories, San Francisco, Sponsor: Shell Development Company

Long-Evans rats (Simonsen Laboratories) were fed technical grade monocrotophos (SD9129, source, purity not given) in the diet at nominal concentrations of 0, 2, 5, 12 or 30 ppm (equivalent to 0.1, 0.25, 0.6 or 1.5 mg/kg bw/d) for 3 generations using 10 rats/sex/group. Diets were mixed and stored refrigerated. Food and water were available *ad libitum*. Females were housed individually, while males were housed in groups of five. Females were mated for 2 weeks, with males rotated once during that time, ie. they serviced two groups of females. The F0 rats were mated when they were 100-days old, after 79 days exposure to the compound.

Randomly selected pups from the first litter were maintained on the diets and mated when 100 days old. Reproductive effects were studied in 3 consecutive generations (F1, F2, F3), each of which was bred to produce one litter.

The number of live pups per litter was determined on the day of birth and on day 5. Litters with more than 10 pups were reduced to 10 on day 5. On day 21, weanlings were counted and weighed, and either euthanised or saved for the next generation. Litter size, mortality of pups and parental weight were determined. Adults rats were weighed, euthanised and a gross necropsy performed. Ten weanlings/sex of the F3 control and 12 ppm, and 5 weanlings/sex of the F3 2 and 5 ppm were selected for examination (all 30 ppm F2 pups died before weaning, and this level could not be continued). Individual body weights, brain, liver and kidney weights were determined. Sections of brain, heart, lung, liver, spleen, pancreas, kidney and testes were preserved for histological examination.

No treatment-related clinical signs were observed at any dose levels. Thinned or missing hair on head, stomach or flanks was seen occasionally in adult females and pups in all treatment groups.

In F0 adults, the bodyweight of females at 30 ppm was statistically significantly decreased ($p<0.01$) at terminal sacrifice, however as the difference was less than 10% it was considered to be of limited biological significance. In the F1 adults, males in both the 12 and 30 ppm, and females in the 30 ppm group had bodyweights significantly lower ($p<0.01$) than controls. In all of these groups, the decrease was greater than 10% and may be considered of biological significance. No significant differences in the F2 adults was found. Based on the decreases in bodyweight, the NOEL can be set at 5 ppm.

Pregnancy rates varied between 80 and 100%, with the exception of F1 dams at 30 ppm, which had a lower incidence of 70%. At 30 ppm pup mortality in the first 2 generations was so high that the experiment was discontinued at this dose after the second generation. The mean litter size was not affected by dose in the F1 generation. In the F2 generation, all treatment groups produced litters significantly reduced in number, with the difference being $p<0.05$ for all groups except the 30 ppm group, which had a significance of $p<0.01$. The mean litter sizes in this generation were 9.3, 7.7, 8.4, 8.3 and 6.4. There is therefore not a clear dose relationship. In the F3 generation, there were no significant differences in litter size between treated and control groups.

Pup survival was significantly ($p<0.01$) decreased at 30 ppm in the first two generations and pup numbers were not sufficient to proceed to a third generation in this group. At 12 ppm, pup survival was significantly decrease in all generations, while at 5 ppm there were significant decreases in the second and third generations. F1a weanlings at 30 ppm showed stunted growth and were emaciated. Pup weights were not altered at 2, 5 or 12 ppm. No abnormalities were observed by gross or microscopic pathological examination of the parental generation or the third generation weanlings. Based on the effects on pup survival seen in the third generation at 5 ppm, the NOEL for reproductive effects can be set at 2 ppm, equivalent to 0.1 mg/kg bw/d. This is lower than the dose at which effects are seen in the adults (5 ppm for bodyweight effects, equivalent to 0.25 mg/kg bw/d).

Eisenlord G, Loquvam GS (1966) Results of long route reproduction study of rats fed diets containing SD 9129 insecticide over three generations. Lab: Hine Laboratories, San Francisco, Sponsor: Shell Development Company

Long-Evans rats (Simonsen Laboratories) were fed technical grade monocrotophos (SD9129, source, purity not given) in the diet at nominal concentrations of 0, 2, 5, 12 or 30 ppm (equivalent to 0, 0.1, 0.25, 0.6 or 1.5 mg/kg bw/day) for 3 generations (10 rats/sex/group). Diets were mixed and stored refrigerated. Food and water were available *ad libitum*. Females were housed individually, while males were housed in groups of five. Females were mated for 2 weeks, with males rotated once during that time, ie. they serviced two groups of females. The F0 rats were mated when they were 100 days old, after 79 days exposure to the compound. All pups from the first litter were discarded at weaning, and the parent rats were mated again 10 days later. Randomly selected pups from the second litter were maintained on the diets and mated when 100 days old. Reproductive effects were studied in 3 consecutive generations (F1b, F2b, F3b), each of which was bred to produce 2 litters.

The number of live pups per litter was determined on the day of birth and on day 5. Litters with more than 10 pups were reduced to 10 on day 5. On day 21, weanlings were counted and weighed. First litter (F1a, F2a) weanling were euthanised, and the second litter (F1b, F2b) either euthanised or maintained for mating to produce the next generation. Litter size, mortality of pups and parental weight were determined. Adults rats were weighed, euthanised and a gross necropsy performed. Ten rats/sex of the F3 control and 12 ppm weanlings, and 5 rats/sex of the F3 2 and 5 ppm weanlings were selected for examination. Individual body weights, brain, liver and kidney weights were determined. Sections of brain, heart, lung, liver, spleen, pancreas, kidney and testes were preserved for histological examination.

In parent rats, bodyweights were significantly ($p<0.05$) reduced in F0 females at 30 ppm. The reduction was not considered to be of biological significance, as it was less than 10% less than control values. F1b females at 12 ppm (highest dose at F1 generation) showed a significant decrease ($p<0.05$), however this was also of limited biological significance, as it was less than 10% different from controls. Weights of F2b males were reduced at 12 ppm. This decrease was statistically significant ($p<0.05$) and of biological significance. Thinned or missing hair was seen occasionally at all dose groups in females.

Litter sizes were not significantly different between treated and control groups in either the F1a or F1b generation. Pup mortality at 30 ppm was so high that this dose level was discontinued beyond the first generation. Litter sizes were significantly reduced in the 5 and 12 ppm treatment groups in the F2a generation (mean litter sizes; 10.5, 9.4, 8.1, 8.8), however no other changes in litter size were seen. Pup mortality was significantly increased in the 12 ppm group in the F1b, F2a and F2b litters. Mortality was also increased in the 30 ppm group in both the F1a and F1b litters. Pup mortality was also increased significantly in the 5 ppm group in the F3b litter. Average litter weights were reduced among F3a weanlings at 12 ppm and among F1a weanlings at 30 ppm. Pup weights were reduced at 12 ppm in F3a weanlings and at 30 ppm in F1a weanlings. Histological and gross examination showed no treatment-related abnormalities in F3b weanlings. As noted, the NOEL for reproductive effects was therefore 2 ppm in the diet, equivalent to 0.1 mg/kg bw/d, and the NOEL for maternal effects was 5 ppm, equivalent to 0.25 mg/kg bw/d.

8 DEVELOPMENTAL STUDIES

8.1 Gavage Teratology Studies

8.1.1 Rat

Fuchs A (1992) Final Report C1414 tech Oral (gavage) teratogenicity study in the rat. Hazleton Deutschland Gmbh 23 HD Project No. 380-195 HD Report No. 1049-380-195 Ciba Geigy Study No. 92 2077 GLP:OECD/USEPA

Monocrotophos technical (purity 77.6%, batch: OP 107001, source: Ciba Giegy Ltd) in distilled water was administered by gavage to Sprague-Dawley Crl(SD) BR (SPF) rats at doses of 0, 0.1, 0.3, l.0 or 2 mg/kg bw/d on days 6 to 15 of gestation using 25 females/group. Doses were selected on the basis of a previous embryotoxicity study, and the concentration of the monocrotophos was checked in the first and last weeks of treatment. Rats were sacrificed on day 20, where day 0 was the first day of gestation, determined by the presence of sperm in the vagina and/or a vaginal plug. Females were housed individually during the treatment phase, with food and water available *ad libitum*. Animals were checked at least once daily for clinical signs and morbidity/mortality. Body weight and food consumption were determined on days 0, 6, 9, 12, 16 and 20 of gestation. A gross pathological examination of dams and fetuses was done on all animals. The ovaries and uteri of the dams were removed, and the following recorded: number of corpora lutei in each ovary, number and position of implantations, number of live and dead foetuses, and early and late resorptions. The uteri of apparently non-pregnant females were immersed in ammonium sulphide to show any implantation sites. Foetuses were examined for any external abnormalities, and individual foetal weight and sex were determined. Approximately half of the foetuses were examined for visceral abnormalities, while the rest were examined for skeletal abnormalities. Dead foetuses were examined as far as possible. Deviations were classed as either malformations (rare and/or probably lethal) or variations (changes seen relatively frequently in controls, or not of functional significance).

There were no maternal deaths during the study. Clinical observations of maternal animals that were related to treatment with monocrotophos included a dose-related increased incidence of tremors from 1 mg/kg bw/d and increased startle reflex at 2 mg/kg bw/d. Statistically significant decreases in maternal bodyweight gains occurred during the exposure period at 1 mg/kg bw/d (day 6 to 9 post coitus) and at 2 mg/kg bw/d (day 6 to 12 post coitus), however the decreases were only 8% in comparison to controls, and are therefore not considered of biological significance. At 2 mg/kg/d, food consumption was very significantly decreased, with consumption only 20% of that of control animals.

Pregnancy rates in all groups were comparable. Pre-implantation losses were comparable to controls and post-implantation losses were not affected by treatment. There were no effects on the number of live and dead fetuses/litter, early and late resorptions or foetal bodyweights attributed to treatment. The proportion of male foetuses was decreased in the two highest dose groups. This was statistically significant ($p<0.05$) at 1 mg/kg bw/d, but not significant at 2 mg/kg bw/d. The percentage of males seen in each group was 56.4, 53.5, 52.4, 44.6 and 46.5%. Therefore there appears to be a dose-related decrease in the percentage of male foetuses seen.

Gross pathological examinations of maternal animals did not reveal any treatment-related effects. Gross pathological examination of foetuses did not reveal any external or visceral

abnormalities that were treatment-related. Increased foetal and litter incidences of incomplete ossification of nasal (foetal/litter incidences (%) C: 0/0, 2.0 mg/kg; 2.23/13.6) and frontal (foetal/litter incidences (%) C: 6.3/14.3, 2.0 mg/kg; 7.46/27.3) bones at 2 mg/kg/d, were likely to be the result of delayed development, which may have been a consequence of maternotoxicity.

The NOEL for maternotoxicity was 0.3 mg/kg bw/d, based on the occurrence of clinical signs at 1 mg/kg bw/d and for foetotoxicity was 1 mg/kg bw/d, based on the delayed ossification seen at 2 mg/kg bw/d.

Lu CC (1984) Technical AZODRIN (SD 9129) teratology study in SD CD rats. Lab: ToxiGenics Ltd Sponsor: Shell Development Company Report WRC RIR-335 GLP - FDA

Monocrotophos technical (WRC Tox Sample No. 55F, purity not given, supplier: Shell Development Company) was administered by gavage to pregnant female Charles River Crl:CD SD(BR) albino rats (source not specified) at doses of 0, 0.3, 1.0 or 2.0 mg/kg bw/d during days 6 to 15 of gestation inclusive. Dosing was based on an earlier dose-ranging study. Either 24 or 25 rats/group were pregnant following confirmed mating. Rats were housed individually, and food and water were available *ad libitum*. Animals were observed at least twice daily for mortality, morbidity or overt signs of toxicity. Females found dead were autopsied, and maternal tissues were stored in formalin as required, along with any foetal tissue obtained. Each rat was examined fully once weekly. Rats were weighed on gestation days 0, 6, 12, 15 and 20. Food consumption was not measured.

All females were killed on gestation day 20. The uterus was removed, weighed and examined to determine the number of implantation sites, resorption sites and foetuses. Resorption sites were classified as early or late, based on foetal structure present. Foetuses were classified as viable or dead. The number of corpora lutea were also recorded. The thoracic and abdominal organs of each female were examined for gross morphological changes. Abnormal tissues were retained for later histopathological examination, and uteri from non pregnant females were placed in ammonium sulfide solution for confirmation of pregnancy status.

Foetuses were removed from the chorion, examined for external developmental anomalies, sexed, weighed and measured. Half of the foetuses from each litter were examined for skeletal abnormalities, and the other half were examined for visceral abnormalities.

Clinical signs of monocrotophos toxicity were seen in all females at 2.0 mg/kg bw/d. These signs included muscle tremors, twitching, staggering gait, salivation and listlessness. Additionally, these animals showed signs of urine staining in the perianal region, crusty eyes and muzzle and lacrimation. There were no specific-treatment related signs in the other groups, although there were isolated occurrences of alopecia in all groups. Maternal body weight was decreased in the high dose group from day 15. As the decrease was greater than 10% it was considered of biological significance. A small statistically significant decrease in the mid dose group was not considered of biological significance.

There were no treatment-related effects in the number of corpora lutea, implantation sites, resorptions (either early or late) or the number of viable foetuses. Mean foetal body weight and crown-rump length were reduced at 2 mg/kg/d. Foetal sex ratios were not altered in treated groups. The percentage of runted foetuses at 1 and 2 mg/kg/day was significantly higher than controls. Delayed ossification of sternebrae was observed at 2 mg/kg/d, however the incidence was within the range previously seen in historical controls in the laboratory. Foetal visceral

examination did not show changes from controls. External foetal examination did not show any significant treatment-related effects. A low incidence of brain malformations was not treatment-related since it was present to a similar extent in control and treated groups.

The fetotoxic effects were considered to be a consequence of maternal toxicity. The NOEL can be set at 1 mg/kg bw/d, based on maternal toxicity (clinical signs and weight loss) and fetotoxic effects (decreased crown-rump length and mean body weight).

8.2 Rabbit

Christian MS, Hoberman AM & Dearlove GE (1987) Developmental Toxicity study of AZODRIN insecticide (technical) in New Zealand White (NZW) rabbits. Lab: Argus Research Laboratories Protocol 619-005, Harkell Laboratory Report Number 014-87. Sponsor: Shell Chemicals.

Monocrotophos technical (purity 77.6%, WRC Tox No 921, 926, source: Shell Chemicals) in sterile water was administered by gavage to NZW [HRA(NZW)SPF] rabbits at 0, 0.1, 1, 3 or 6 mg/kg bw/d on days 6 to 18 of gestation following artificial insemination, using 20/group. Clinical signs and general appearance were checked twice daily. Rabbits were observed for compound related effects 15, 30 and 60 min after dosing, and once hourly for the next 3 h. Rabbits were also observed twice daily during the post dosing period. Body weight was recorded daily during gestation, and food consumption was also determined. Rabbits were sacrificed on day 28 (day 0 first day of gestation). A gross pathological examination of dams and fetuses was performed on all animals dying and sacrificed at the end of the study, as well as on any aborted foetuses (where possible). Any gross abnormalities identified were preserved in formalin for later histopathological examination if required. Post-implantation losses, early and late resorptions, numbers of live and dead foetuses, foetal bodyweights, sex ratios, and numbers per/litter were also determined.

Doses were selected on the basis of a dose-range finding study in which NZW rabbits (5/group) were given monocrotophos technical (source, batch no, purity not specified) at 0, 0.1, 0.3, 1, 1.5, 2, 3 or 5 mg/kg bw/d on days 6 to 18 of gestation. In this study, maternal effects evident at 5 mg/kg/d included increased mortality and incidences of clinical signs (faecal changes, excess salivation, rales, constricted pupils, shallow breathing and decreased motor activity), and slight decreases in bodyweights. Body weight decreases were less than 10% in comparison to controls, and were not considered of biological significance. Gross pathological examination of maternal animals revealed clear fluid in the mouth and nose, and faecal staining of the fur. Foetal bodyweights were decreased more than 10% in comparison to controls at 5 mg/kg bw/d in this trial.

Deaths occurring during the main study included one doe at 3 mg/kg bw/d and 13 does at 6 mg/kg bw/d. Deaths occurred after 6 days of administration and were preceded by signs of excitation or depression, diarrhoea, weight loss and decreased food consumption. Clinical observations in other dams at 3 and 6 mg/kg bw/d included faecal changes (diarrhoea, mucoid faeces and dried or no faeces), and at 6 mg/kg bw/day there were increased incidences of transient signs of toxicity occurring from 30 minutes after dosing and persisting for up to 5 h. Transient signs included hyperpnoea, decreased motor activity, excess salivation, rales, tremors, impaired or loss of righting reflex, constricted pupils and ataxia. Pathological examination of animals in the high dose group revealed increased incidences of ulceration of the stomach and duodenum, enlarged and/or discoloured gall bladders, and pulmonary oedema.

A statistically significant dose-related decrease in maternal bodyweight gains occurred during the dosing period from 3 mg/kg/d, however the decreases observed were less that 10% of controls, and were considered not to be of biological significance. At 6 mg/kg/d food consumption was decreased.

Pregnancy rates between groups were comparable. An increased incidence of abortions occurred in some treatment groups (0/16, 2/18, 1/17, 0/18 and 1/19), however, the incidences were within the historical control range. Three does given 3 mg/kg/d had premature deliveries, and because the incidence (16.7%) exceeded that of the historical controls (2.0 %) and it occurred in rabbits displaying overt signs of toxicity it was considered to be treatment-related. The high mortality at 6 mg/kg/d precluded evaluation of this parameter.

Does given 6 mg/kg/d had non-statistically significant increases in the mean number of total (early and late resorptions) and late resorptions, and slightly lower uterine weights. Also at this dose, live foetal bodyweights were decreased and the mean percent of dead or resorbed conceptuses per litter were increased. No other foetal parameters were affected at any dose.

There were dose-related increased foetal and litter incidences of agenesis of the intermediate lobe of the lungs (foetal/litter incidences (%) 0/0, 0/0, 0/0, 0.8/6.7, 6.7/33.3) which also exceeded the incidence of a similar birth defect of the lung (agenesis of the diaphragmatic lobe) in the historical control data submitted (foetal/litter incidences (%): 0.04/0.3). There were increased litter and foetal incidences of irregular ossification of the parietal bones of the foetal skull (foetal/litter incidences (%) 2.3/6.2, 0.8/6.7, 2.0/12.5, 0.8/6.7, 6.7/50) at 6 mg/kg bw/day which also exceeded the incidences in the historical control data (foetal/litter incidences (%): 0.04/0.3). The above abnormalities most likely resulted from delayed development as a consequence of maternotoxicity. While there were increased foetal and litter incidences of external gross and other soft tissue and skeletal alterations occurring in treatment groups, the incidences were low or only marginally increased from concurrent controls and were not considered dose-related.

The NOELs for maternotoxicity and foetotoxicity were 1 mg/kg bw/d, based on the maternal death and clinical signs seen at 3 mg/kg bw/d, and the increased incidence of agenesis of the intermediate lobe of the lung seen at 3 mg/kg bw/d.

Dix KM & Wilson AB (1972) Toxicity studies with AZODRIN: Teratology experiments in rabbits, given AZODRIN orally. Shell Research Ltd, Sittingbourne. TLGR.0031.72

Groups of pregnant female banded Dutch rabbits (Hylyne Commercial Rabbits, Northwich, Cheshire) were administered oral doses of a 40% formulation of monocrotophos in hexylene glycol (supplier:Woodstock Agricultural Research Centre) (a 5% solution in corn oil, in gelatin capsules) at 0, 0.7 or 2 mg formulation/kg bw/d from gestation days 6 to 18 inclusive using 32 rabbits as controls and 16/dose group. One group of rabbits received 37.5 mg/kg/d thalidomide as a positive control. 10 rabbits were mated on each of 8 days, with 4 animals/day allocated as negative controls, 2/day/monocrotophos treatment group, and 2/day as positive controls. A preliminary study was done which indicated that the maximum dose of monocrotophos tolerated by pregnant rabbits (based on general health and body weight) was 2 mg/kg bw/d.

Rabbits were observed daily for general health, and were weighed at the time of mating and on days 6, 9, 12, 15, 18 and 28 of gestation. Food and water were available *ad libitum* throughout the study. Animals were killed on gestation day 28, and the number of live foetuses, late foetal deaths and resorption sites in the uterus of each female were noted. Live feotuses were place in

an incubator to estimate survival during the first 24 h. They were observed hourly for the first 7 h, then again at 24 h. At the end of the 24-h observation period, all foetuses were examined, weighed and crown to rump measurement made. Foetuses showing obvious visceral abnormalities were dissected. Foetuses with skeletal abnormalities were examined by the alizrin technique. Abdominal viscera were examined during the preparation of the skeletons.

Maternal bodyweights at 0.7 and 2 mg/kg/d were statistically significantly decreased during dosing but returned to normal by day 28. Decreases seen were less that 10%, and therefore of questionable biological significance. There were no other toxic signs. There were no treatment-related effects on number of pregnant dams surviving to term or on the mean live litter size. There was a slight increase in the number of resorptions and early foetal deaths seen at 2 mg/kg bw/d monocrotophos, however these were not statistically significant. Survival of foetuses over the first 24 h after removal was not affected. There was an increase in the number of foetuses with an extra rib in the monocrotophos treated animals, with the incidences being 18%, 24% and 27% for control and treated groups. This is within the range considered normal for these rabbits, with frequencies of up to 36.9% accepted as normal variation. Examination of foetuses showed no treatment-related effects on the extent of major or minor abnormalities, whilst in the positive control group there was a significantly higher number of thalidomide litters with abnormal foetuses. One high-dose foetus had a cleft palate; this was the only major abnormality seen following treatment with monocrotophos.

The 40% formulation of monocrotophos was not considered embryotoxic or teratogenic in rabbits up to 2 mg/kg bw/d, and the NOEL for teratogenic effect was therefore 2 mg formulation/kg bw/d equivalent to 0.8 mg active/kg bw/d.

9. GENOTOXICITY STUDIES

The genotoxicity findings are summarised in the table below.

Assay	Bacterial strain or Cell type	Dose levels	Metabolic activation	Results	References
Gene mutation	*S. typhimurium* TA100 TA98 TA1535 TA1537 TA1538	150 - 400 µg/plate	+.-	 weak +,+ -,- -,- - - - -	Moriya et al (1983)
	S. typhimurium TA100 TA98 TA102 TA1535 TA1537	10 - 8000 µg/0.1mL	not stated	 weak + - - - -	Hool & Arni (1980)*
	S. typhimurium TA1535 TA1536 TA1537 TA1538	Spot test	not given	 - - - -	Carere et al (1978)
	S. typhimurium TA100	not given	not given	-	Shirasu et al (1984*)
	S. typhimurium TA1535 TA1536 TA1537 TA1538	not specified	not stated	 - - - -	Dean et al (1974)
	S. typhimurium TA1535 TA1537 TA1538 TA100	1-1000 µg/plate	+,-	 -,- -,- -,- -,-	Waters et al (1977)
	S. typhimurium TA98 TA100 TA102 TA1535 TA1537	20 - 8000 µg/0.1mL	+,-	 -,- weak +,+ -, weak+ -,- -,-	Hool (1986)
	E.coli WP2	not specified	not stated	-	Dean (1972)
	E.coli - streptomycin-dependent	0.1 mL of 1, 10 & 100%	not done	-	Hurni & Ohder (1970)
	E.coli WP2	1 to 1000 µg/plate	+,-	-,-	Waters et al (1977)

	Saccharomyces cerevisiae D4	up to 50 mg/mL	not done	-	Dean et al (1974)
	Saccharomyces cerevisiae D7	not stated	+,-	+,+	Mortelmans et al (1980)
	Saccharomyces cerevisiae D3	5%	not stated	+	Simmon et al (1977)*
	Saccharomyces cerevisiae	5% w/v	+,-	+,+	Waters et al (1977)
	Aspergillus nidulans 35	not specified	not stated	-,-	Morpurgo et al (1977)
	Aspergillus nidulans D3 D7	0 - 1 mM	 +,- +,-	 -,- +, weak +	Vallini et al (1983)
	Mouse lymphoma cells L5178Y	0 - 1000 µg/mL	+,-	+,+	Jotz et al (1985)
Host mediated	*S.cerevisiae* in male CFI mice	0-12 mg/kg bw		-	Dean et al (1974)
Sister chromatid exchange	CHO cells	0.0025% - 0.2%	+,-	+, +	Evans & Mitchell (1980)
	CHO cells	0 - 800 µg/mL	+, -	+,+	Lin et al (1987)
	CHO cells	25 - 400 µg/mL	+,-	+,+	Wang et al (1987)
	RTE cells	12.5 - 100 µg/mL	-	weak +	Wang et al (1987)
	Human lymphoid cells LAZ-007	0 - 20 µg/mL	+,-	-,-	Sobti et al (1982)
	Human lymphocytes from peripheral blood	0.1 - 0.8 µg/8 mL	-	+	Rupa et al (1988)
Chromosome aberration	Human leucocytes from peripheral blood	10^{-3} - 10^{-9}	-	+	Vaidya & Patankar (1982)
	Human lymphocytes from peripheral blood	0.1 - 0.8 µg/8 mL	-	+	Rupa et al (1988)
	Mouse - CFI	0 - 4 mg/kg bw		-	Dean (1973a)
	Hamster	10.5 mg/kg bw		-	Duma et al (1977)
	Mouse, Swiss male	0 - 2 mg/kg bw		+	Vaidya & Patankar (1982)
	Rat - male Wistar	0 - 2 mg/kg bw		+	Adhikari & Grover (1988)
	Mice, Swiss male	0 - 5.6 mg/kg bw		+	Prabakaran (1996b)
	Hamster	1.4 - 5.6 mg/kg bw		-	Strasser (1986)

Micronucleus test	Mouse - Swiss, male	0 - 8 mg/kg bw		-	Kirkhart (1980)
	Mouse - Swiss, male	0 - 2 mg/kg bw		+ at 1.5, 2 mg/kg bw	Vaidya & Patankar (1982)
	Mouse - Swiss	0 - 5.6		-	Prabakaran (1966a)
	Mouse	not given		-	Water et al (1982)*
	Mouse - Swiss	1.25 - 5 mg/kg bw		-	Bhunya & Behera (1988)*
	Mouse - Tif:MAGf	9 mg/kg bw		-	Herner (1992a)*
Nuclear anomalies	Hamster	1.4 - 5.6 mg/kg bw		-	Strasser et al (1986)
Dominant lethal assay	Mouse - CFI	0 - 4 mg/kg bw		-	Dean (1973b)
	Mouce -ICR	0 - 9 mg/kg bw		-	Waters et al (1977)
UDS synthesis	Human foetal lung fibroblasts	not stated	+,-	-, +	Waters et al (1980)
	Human diploid fibroblasts	10^{-3} - 10^{-7} M	+, -	+,+	Waters et al (1977)

* represents studies not reviewed, but cited in Skripsky & Loosli (1994)

9.1 Gene Mutation Assays

Carere A, Ortali VA, Cardamone G & Morpurgo G (1978) Mutagenicity of dichlorvos and other structurally related pesticides in Salmonella and Streptomyces. Chem Biol Interact 22: 297 - 308

The mutagenic activity of azinphos methyl, diallate, dichlorvos, EPTC, fenchlorphos, mevinphos, monocrotophos, noruron, parathion methyl, triallate, trichlorphon and vegadex was assessed using *Salmonella typhimurium* strain TA1535, TA1536, TA1537 and TA1538 and *Streptomyces coelicolor*. The doses of the pesticides used in each case was not specified. Dichlorvos and trichlorphon were negative with *Salmonella* using a Spot test, however were found to be mutagenic using a liquid culture. Both chemicals were mutagenic in a spot test with *Streptomyces*. Of the carbamates tested, EPTC and noruron were not mutagenic. Triallate was weakly mutagenic in Streptomyces, while triallate and vegadex were powerful mutagens in both bacteria. None of the five organophosphorus insecticides tested (including monocrotophos) were mutagenic in either bacteria.

Dean BJ (1972) The mutagenic effects of organophosphorous pesticides on microorganisms. Arch Toxicol 30:67 - 74

The mutagenic effects of a range of pesticides, including dichlorvos, tetrachlorvinphos, dicrotophos, crotoxyphos, malathion, chlorfenvinphos, monocrotophos and parathion-methyl, as well as a range of other compounds was investigated in *Escherichia coli* WP2, *Serratia marcescens* HY/ 13 and *Serratia marcescens* HY/ 21. The doses used in these trials were not specified, and it was not indicated whether any metabolic activation system was used. The

organophosphorus pesticides (including monocrotophos) did not produce reverse mutation in *E coli* on solid medium. Monocrotophos was not tested against either strain of *Serratia*. Dichlorvos was positive at 25, 50 and 100 mg/mL. No positive controls were used in this trial.

Dean N, Doak, S Somerville HJ & Whitebread C (1974) Toxicity studies with AZODRIN. Effect of AZODRIN on micro-organisms in the host mediated assay and in vitro. Shell Research Ltd, Sittingbourne. TLGR.0030.74

The mutagenic effect of technical monocrotophos (purity 77.3% w/v in hexylene glycol) and analytical grade monocrotophos (purity 99%) (both supplied by Chemical Toxicology Division, Tunstall Laboratories) was assessed using four strains of *Salmonella typhimurium* (TA1535, TA1536, TA1537 and TA1538) and *Serratia marcescens* HY/ 13 and HY/ 21. The dose of monocrotophos used was not specified. There was no indications of reversions with any strain of *S. marcescens* where the positive controls (N-methyl-N'-nitro-N-nitroguanidine (NTG)) showed reversions. With *S. typhimurium*, there were no positive results with monocrotophos. Positive results occurred with NTG with TA1535, TA1537 and TA1538. Positive results also occurred with ethyl methanesulphonate with TA1535 and also TA1538. Thus this study did not show monocrotophos to be mutagenic in either bacterial system.

In a study of mitotic gene conversion in *Saccharomyces cerevisiae* strain D4, analytical grade monocrotophos (> 99% purity) and a 77.3% formulation in hexylene glycol did not increase the frequency of mitotic gene conversion even at final concentrations of 10 mg/mL (1%). At higher concentrations up to 50 mg/mL an increase was seen but this was also accompanied by reduced survival of the yeast cells. A positive control (ethyl methanesulphonate) caused an increase in conversions at both ade2 and trp5.

Male CFI mice (source not specified) were dosed orally with analytical grade monocrotophos (>99% pure, in water) at 0, 2, 4, 8 or 12 mg/kg bw followed by an IP injection of *Saccharomyces cerevisiae* strain D4 cells. Additionally, a positive control of EMS and a negative control of water were carried out. There was no change in the frequency of mitotic gene conversion in the yeast cells related to treatment with monocrotophos.

Moriya M, Ohta T, Watanabe K, Mivazawa T, Kaot K & Shirasu Y (1983) Further mutagenicity studies on pesticide in bacterial reversion assay systems. Mutat Res 116:185 - 216.

The mutagenic potential of monocrotophos was assessed in *Salmonella typhimurium* (strains TA100, TA98, TA1535, TA1537, TA1538) and in *E. coli* (WP2 hcr), using the Ames method. Monocrotophos produced positive results in E coli and in the TA100 strain of *Salmonella*. Results were comparable either with or without metabolic activation. These results were only seen at doses of greater than 1 mg/plate, and there were only 0.0064 revertants/nmole. Monocrotophos was therefore only weakly mutagenic in this trial.

Hurni H & Ohder H (1970) Report on the mutagenic effect of technical monocrotophos. Project No Tif 261

Monocrotophos technical (Ciba Geigy Ltd) was tested for mutagenic potential with streptomycin-depended *Escherichia coli* by the paper disc method. Monocrotophos was tested at 1, 10 and 100%. The negative control was distilled water, while the positive control was betapropiolactone. Bacteria were cultivated on nutrient agar supplemented with streptomycin, and multiplied for 48 h. Cells were then centrifuged, washed and resuspended at between $10^{8.5}$ to 10^{9} cells/mL. This suspension was then inoculated onto agar, with 0.1 mL of bacterial suspension applied per plate. The paper disc was then applied, and moistened with 0.1 mL of

the test chemical. The media was incubated for 4 days under controlled conditions. No mutant colonies were seen in the negative control or at any dose of monocrotophos, in comparison to 170 mutant colonies at 10% betapropiolactone. Therefore, monocrotophos was negative in this test of mutagenicity.

Waters MD, Simmon VF, Mitchell AD & Jorgenson TA (1977) Evaluation of selected pesticides as chemical mutagens. In vitro and in vivo studies. US EPA Office of Research and Development. Contract no. 68-01-2458

Monocrotophos technical (batch H, 9-SCL-77, purity 55%, source: Shell Chemical Co) was tested for mutagenic activity in *Salmonella typhimurium* strain TA 1535, TA 1537, TA 1538 and TA100, *Escherichia coli* WP2, W3110 and p3478 and *Bacillus subtilis* H77 and M45.

S. typhimurium was incubated with 1 to 1000 µg/plate of monocrotophos and plated out for 2 days on minimal medium, either with or without the supernatant fraction of liver from Aroclor stimulated mice. The his+ revertants were counted and recorded. Monocrotophos was negative in this test both with and without metabolic activation.

E. coli WP2 was tested for tryptophan independence in a similar method to *S typhimurium*, both with and without metabolic activation, and was negative.

The survival ratios seen in *E coli* W3110/p3478 and *B subtilis* H77/M45 were tested when incubated with monocrotophos. There was no effect on survival either with or without metabolic activation, and monocrotophos was determined to be negative in this test.

In these bacterial mutagenicity tests, monocrotophos was shown to be negative for gene mutation effects.

Hool (1986) Salmonella/Mammalian-microsome mutagenicity test. Test No 850810 Ciba-Geigy Ltd, Basle Switzerland

Monocrotophos technical (batch OP 506944, purity 78.4%, source: Ciba Geigy Switzerland) in acetone was tested for mutagenic potential using *Salmonella typhimurium* strains TA 98, TA 100, TA 102, TA 1535 and TA 1537. The test chemical was incubated with the bacteria both with and without metabolic activation. The negative control was acetone, and the positive controls used varied depending on whether metabolic activation was used or not. Details are elaborated in the following table:

Positive control agents used with *S. typhimurium* strains.

Strain	Positive control without metabolic activation	Positive control with metabolic activation
TA 98	Daunorubicin-HCl 5 or 10 µg/0.1mL phosphate buffer	2-aminoanthracene 5 µg/0.1mL DMSO
TA 100	4-nitroquinoline-N-oxide 0.125 or 0.25 µg/0.1mL phosphate buffer	2-aminoanthracene 5 µg/0.1mL DMSO
TA 102	mitomycin-C 0.5 or 1.0 µg/0.1mL bidistilled water	2-aminoanthracene 20µg/0.1mL DMSO
TA 1535	sodium azide 2.5 or 5.0 µg/0.1mL bidistilled water	cyclophosphamide 250 µg/0.1mL phosphate buffer

TA 1537	9(5) aminoacridine hydrochloride monohydrate 50 or 100 µg/0.1mL DMSO	2-aminoanthracene 5 µg/0.1mL DMSO

Monocrotophos was incubated in four tests: an initial toxicity test, with doses ranging from 20 to 5000 µg/0.1 mL, and 3 mutagenicity tests. The first test used concentrations of 20, 78, 313, 1250 or 3000 µg/0.1 mL. The second test used concentrations of 500, 1000, 2000, 4000 or 8000 µg/0.1 mL, and the third used concentration of 10, 30, 90, 270, 810, 2430 or 7290 µg/0.1 mL. Each concentration was tested in triplicate, and the arithmetic mean of the results was used for evaluation. The samples were incubated for 48 h prior to reading. The supernatant fraction of livers from Aroclor induced rats were used as a metabolic activation mixture.

Monocrotophos was negative when tested with TA 98, TA 1535 and TA 1537 at all doses, both with and without metabolic activation. Monocrotophos had a weakly positive effect on TA100 without metabolic activation at doses between 2430 and 8000 µg/0.1 mL; with metabolic activation doses of 7290 µg/0.1mL and higher were required. Monocrotophos was weakly positive with TA 102 at doses from 2430 µg/0.1 mL in the absence of metabolic activation, however showed no activity with metabolic activation. Monocrotophos was therefore determined to be a very weak mutagenic agent in this system.

Mortelmans KE, Riccio ES & Shepherd GF (1980) In vitro detection of mitotic crossing-over, mitotic gene conversion , and reverse mutation with S. cerevisiae D7 for seven pesticides. Project no LSU 7558-20 Lab: Stanford Research Institute Sponsor: Shell Chemicals.

Monocrotophos (purity 58.4%, batch no. 9-SCL-77, source: Shell Chemicals) in DMSO was tested for mutagenicity using *Saccharomyces cerevisiae* D7, a eukaryote which can be used to detect mitotic crossing over, gene conversion and reverse mutation. The supernatant fraction of a liver homogenate from Aroclor induced adult male rats was used as a metabolic activation mixture. Yeast cell were incubation with the test substance for 4 h prior to plating out on media to detect any mutagenic change. Mitotic crossing over was tested by plating on material similar to the culture media, gene conversion by plating on tryptophan deficient culture media, and reverse mutation by plating on material lacking isoleucine. In an initial test, monocrotophos was positive for crossing over at 2%, for gene conversion at 3% and for reverse mutation at 2%. The compound was less active following incubation with the metabolic activating substance. In a second test, with much lower activity in the negative control, monocrotophos was positive at concentrations of 1% without activation.

Morpurgo G, Aulicino F, Bignami M, Conti L & Velcich A (1977) Relationship between structure and mutagenicity of dichlorvos and other pesticides. Atti Acad Naz Lincei Cl Sci Fish Mat Nat Rend 62(5):692 - 701.

Monocrotophos technical (souce: Shell Chemical Co, purity and batch no. not specified) was tested for mutagenicity using two strains of *Aspergillus nidulans*, strain 35 (a haploid strain) and strain P (a diploid strain). The dose of monocrotophos used was not specified. Point mutations were identified using 8-azaguanine resistance, cross over induction tested by the appearance of "fpa"-resistant green colonies. Non-disjunction was tested in two ways: firstly by measuring induction in the spot test of yellow "fpa"-resistant colonies, and secondly by counting yellow or dark green sectors induced in colonies. Monocrotophos was negative for mutagenicity in all tests.

Vallini G, Pera A & Bertoldi M de (1983) Genotoxic effects of some agricultural pesticides in vitro tested with Aspergillus nidulans. Environ Poll (Series A) 30:39 - 58

Two separate studies were carried out in *Aspergillus nidulans* (diploid strains D3 and D7) using monocrotophos (Nuvacron, source: Ciba-Geigy Italia SpA, Milano; purity not specified). The frequency of mitotic gene conversion was investigated using the D3 strain, and the frequency of mitotic crossing over and non-disjunction were investigated using the D7 strain. The fungi were cultured, and conidia collected after 3 - 4 days incubation. The conidia solution was incubated with 1 mL of pesticide solution at 0, 0.25, 0.5 or 1 mM of monocrotophos for 3 - 4 h, either with or without a metabolic activation solution. Gene conversion was detected by plating on Czabek medium and Czabek plus PABA medium. Green colonies on the minimal medium were indicative of gene conversion. Mitotic crossing over was detected by plating on pimaricin-supplemented medium. As pimaricin resistance is recessive, this detects the incidence of crossing over. Non-disjunction results in monosomic or trisomic fungi, and was detected by plating on pimaricin medium.

In strain D3, monocrotophos did not induce mitotic gene conversion in the presence or absence of metabolic activation. In conidia of strain D7 treated for 4 hours with 0.25 or 0.5 mM technical grade monocrotophos, the compound produced mitotic crossing over and mitotic non-disjunction, effects which were reduced in the presence of metabolic activation. The effects were not observed at 1 mM monocrotophos.

Jotz MM & Mitchell AD (1980) An evaluation of mutagenic potential of monocrotophos employing the L5178Y Tk +/- mouse lymphoma assay. Project No LSU-7558 Lab: Stanford Research Institute. Sponsor: Shell Chemicals.

Monocrotophos technical (purity 58.4%, lot number 9-SCL-77, source: Shell Oil Company) was tested for mutagenic activity *in vitro* using L5178Y mouse lymphoma cells heterozygous for thymidine kinase. Monocrotophos concentrations ranged from 0 - 1000 µg/mL, diluted in DMSO. Metabolic activation was tested using an Aroclor 1254-induced rat liver homogenate as the activation system. The positive control compounds were ethylmethane sulfonate (EMS) and 3-methylcholanthrene (3-MCA) which induce mutagenesis without and with metabolic activation respectively. Each compound was tested in the presence and absence of the metabolic activation preparation. Duplicate samples were used for each test compound dilution and for the negative and positive controls, with each sample using 6 000 000 fresh cells in 10 mL of medium. Samples were incubated for 4 h, followed by removal from the test solution by a series of low speed centrifugations, removal of the supernatant and resuspension in fresh medium. The cells were then maintained in a roller drum for 2 days for expression of any mutations. Cell growth was monitored daily by adding 1 mL of cell suspension to 9 mL of trypsin, incubating for 10 min, and then counting the cells. After the expression period, the cells were seeded in soft agar medium (both selective to determine mutation, and nonselective to determine viability). Cells were cultivated for 11 days, followed by counting of cell colonies. Mutation frequency was determined by dividing the number of mutant cells per mL of original suspension culture by the number of viable cells per mL of original suspension.

Dose ranging studies indicated that the appropriate concentration to use was approximately 50 to 900 µg/mL without metabolic activation and from 300 to 1000 µg/mL with metabolic activation. In the absence of metabolic activation, mutation frequency increased in a dose related manner. Mutation at approximately twice the frequency in negative controls were seen at 200 µg/mL and higher. There was no significant decrease in viability. With metabolic activation there was a similar pattern of dose related increase in mutation, however it was less marked. Mutations at twice the frequency of that seen in negative controls was seen at 720

µg/mL and higher. Survival appeared to be decreased at the highest doses of monocrotophos tested. Therefore it appears that monocrotophos induces mutation in L5178Y mouse lymphoma cells *in vitro*.

9.2 Chromosomal Aberration Tests

Evans EL & Mitchell AD (1980) An evaluation of the effect of monocrotophos on sister chromatid exchange frequencies in cultured Chinese hamster ovary cells. Project no. LSU-7558 Lab: Stanford Research Institute, Menlo Park. Sponsor: Shell Chemicals.

Monocrotophos technical (purity 58.6%, source: Shell Oil Company,) was dissolved in DMSO. This material was further diluted in culture medium to produce the concentrations tested, resulting in the maximum DMSO concentration being 1%, which was not cytotoxic. Chinese hamster ovary cells were cultured in complete medium, seeded and grown for 1 to 2 days prior to introduction of the test compound. The metabolic activation system was an Aroclor 1254-induced rat liver supernatant fraction. The positive controls were ethyl methanesulfonate (EMS) and dimethylnitrosamine (DMN). Negative control was DMSO diluted in culture media.

Cytotoxicity evaluations with and without metabolic activation were done with monocrotophos solutions of 0.0008% to 0.5%. Cells were harvested after 24 h, with the last 2.5 h in colchicine. Cells were examined to determine the number of divisions during exposure. The highest concentrations permitting two cell divisions in 24 h were 0.04% without metabolic activation, and 0.2% with metabolic activation.

In the test without metabolic activation, monocrotophos was tested at 5 serial dilutions from 0.0025% to 0.04%. With metabolic activation, monocrotophos was tested at 5 dilutions from 0.0125% to 0.2%. Cells were harvested after 24 h without metabolic activation, and after 4.5 h with metabolic activation, with the last 2.5 h with the addition of colchicine. For each test, 50 cells/sample were assessed for the number of SCEs and for the no. of chromosomes/cell.

The frequency of SCEs was increased following exposure to monocrotophos, in a dose-related manner. The maximum effect without metabolic activation was seen at 0.02% (the sample at 0.04% was unable to be evaluated), and was 19 SCEs/cell (negative control 13 SCE/cell, positive control 31 SCE/cell). The maximum effect with metabolic activation was seen at 0.2%, and was 27 SCEs/cell, with negative control at 14 SCE/cell, and positive control at 42 SCE/cell. No effect was seen at 0.005% without metabolic activation, or at 0.025% with metabolic activation. Monocrotophos was determined to be weakly mutagenic in this assay.

Lin MF, Wu CL & Wang TC (1987) Pesticide clastogenicity in Chinese hamster ovary cells. Mutation Research 188, 241 - 250

Monocrotophos technical (purity 78%, source: Shell Chemical Co. Taiwan) was incubated with Chinese Hamster ovary (CHO) cells at 0, 50, 100, 200, 400 or 800 µg/mL, either with or without metabolic activation. The metabolic activation solution was prepared from the supernatant fraction of the liver of Aroclor-induced Sprague Dawley rats, and was tested with the CHO cells to determine the optimum concentration for testing. Cyclophosphamide was used as the positive control. All test solutions were incubated with the CHO cells for 18 h, with colcemid added 2 h before the end of incubation. In examining the sample, at least 100 metaphase cells were randomly sampled and examined for chromosome aberrations.

At 200 and 400 µg/mL monocrotophos without metabolic activation, there was a significant increase in aberrant cells in comparison to controls, with the percentages for the doses being 0,

2, 2, 24 and 48%. Abnormalities included chromosome gaps, breaks and exchanges. Survival at 800 µg/mL monocrotophos without metabolic activation was too low to allow the percentage of aberrant cells to be assessed (only 2% of cells survived). With metabolic activation, the percentage of abnormalities was decreased, and the survival at the highest dose was increased. The percentages of aberrant cells were 2, 0, 0, 15, 36 and 87%. Based on these findings, monocrotophos was positive for chromosomal damage in this CHO cell test.

Wang TC, Lee TC, Lin MF & Lin SY (1987) Induction of sister chromatid exchanges by pesticides in primary rat tracheal epithelial cells and Chinese hamster ovary cells. Mutation Research 188:311 - 321

Monocrotophos technical (purity 78%, source not specified) in DMSO was tested for cytotoxicity and induction of sister chromatid exchange using Chinese Hamster ovary (CHO) cells and rat tracheal epithelial (RTE) cells. The RTE cells were obtained from an 8 week old rat, and grown under controlled conditions.

CHO cells were grown overnight, then incubated with the pesticide mixture either with or without metabolic activation for 24 h. Monocrotophos was included at 25, 50, 100, 200 or 400 µg/mL. Colcemid was added 2 h before the end of incubation. There was a significant increase in sister chromatid exchange in the two highest doses either with or without metabolic activation. No information on cell survival was provided.

RTE cells were grown for 24 h, then incubated with monocrotophos for 32 h at 12.5, 25, 50, or 100 µg/mL. At 29 h colcemid was added. The only significant increase in sister chromatid exchange was seen at 100 µg/mL, and at this dose cell survival was 81.1%. In this trial, monocrotophos induced chromosomal damage in CHO cells, but was negative in RTE cells.

Sobti TC, Krishan S & Pfaffenberger CD (1982) Cytokinetic and cytogenetic effects of some agricultural chemicals on human lymphoid cells in vitro: organophosphates. Mutat Res 102:89 - 102.

LAZ-007 human lymphoid cells of B cell origin were cultured in the presence of 0-20 µg/mL technical grade monocrotophos (source Chemical Services Inc. Westchester, PA). Controls were cultured in 0.1% ethanol. Phenobarbitol-induced rat liver microsomal S9 was used to test the effect of metabolic activation. Test chemicals were incubated with cell lines for 48 h, and the survival rate, mitotic index and frequency of sister chromatid exchange was measured. Monocrotophos significantly decreased cell survival, with 57% survival at 0.02 µg/mL, 39% at 0.2 µg/mL, 31% at 2 µg/mL and 11% at 20 µg/mL. The mitotic index was reduced by monocrotophos. The frequency of sister chromatid exchanges was increased significantly in the presence of 2 and 20 µg/mL monocrotophos. The increases were less than twice control values, and given the low cell survival rates, this was not considered to be an indication of a positive effect.

Vaidya VG & Patankar N (1982) Mutagenic effect of monocrotophos - an insecticide in mammalian test systems. Ind J Med Res 76:912 - 917

Human peripheral blood lymphocytes in culture were stimulated with phytohaemagglutinin and incubated with technical grade monocrotophos (69% purity; source: Agrochemical Division of Ciba Geigy, Bombay) at concentrations from 10^{-9} to 10^{-3} M for 50 hours at 37°C. Colchicine was added at 25 ug/mL 4 h prior to harvest. At the highest concentration of monocrotophos, no dividing cells were seen. The percentage of abnormalities ranged from 7% at 10^{-4}M to 1% at 10^{-9}M, with the abnormalities mainly being chromatid gaps, chromatid breaks and terminal deletions.

Monocrotophos technical (69.4% purity, Agrochemical Division of Ciba Geigy, Bombay) was given IP to male Swiss mice (3/group) at doses of 0, 1, 1.5 or 2 mg/kg bw at 0 and 24 hours and the mice killed 6 h after the second dose. After analysis of 1000 erythrocytes per animals an increased incidence of micronuclei (2-3 times control) was observed at 1.5 and 2 mg/kg bw monocrotophos. No change in the frequency of micronuclei was seen at 1 mg/kg bw.
Male Swiss mice (source not specified) were injected IP with 0, 1, 1.5 or 2 mg/kg bw technical grade monocrotophos (69%, in water; source Agrochemical Division, Ciba Geigy Bombay) and killed after 24 h. Three h prior to euthanasia, mice were injected with 0.25 mg/kg bw colchicine IP. Chromosome preparations from femoral bone marrow showed a slightly higher incidence of chromatid gaps and breaks in treated animals versus controls. The overall incidence was low, with the highest rate of abnormalities being 4%, seen at 2 mg/kg bw.

Based on the effects on the incidence of micronuclei seen at 1.5 mg/kg bw, and the slight increase in chromatid gaps and breaks seen at 2 mg/kg bw, monocrotophos is determined to be a weak mutagen in these test systems.

Rupa DS, Laksham Rao PV, Reddy PP & Reddi OS (1988) In vitro Effect of Monocrotophos on Human Lymphocytes. Bull Environ Contam Toxicol 41:737 - 741
Monocrotophos (purity 36%, source Khaltan & Co, Calcutta India) in DMSO was cultured with lymphocyte cells obtained from a healthy male donor at doses of 0.1, 0.2, 0.4 and 0.8 µg/8 mL of culture media for 24, 48 or 72 h. Two negative controls were used; one with and one without DMSO. Colchicine was added 2 h before harvesting cultures. Two sets of cultures were maintained; the first to investigate chromosomal aberrations, and the second to consider sister chromatid exchange.

Four hundred cells/dose and time were examined to investigate chromosomal aberrations. Fifty cells were examined for sister chromatid exchanges. There was a significant increase in the incidence of aberrant cells at the 2 highest doses of monocrotophos at each time interval. The incidence of sister chromatid exchange was increased significantly ($p<0.05$) at all doses of monocrotophos at all time periods. Monocrotophos was therefore positive for chromosome damage in this test using human lymphocyte cells.

Kirkhart, B(1980) Micronucleus test on monocrotophos. Project No LSU 7558-19 Lab: Stanford Research Institute. Sponsor Shell Chemicals.
Monocrotophos technical (Lot no. 9-SCL-77, Shell Co, purity not given) was administered by IP injection at 0, 2, 4 or 8 mg/kg bw at 0 and 24 h to male Swiss mice (Simonsen Laboratories, Ca) using 24/group. Negative control animals received IP injections of DMSO while positive controls were given trimethyl phosphate in DMSO at 5 mL/kg bw. Doses were based on toxicity data from the Registry of Toxic Effects of Chemical Substances (RTECS). Femoral bone marrow smears were made at 48, 72 and 96 h after the first dose. Five hundred polychromatophilic erythrocytes (PCEs) per animal were evaluated for the presence of micronuclei. There was no increase in micronuclei formation observed in femoral bone marrow following administration of monocrotophos at any dose, or at any of the three time periods.

Prabakaran P (1996a) Micronucleus Test of Monocrotophos Technical to Mice. Report no 853/JRF/TOX/96. Lab: Jai Research Foundation. Sponsor: United Phosphorus Limited, Mumbai India. GLP: OECD/US EPA
Monocrotophos technical (batch no. 307, purity 74.4% source: United Phosphorus Ltd) was administered PO by gavage to Swiss albino mice (Jai Research Foundation) at doses of 0, 1.4,

2.8 or 5.6 mg/kg bw in a single dose using 5 mice/sex/group. Mitomycin-C was used as a positive control at 4 mg/kg bw by single IP injection using 5 mice/sex/group. The positive control test was performed at a different time to the monocrotophos trial.

Mice were dosed and euthanised an unspecified time later. The femoral bone marrow was removed, placed in 3 mL foetal calf serum and centrifuged. The cell pellet was removed and smeared onto a clean slide prior to fixing in methanol and air drying. A minimum of 2000 erythrocytes were examined per animals for the presence of micronuclei, and other signs of chromosomal damage.

Mice in the high dose group exhibited abdominal breathing and mild tremors following dosing. Male mice at 5.6 mg/kg bw had a statistically significant increase in the incidence of micronuclei, however this increase was very small in comparison to effects seen in the positive control animals. No effects were seen in female mice. No other evidence of chromosomal damage was seen in this test. Based on the small effect seen in high dose males, monocrotophos is determined not to cause chromosomal damage at doses up to 2.8 mg/kg bw.

Dean BJ (1973a) Toxicity studies with AZODRIN; Chromosome studies on bone marrow cells of mice after a single dose of AZODRIN. Shell Research Ltd, Sittingbourne. TLGR.0014.73

Analytical grade monocrotophos (>99% pure, batch TSL/62/70/P, supplier not stated) prepared as a 0.4 mg/mL solution in DMSO was administered to CFI mice (source: Shell Research Ltd, Sittingbourne) at doses of 0, 2 or 4 mg/kg bw, using 8 mice/sex/group. Each animal was injected with 0.01 mL/g bw of a 0.04% Colcemid solution (obtained from Ciba Laboratories Ltd, Horsham Sussex) 90 min before the end of the experiment. Mice were killed 8 or 24 h after dosing, and the femurs removed. Chromosome preparations of bone marrow cells showed no significant differences in the incidence of chromatid gaps or polyploidy. Of the 4,692 cells examined from 47 mice, 7 cells showed single chromatid gaps. No chromosome breaks or other chromosome aberrations were observed. There was no positive control used in this trial. Monocrotophos was negative for mutagenic effects in this trial.

Duma D, Raicu P, Hamar M & Tuta A (1977) Cytogenetic effects of some pesticides on rodents. Rev Roum Biol Anim 22(1):93 - 96

Monocrotophos (Nuvacron; source and formulation details not specified) was administered to hamsters at 10.5 mg monocrotophos/kg bw (route not specified). Bone marrow smears were performed 24, 48 and 72 h after administration. There was a decrease in the mitotic index at 48 h, which was presumed to be related to an inhibition of cell division. No chromosome abnormalities were observed.

Adhikari N & Grover IS (1988) Genotoxic effects of Some Systemic Pesticides: In Vivo Chromosomal Aberrations in Bone Marrow Cells in Rats. Env and Molecular Mutagenesis 12:235 - 242

Monocrotophos (source, batch no, purity not specified) in DMSO was injected IP into male Wistar rats (source: Animal House, Haryana Ag University, Hisseri, India) at doses of 0, 0.5, 1 or 2 mg/kg bw/day for 2 consecutive days using 5/group. The positive control was ethyl methane sulphonate. Animals were killed 6 h after the last dose, having received 4 mg/kg bw colchicine 2 h before euthanasia.

Following euthanasia, the femurs were removed and the bone marrow aspirated in saline. Following centrifugation, the cell pellet was resuspended in 1% sodium citrate. Cells were later

placed in a methanol/acetic acid fixative prior to preparation on slides. At least 40 - 50 metaphases per animal were examined to determine chromosomal damage.

The percentage of abnormal cells produced were 4.5, 4.4, 5.7 and 8.7%. Therefore there was a significant increase in the percentage of abnormalities seen at 2 mg/kg bw in comparison to that seen with control animals. Thus, monocrotophos was positive for chromosomal damage in this trial.

Prabakaran P (1996b) Chromosomal aberration study of monocrotophos technical to mice. Report no 852/JRF/TOX/96. Lab: Jai Research Foundation. Sponsor: United Phosphorus Limited, Mumbai India. GLP: OECD/US EPA

Monocrotophos technical (batch no. 307, purity 74.4%, source United Phosphorus Limited) was administered PO by gavage to Swiss albino mice (Animal House, Jai Research Foundation) at doses of 0, 1.4, 2.8 or 5.6 mg/kg bw, using 5 mice/sex/group. Mitomycin-C was used as a positive control, and was administered at 4 mg/kg bw by IP injection. The positive control test was done at a different time to the main test. On the day following treatment, mice were given 4 mg/kg bw colchicine IP, and euthanised 4 h later.

The femurs were removed, and femoral bone marrow extracted. The cells were suspended in phosphate buffered saline and centrifuged. The cells were then resuspended in hypotonic potassium chloride, incubated for 30 minutes and then recentrifuged. Cells were fixed in a methanol/acetic acid mixture, smeared onto slides and air dried.

A minimum of 500 cells/animal were examined. The mitotic index was determined. A minimum of 50 metaphase cells were examined per animal. These were scored for chromosome gaps, breaks or fragments, and the number of chromosomes present. The number of aberrant cells were determined.

In males, the mitotic index was statistically significantly ($p<0.05$) decreased in comparison to controls at the 2 lowest doses, although it was normal at the highest dose. There was no increase in the number of aberrant cells in males. In females, there was no change in the mitotic index at any dose, however the number of aberrant cells was statistically significantly increased ($p<0.05$) at the highest dose. Aberrant cells were approximately 6 times more common in these animals than in the negative control. The high dose abnormality was approximately 25% of that seen in the positive controls. It appears that monocrotophos can induce chromosomal damage at doses above 2.8 mg/kg bw, based on the effects seen in female mice.

Strasser F, Langauer M & Arni P (1986) Nucleus anomaly test in somatic interphase nuclei of Chinese hamster. Test 850809 Ciba Geigy Limited, Basle Switzerland

Monocrotophos technical (Batch OP 506944, purity 78.4%, source Ciba Geigy Ltd, Basle, Switzerland) was administered in distilled water by oral gavage to Chinese hamsters (random outbred strain, Ciba Geigy, Tierfarm, Switzerland) in an initial tolerance test (2/sex/group), followed by a mutagenicity test (6/sex/group).

In the tolerance test, the first doses used were 6, 30 or 150 mg/kg bw. The doses were progressively reduced until the doses suitable for the mutagenicity test is derived. The highest dose survived by all animals in the tolerance test was the highest dose used in the mutagenicity test; the two lower doses were derived from this. The doses used in the mutagenicity test were determined to be 1.4, 2.8 or 5.6 mg/kg bw/day, administered on 2 consecutive days.

The positive control for the mutagenicity test was cyclophosphamide at 120 mg/kg bw, while distilled water was used as the negative control. Twenty four h after the second dose, all animals were euthanised. A slide preparation of the femoral bone marrow was made, and 1000 bone marrow cells/animal examined. Anomalies tested for were single Jolly bodies, fragments of nuclei in erythrocytes, micronuclei in erythroblasts, micronuclei in leucopoietin cells and polyploid cells. The percentage of cells showing abnormalities were not significantly different in monocrotophos treated animals than in the negative controls. Positive control animals showed a marked increase in anomalies. It was determined that in this test monocrotophos was negative for mutagenicity.

Strasser F (1986) Chromosome studies on somatic cells of Chinese Hamster. Test No 850808 Ciba Geigy Ltd, Basle, Switzerland. GLP: USEPA

Monocrotophos technical (batch no. OP 506944, purity 78.4%, source Ciba-Geigy Switzerland) in distilled water was administered PO by gavage to Chinese hamsters (random outbred strain; Ciba-Geigy Tierfarm, Sisseln) on two consecutive days at 1.4, 2.8 or 5.6 mg/kg bw using 4/sex/group. The appropriate doses had been determined by a tolerance test, using hamsters of a similar strain with 2/sex/group. The tolerance test involved a progressive decreasing of dosage to find the maximum dose which could be tolerated for 2 days consecutive administration.

The negative control for the trial was distilled water, the positive control was cyclophosphamide at 64 mg/kg bw. After the second dose, animals were give 10 mg colcemide/kg by IP injection, and were euthanised 4 h later. The bone marrow from both femurs was extracted, and suspended in a salt solution diluted with water to form a hypotonic solution. This was then centrifuged, and the cells resuspended in a methanol:acetic acid mixture and left overnight. The cells were then recentrifuged and suspended, and plated out onto wet slides for examination. At least 100 metaphases per animal were examined for chromosome aberrations, including breaks, exchanges, deletions, fragments, gaps, decay or numerical aberrations. In animals treated with monocrotophos, there was one incidence of a chromatid gap at the low dose, a chromosome break at 2.8 mg/kg bw, and a minute at the high dose. These were considered to be of a frequency which could arise naturally and were not considered to be related to treatment. Therefore, monocrotophos did not induce chromosome damage in this test.

9.3 Other Mutagenicity Tests

Dean BJ (1973b) Toxicity studies on AZODRIN: Dominant lethal assay in male mice after a single oral dose of AZODRIN. Shell Research Ltd, Sittingbourne. TLGR.0027.73

Monocrotophos (SD9129, purity >99%, batch no TSL/62/70/P, supplier: Woodstock Agricultural Research Centre, Sittingbourne, UK) in dimethyl sulfoxide was administered by gavage to male CFI mice (Tunstall Laboratory) at doses of 0, 1, 2, or 4 mg/kg bw, using 12 mice/group (control 24 mice, dosed with dimethyl sulfoxide). Following dosing, each male was caged with 3 randomly selected females for 7 days. This procedure was repeated weekly with new females for a total of 8 weeks. Mating was presumed to have occurred by midweek; 13 d after the presumed mating, females were euthanised and uterus removed. Nonpregnant females were noted, and the number of early foetal deaths, live foetuses and late foetal deaths were recorded.

There were no clinical signs seen in the males related to dosing with monocrotophos. The percentage pregnancies in females ranged between 59 and 81%, with no treatment related changes observed, either in the weekly figures or averaged over the 8 weeks. The total number

of foetal implants did not vary with treatment, either on a weekly basis or averaged over the trial. Additionally, the number of early foetal deaths did not vary with treatment. Thus there were no dominant lethal mutations detected in male CFI mice following dosing with 1, 2 or 4 mg/kg bw monocrotophos. No positive control was used in this study.

Waters MD, Simmon VF, Mitchell AD & Jorgenson TA (1977) Evaluation of selected pesticides as chemical mutagens. In vitro and in vivo studies. US EPA Office of Research and Development. Contract no. 68-01-2458

Monocrotophos technical (batch H, 9-SCL-77, purity 55%, source: Shell Chemical Co) in corn oil was fed to male ICR/SIM mice (Simonsen Laboratories, Gilroy, CA) at 0, 15, 30 or 60 ppm (equivalent to 0, 2.25, 4.5 or 9 mg/kg bw/day) for 7 weeks before mating with untreated females. Doses were determined following a trial to determine the acute oral LD50 for this particular strain of mice, which was determined to be 17 mg/kg bw. Males were maintained with 2 females for a period of 7 days. Mating was confirmed by the presence of a vaginal plug. At the end of 7 days, 2 new females were placed with the male. This continued for 8 weeks. Each female was killed at the estimated midterm of the pregnancy, and was examined for implantations, and early and late resorptions. A positive control group, each of whom received 0.2 mg/kg bw treithylenemelanine (TE) by IP injection 2 h before mating commenced was maintained. Monocrotophos was negative for mutagenicity in this trial, showing no increase in dead implants or change in the number of implantations in comparison to the negative control. The positive control showed a significant increase in the average dead implants, and decrease in the average implants per female in comparison to the negative control.

Monocrotophos technical (batch no H, 9-SCL-77, purity 55%, source: Shell Chemical Co) was incubated with human diploid fibroblasts (WI-38 cells). The cells had been prepared by incubation in 0.5% serum, and were synchronised in the same phase of the mitotic cycle. Monocrotophos solutions ranging in concentration from 10^{-3} to 10^{-7} M were prepared, and the cells were incubated with this solution and 1µCi/mL of ^{3}H-TdR for 3 h. The pesticides were also incubated in a similar manner with the addition of the supernatant fraction of mouse liver homogenate for metabolic activation. Incubation only occurred for 1 h in this case, to prevent any toxicity from the liver homogenate altering the results. Both groups of samples were later incubated with unlabeled thymidine. Two positive controls were used. These were 4-nitroquinolone, which produces UDS without metabolic activation, and dimethyl nitrosamine, which only produces UDS with metabolic activation. Monocrotophos was found to be positive in solutions at concentration of 10^{-3} M without metabolic activation, and at concentrations of 10^{-2} M with metabolic activation. The frequency of UDS were approximately twice that seen in the negative controls, whereas the positive controls produced approximately 10 times the frequency of UDS.

Thus monocrotophos was negative for mutagenicity in the dominant lethal assay, however was weakly positive in a UDS test with human diploid fibroblasts.

Waters MD, Simmon VF, Mitchell AD & Jorgenson TA (1980) An overview of short-term tests for the mutagenic and carcinogenic potential of pesticides. J Environ Sci Health B 15(6): 867 - 906

In cultures of human foetal lung fibroblasts a 60% formulation of monocrotophos in acetone (Azodrin-5) at unspecified doses caused UDS, an effect which was abolished in the presence of metabolic activation. The protocol for this was not elaborated, and as the magnitude of the doses was not indicated few conclusions can be drawn from this.

A 60% formulation of monocrotophos in acetone (Azodrin-5; source not given) was included at 0, 2 or 3 ppm in feeding solution for *Drosophila melanogaster*. No details of the protocol for the sex linked recessive lethal test were supplied. Monocrotophos was reported to have no effect in this test. The fact that monocrotophos is an insecticide suggests that this test is not a useful means of evaluating its toxicity.

10 NEUROTOXICITY

10.1 Mice

Gupta M & Bagchi GK (1982) Behavioural pharmacology of FURADAN and NUVACRON in mice. Ind J Hospital Pharmacy, July/August 136 - 141

Monocrotophos (source, purity unspecified), dissolved in 0.9% sodium chloride was administered IP to male albino mice (UK Horn Huderfield strain: source unspecified) at doses of 0, 2, 3 or 4 mg/kg bw using 15/group. Righting reflex, corneal reflex, pinna reflex and grip strength were measured and a traction test was done. Awareness was evaluated by assessing the movement of the mice when held by the scruff of the neck in a variety of positions, including a normal walking position, vertically or supine. A somersault test, involving tossing the mouse so that it performs 2 or 3 somersaults, and evaluating how the animal lands (on all 4 feet, on one side etc) was performed. Motor activity was assessed using a photo-actometer. The incidences of stereotypy and catalepsy were also evaluated.

Doses of 2 mg/kg bw monocrotophos had a significant effect on awareness in comparison to controls, with the effect being between 'strong effect' and 'very strong effect'. There was no effect on performance in the somersault test. Motor activity was slightly altered by monocrotophos, but not in a dose-related manner. The pinna reflex was absent in all treated groups. There was also a dose related decrease in gripping time, with controls gripping for 35 seconds, and the high dose group gripping for 26 seconds. Mice treated with monocrotophos were inactive for 30 - 60 min after administration, with behaviour gradually returning to normal over 240 min. Therefore there appears to be short term effects on the neurological system in mice; the time frame is consistent with these being related to ChE inhibition.

10.2 Rat

Rose GP & Dewar AJ (1980) Toxicity studies on the RIPCORD/AZODRIN formulation EF 5254: Biochemical and functional studies on the neurotoxicity of the formulation EF 5254 in the rats. Shell Research Ltd, Sittingbourne. TLGR.79.027.

The neurotoxicity potential of a combined formulation of monocrotophos and a pyrethroid was assessed using Wistar rats (Tunstall Breeding Unit). The formulation (EF 5254) contained 50 g/L pyrethroid (unspecified) and 200 g/L monocrotophos in hexylene glycol and Shellsol AB. The LD50 of this formulation had been established at 96 mg/kg bw. Five treatment groups, of 8 rats/sex/group were established. Group 1 received blank formulation at 25 mg/kg bw/day, group 2 received EF 5254, group 3 received EF5254 without monocrotophos and group 4 received EF 5254 without the pyrethroid. Group 5 was a positive control group, receiving a dose of pyrethroid (unspecified) known to be neurotoxic (150 mg/kg bw). Rats were observed for clinical signs, and were also tested for neuromuscular function by being placed on an inclined board; as the angle of the board was increased, the angle at which the rat began to slip was measured.

Three weeks after dosing commenced, rats were euthanised. The right and left sciatic nerves were dissected out to the distal phalangeal branches of the posterior tibial nerves. These nerves were then sectioned into proximal and distal portions. The right and left trigeminal ganglia were dissected free after removal of the brain. The levels of -galactisodase and -glucuronidase in these tissues were analysed.

No mortalities were seen in groups 1 or 3. In the group receiving EF5254, 6/8 females died while all males survived. In group 4, 4/8 females and 2/8 males died, while 1 male in the positive control group died. Males receiving EF 5254 either with or without monocrotophos showed decreased body weights, as did control animals. There were no significant effects on body weights in female animals. Clinical signs observed in animals receiving EF 5254 were ataxia, tiptoe walk, hyperexcitability, convulsions, tremors, unkempt appearance and lethargy. Animals receiving monocrotophos alone showed tiptoe walk, tremors, lethargy and an unkempt appearance. Positive control animals showed ataxia, splayed gait, tiptoe walk, hyperexcitability and lethargy, with one animal showing tremors. There were transient decreases in neuromuscular function in animals receiving EF 5254, monocrotophos and in positive controls. These changes were only significant in female animals.

Analysis of enzyme levels in sciatic nerve revealed a significantly increased ($p<0.01$) level of both -galactisodase and -glucuronidase in the proximal nerve section following EF 5254 administration. Following monocrotophos administration, glucuronidase levels were significantly increased in both the proximal and distal sciatic nerves ($p<0.01$), with a lesser increase ($p<0.05$) in galactosidase levels in both sections. Positive control animals showed increases in both enzymes in both the proximal and distal sections of the nerves. In the trigeminal ganglion, both enzymes were increased following EF 5254 administration, and in the positive control group. Levels of glucuronidase were increased following monocrotophos treatment.

Therefore there is an indication that monocrotophos may cause changes to sciatic nerve enzyme levels similar to those seen following a known neurotoxic pyrethroid. It must be noted however, that the dose of monocrotophos used was relatively high, at around 1/4 of the LD50, and produced deaths and obvious clinical signs. It is of interest that despite the transient changes in neuromuscular function assessed clinically, there was no histopathological examination of the nerves.

Wolthuis GL, Hoodendijk EMG & Vanwersch RAP (1982) Behavioural effects in rats of low doses of insecticides in relation to brain and blood cholinesterase activity. Addendum to the first interim report. Shell Project 7-1-81. Rijswijk Medical Biological Laboratory TNO

Monocrotophos (purity 95%, source: Chrompack BV Middleberg, the Netherlands) was administered to male Wistar rats (MAG/MBL) (source not specified) in a number of experiments. The LD50 of the chemical was determined to be 6.1 mg/kg bw in this strain.

The experiment aimed to determine the peripheral effects of monocrotophos by measuring the respiratory minute volumes and the effects on the neuromuscular junction. This was measured by tetanically stimulating the sciatic nerve, and measuring the contraction of the gastrocnemius soleus muscle. Initially, 12 mg/kg bw was administered. This produced respiratory arrest within 30 min. Neuromuscular transmission was significantly inhibited at this time.

When rats were pretreated with 50 mg/kg bw of atropine sulphate or atropine methylnitrate, the dose of monocrotophos required to induce respiratory failure increased to 15 times the LD50, or approximately 92 mg/kg bw. At this dose, respiratory failure was induced by inhibition of neuromuscular transmission.

A diaphragm strip prepared from a rat which died in one of the above experiments was mounted in a laboratory preparation, and was washed free of monocrotophos. Neuromuscular transmission was restored following washout of the pesticide; a second dose of pesticide resulted in a return of inhibition. These trials indicated that monocrotophos has primarily a peripheral

action. This is supported by the significant preventative role of atropine which is unable to cross the blood-brain barrier.

Following the above trials, a number of behavioural trials were performed. A runway performance test using trained rats (9/group) was done. Treated rats were injected SC with approximately 2 mg monocrotophos/kg bw, and control rats injected SC with saline. The reaction time (time from box being opened to rat entering the runway) and the running time were measured using photoelectric beams. Monocrotophos had no effect on the running times in this trial. The reaction time 60 minutes after injection was increased related to monocrotophos dosing; this was related to one rat taking 3 seconds to leave the box.

Open field behavior was measured in rats receiving 0, 0.6, 1.2 or 1.8 mg/kg bw monocrotophos by SC injection, with open field measurements commencing 30 min after injection. Animals were euthanised approximately 2 h later, and the ChE activity in plasma, blood and brain determined. A dose of 0.6 mg/kg bw had no effect on open field behavior, however the higher two doses decreased the distance moved and areas entered in the trial. All doses administered produced significant ChE inhibition in the plasma, blood and brain.

Rats were injected daily with 0.18 mg/kg bw monocrotophos or saline (control). Two rats/group received IP injection, while 3 rats/group received SC injection. Rats were injected 5 days/week for 3 weeks, with body weight and tail length determined at the end of the trial. Open field behavior was measured on days 5 and 12, and was unaffected by this dose of monocrotophos. ChE inhibition was measured 1, 2 and 3 h after injection in rats receiving SC injections. There was significant inhibition of plasma, blood and brain ChE at all three times at this dose.

10.3 Hens

Jenkins LJ (1981a) 14 day neurotoxicity study of AZODRIN in chicken hens. Lab: Food and Drugs Research Laboratories, Report 6535-1 Sponsor: Shell Development Company, Houston WRC RIR-147

Monocrotophos technical (source, purity not specified) was administered by oral gelatin capsules to White Leghorn hens (Babbock Farms, Ithaca NY) at doses of 0, 0.03, 0.1, 0.3 or 1.0 mg/kg bw/d for 14 d using 5 hens/group. There were also 2 positive control groups, receiving 50 or 100 mg/kg bw/d tri-ortho-cresyl-phosphate (TOCP). Negative control animals received a capsule containing ground feed. Capsules were prepared on the day of dosing. Blood was collected from all hens on the d before dosing and on d 1, 7 and 14. Plasma and erythrocyte ChE activity were determined. After 14 d, the hens were euthanised, and the whole brains collected and weighed. The brain ChE activity and neurotoxic target esterase (NTE) levels were determined. Any animals in a moribund condition were sacrificed, and examined as for animals at the end of the study.

Hens were examined daily for clinical signs, including observations of walking. Delayed neuropathy signs were scored as follows: 0 = no detectable signs, 1 = slight unsteadiness on walking, 2 = marked staggering and occasional falling, 3 = advanced neurotoxic signs, extreme difficulty in walking, falling often, 4 = unable to walk, standing with difficulty, 5 = complete motor paralysis of legs, lying on side.

Hens in the high-dose monocrotophos group were euthanised on day 3. Two of the animals were unable to stand, while the other three were unable to walk or obtain food or water. These effects were considered to be due to acute compound related effects, rather than to any delayed

neuropathy. Body weight of hens receiving 0.3 mg/kg bw/d monocrotophos was significantly decreased at the end of the study. No other body weight changes were noted. Egg production was significantly ($p<0.05$) decreased in all treatment groups except the lowest dose. Significant plasma ChE inhibition was seen at 0.1 mg/kg bw/d of monocrotophos, while brain ChE activity was only inhibited by doses of 1.0 mg/kg bw/d. No NTE inhibition was seen at any dose level of monocrotophos, while the positive control produced 90% inhibition. Neurological scoring indicated that, although there was a slight increase in scores at 0.3 mg/kg bw/d (due to a score of 1 in one individual), there was no significant change related to treatment. The animals on 1.0 mg/kg bw/d had high neurological scores prior to their euthanasia, however this was considered to be due to the acute effects of the compound. Therefore there was no evidence in this study of delayed neuropathy related to treatment with monocrotophos.

Owen DE & Butterworth STG (1978) Toxicity of organophosphorus insecticide AZODRIN. Investigation of the neurotoxic potential of AZODRIN-5 to adult domestic hens. Shell Research Ltd, Sittingbourne. TLGR.0066.78

Warren Studdler laying hens (Lervill Farms Ltd, Ken) were premedicated with intramuscular injections of 17.4 mg/kg bw atropine sulphate (British Drug Houses Ltd, Dorset) and 50 mg/kg bw pralidoxime chloride (Ayerst Lab, NY). One h later, one group received 6.7 mg/kg bw of a 60% formulation of monocrotophos in acetone (Azodrin-5, source: Shell Biosciences Laboratory, Sittingbourne) given orally in gelatin capsules. This dose had previously been established as the LD50 for hens; the birds were premedicated to increase survival. A positive control group received 0.5 mL/mg tri-o-tolyl phosphate (TOTP) (source: Kodak Ltd) by gavage and a third control group was not treated. There were initially 6 hens/group, however an addition 8 hens were added to the monocrotophos group after the first treatment. Hens surviving the first injection of monocrotophos were given a second injection 3 weeks later. All animals were euthanised either 3 weeks after their final dose, or when they showed a persistent progressive ataxia.

All animals were observed daily for clinical signs, and tested for the ability to land without staggering after induced flight. On autopsy, the brains, sciatic nerve and spinal cord were removed and preserved in formalin. Sections of the cervical, thoracic and lumbar spinal cords were prepared for histopathological examination, as were section of the sciatic nerve, cerebellum and medulla oblongata.

In the monocrotophos treated group, 3 birds died within 4 days of the first dose, and one after the second dose. Five of the 8 new birds introduced also died. Overall, 5 hens survived 2 doses of monocrotophos. These birds showed no persistent ataxia or histological lesions of the nervous system and were similar to the control group. The positive controls showed ataxia, loss of coordination and loss of balance. There were foci of axonal and myelin degeneration in the spinal cord and sciatic nerves of these animals.

Shellenberger TE (1965c) Letter Report No 7 Ref Project B-4843. Stanford Research Institute, Menlo Park.

Monocrotophos (source, purity not given, code 7-3-0-0) was fed in the diet to White Leghorn hens (source not specified) at doses of 0, 1, 10 or 100 ppm monocrotophos using 10/group. A positive control group received 1000 ppm tri-orthocresyl phosphate (TOCP). After 4-weeks feeding, 5 birds/group were euthanised, and examined for gross pathology. Sections of brain, spinal cord (cervical and lumbar) and sciatic nerve were preserved for histopathological examination. The remaining birds were fed untreated diets for 4 weeks. Body weight, food

consumption and egg production were recorded on a weekly basis. Hens were examined daily for any clinical signs.

Body weights were reduced in birds on 10 ppm (by 10 - 12%) and 100 ppm (by 20 - 35%). Egg production was markedly decreased in the 10 and 100 ppm groups, with egg production ceasing totally during the 2nd week of feeding. Egg production was slightly decreased by week 4 in the 1 ppm group. Abnormal clinical signs were observed in the 100 ppm treatment group, with tremors seen after 10 - 12 days of feeding. The birds were able to stand and walk at this stage. Positive control animals showed significant neurotoxic signs after 16 -17 days, including leg weakness and an inability to stand. There were no abnormalities seen on gross pathological examination. On histopathological examination, signs of demyelination were seen at a relatively high frequency in control animals. The frequency was similar in birds treated with monocrotophos. The demyelination was more consistent and severe in the positive control animals.

Jenkins LJ (1981b) Neurotoxicity evaluation of AZODRIN insecticide: Subchronic oral administration in hens. Lab: Food and Drugs Research Laboratories (Report Np 6535-11) Sponsor: Shell Development Company, Houston WRC RIR-148

Monocrotophos (sample no. #14200-5555B, source: Shell Development Company, purity not given) was administered orally in gelatin capsules at doses of 0, 0.03, 0.1, or 0.3 mg/kg bw/d for 78 d to White Leghorn hens (10/group). Additionally, 10 hens received daily oral doses of 7.5 mg/kg bw/d TOCP as a positive control. From days 79 to 96 (the end of the study), the 0.3 mg/kg bw/d group received 0.5 mg/kg bw/d monocrotophos and the dose of TOCP given to the positive control group was increased to 10 mg/kg bw/d, as only one positive control hen had exhibited a positive neurological response at this stage. Hens were housed individually, and food and water were available ad libitum. Hens were observed once daily for clinical signs and mortality, and records of egg production were made throughout the study. All birds were observed walking daily, even where this had to be induced. Clinical signs of neuropathy were rated on a scale from 0 to 5, with 0 being no detectable symptoms, and 5 being complete motor paralysis of legs, lying on side. Blood was collected from all hens on d -1, 1, 30, 58 and just prior to sacrifice. Plasma and erythrocyte ChE activities were determined. At the end of the study, all hens were euthanised, and perfused with saline and a formaldehyde/glutaraldehyde solution for approximately 23 min. The brains, optic and cranial nerves, vertebral columns and the sciatic, tibial and perineal nerves were removed. The vertebral column was decalcified prior to removal of cervical, thoracic and lumbar section of spinal cord and dorsal root ganglion to minimise artifacts produced by tissue stretching. The prepared slides were examined by two histopathologists, and all lesions were graded on numerical scales as to severity.

There were no treatment related effects on body weight seen in this study. Hens in the 0.3 mg/kg bw/d group showed decreased egg production during days 14 - 41, however this then returned to normal levels until the end of the study. There were no clinical signs of neuropathy seen in any animals treated with monocrotophos; a small number of positive control animals showed gait deficits. There was significant inhibition of plasma ChE seen in animals in the mid and high-dose treatment groups from day 30 until the end of the end of the study. Erythrocyte ChE was inhibited only in the high dose group. There were no clinical or histopathological indications of delayed neurotoxicity in hens receiving monocrotophos, whereas those receiving 10 mg/kg TOCP showed neurotoxic signs, particularly swollen axons.

11 HUMAN STUDIES

11.1 Oral or dermal administration

Verberk MM (1977) Incipient cholinesterase inhibition in volunteers ingesting monocrotophos or mevinphos for one month. Toxicol appl Pharmacol 42:345 - 350

Groups of 6 male students, aged 20-30 and weighing 60-90 kg, received daily capsules providing 0, 0.0036 or 0.0057 mg/kg bw monocrotophos (>99% pure, in corn oil, acetone, source not specified) for 28 days. Baseline ChE levels were determined prior to administration. Plasma and erythrocyte ChE activity was determined twice weekly throughout the trial, and for 2 weeks after completion of dosing. ChE inhibition was measured colorimetrically. ALT, AST and AP levels were determined prior to the completion of dosing.

There were no toxic signs observed, and no changes in ALT, AST or AP activities. Erythrocyte ChE activity was not altered by either dose of monocrotophos. Plasma ChE levels in the 0.0036 mg/kg bw/d group decreased by 15% during the first 18 days and remained at that level. This was not considered to be a significant depression of ChE activity. In the 0.0057 mg/kg bw/day group there was a continuous decrease in plasma ChE throughout the study to a low of 24% inhibition at 28 days. Based on the inhibition of plasma ChE activity seen at 0.0057 mg/kg bw/d, the NOEL may be set at 0.0036 mg/kg bw/d.

Verberk MM (1972) Cholinesterase inhibition in man caused by 30 days administration of monocrotophos (translation). Coronel Laboratories, University of Amsterdam

Monocrotophos (purity 99%) in 90% maize oil and 10% acetone was administered to young male volunteers in two preliminary trials, followed by a longer-term study. In the first trial, 0.015 mg/kg bw/d was administered in gelatin capsules to 8 individuals. Plasma ChE levels had decreased to 65% of pretest levels within 7 days of commencing treatment. In a subsequent trial, 6 individuals were given 0.0036 mg/kg bw/d orally for 21 days. The plasma ChE activity decreased initially, then stabilised with approximately 15% inhibition in comparison to pretest levels. This trial did not maintain a control group, and the results were therefore considered to be of limited use.

In the definitive trial, 3 groups of 6 young male volunteers were administered 0, 0.0036 or 0.0059 mg monocrotophos/kg bw/d by gelatin capsule. The control group received gelatine capsules containing vehicle alone. The ChE activity of each of the volunteers was determined 4 times pretest, during administration, and 4 times up to 12 days after cessation of dosing. A limited blood test (Hb, sedimentation and leucocyte count) and urine test (albumin and sedimentation) were performed pre-test. The AST, ALT and AP levels were determined 10 days pre-test, at the end of administration and 12 days post test.

Plasma and erythrocyte ChE levels were not significantly changed in the low dose group. In the high-dose group, there was significant inhibition of plasma ChE activity from days 18 to 28 of the trial, with levels decreasing to 72% of pretest values. Erythrocyte ChE activity was not affected. Plasma ChE levels had returned to normal by day 9 after cessation of dosing. Based on the effect on plasma ChE seen at 0.0059 mg/kg bw/day, the NOEL can be established at 0.0036 mg/kg bw/d.

Feldman RJ & Maibach HI (1974) Percutaneous penetration of some pesticides and herbicides in man. Toxicol Appl Pharmacol 28:126 - 132

^{14}C Labeled monocrotophos (source: either New England Nuclear Corporation, Boston Mass, or Amersham Searle Corporation, Skokie Illinois) was used. An IV dose of 1 Ci/mL was

prepared with a propylene glycol solvent, with 1µCi administered to each volunteer (six normal male volunteers; age not specified). Urine was then collected for 5 days. The first 12 h was divided into three 4-h time periods. A 12-h sample was then collected. Samples were then collected every 24 h. The radioactivity present in the urine was determined and was used to quantify the urinary excretion following parenteral administration. The percentage of administered dose excreted following IV administration was used in a trial to determine the degree of absorption following a dermal dose. For dermal application, the dose was dissolved in acetone and applied to either one or both forearms, depending on the skin surface required to reach the dose required. The skin was air dried after application, and subjects were asked not to wash the skin for 24 h after application. Urine collection proceeded following the protocol used for IV administration.

Following IV administration of monocrotophos 68% of the administered dose was excreted in the urine in the 5 d following administration. The half life was determined to be 20 h. Following dermal application of monocrotophos, approximately 15% was excreted in urine in 5 days, indicating incomplete skin absorption of monocrotophos from the forearm skin. This indicated that approximately 22% of the administered dose of monocrotophos was absorbed in this trial.

11.2 Field studies

Guthrie FE, Domanski JJ, Chasson AL, Bradway DE & Monroe RJ (1976) Human subject experiments to estimate reentry periods for monocrotophos-treated tobacco. Arch Environ Contam 4: 217 - 225

Groups of 13-15 volunteers wearing long trousers and short-sleeved shirts worked for 8 h periods in tobacco fields treated 48, 72 or 96 h earlier with 0.5 pounds/acre of monocrotophos. At 48 h, group mean plasma and erythrocyte ChE activities were reduced by 4% and 9% respectively relative to pre-exposure. Rainfall in excess of 25 mm prior to the 72 and 96 h re-entry resulted in plasma and erythrocyte ChE inhibition being little changed from pre-exposure levels. Urine collected from volunteers 3-6 h after each exposure did not identify the presence of any dimethyl phosphate (major metabolite).

Mice (10-15) physically exposed to tobacco leaves taken directly from treated fields (as above) for 10 h/d had almost complete plasma ChE inhibition (99%) immediately after spraying. This inhibition was reduced to 42% (relative to pre-exposure) after 24 h and 40% after 48 h post spraying. This latter result at 48 h contrasts markedly with a second group of mice that had 75% plasma ChE inhibition 48 h after exposure. The reason for this discrepancy in unclear. As expected, the 72 h and 96 h levels of plasma ChE inhibition were both substantially reduced by only 6% (relative to pre-exposure) because the rain had washed the monocrotophos from the leaves.

Van Sittert NJ & Dumas EP (1990) Field study on exposure and health effects of an organophosphate pesticide for maintaining registration in the Phillippines. Med Lav 81.6: 463 - 473

This published report investigated the extent of monocrotophos exposure for 21 spraymen involved in manually spraying rice with pressurised backpack sprayers in the Phillippines. All 28 recruited volunteers (ie. including 7 controls) had no occupational exposure for at least 3 weeks before the study. In the spray operation, groups of 2 spraymen were randomly allocated 11 plots (1 of the 11 plots had only 1 sprayman) that were to be sprayed for 5 h/d over 3 consecutive days. Each group sprayed 1-1.5 ha/d with 120-240 L of 0.09-0.18% (w/v)

monocrotophos. During filling and spraying operations, spraymen were clad in normal work clothes, ie. long sleeved shirt and long trousers but without any footwear. The extent of exposure was monitored by two separate methods, namely quantifying the monocrotophos metabolite, dimethylphosphate (DMPO) in pooled urine collected over 24 h the day before, during and the day after spraying, and determining ChE activity in whole blood and erythrocytes (plasma ChE inhibition was calculated by difference). ChE activities were determined 2 h after spraying on days 1, 2 and 3 and 21 h after the third spray, ie. on day 4. DMPO concentration was determined using GC-LC methodology (detection limit 5 µg/mL). ChE activity was measured using a colorimetric assay.

Only one sprayman reported a short episode of blurred vision (duration not reported) following spraying. Excretion of urinary DMPO (expressed as monocrotophos equivalents) increased with successive daily exposure from a median of 0.07 mg/24 h (range, <0.04 to 0.58) on the day before spraying to 0.64 (range, <0.04 to 1.9), 0.74 (range, <0.04 to 5.1) and 1.9 (range, 0.09 to 6.3) mg/24 h respectively on the 3 spraying days. Even on the day following exposure, a median of 0.76 mg/24 h (range 0.07 to 3.5) was observed, indicating a relatively long half life. A mean half life of 18 h was estimated from excreted DMPO levels.

Mean percent inhibition of ChE activity in plasma and erythrocytes relative to activity in the control group are shown below in Table 1. The mean values shown in square brackets are calculated relative to pre-exposure levels in the same individual.

Table 1: Cholinesterase Inhibition - (expressed as % reduction)*

Day	Plasma	Erythrocyte
0	-8	-7
1†	-5 [-5]	-6 [12]
2†	14 [44]	8 [11]
3†	60 [75]	9 [43]
4	54 [71]	8 [36]

* negative values are where test ChE activity exceeds controls; † spraying day.

Although the exact extent of monocrotophos exposure cannot be determined from either urinary excretion of DMPO or ChE inhibition, it is clear that it is substantial. Although there were few overt clinical signs in the presence of significant ChE inhibition, the possibility of impaired nerve conduction or electromyographic effects were not investigated.

Shell Development Company (1968) Dermal exposure to Azodrin insecticide resulting from aerial application. Modesto, Shell Development Company. M-37-68

An experiment to determine the degree of exposure of workers assisting in the aerial application of monocrotophos was conducted. A 30-acre cotton field was treated with approximately 1.2 L/ha of Azodrin 5 (a 60% formulation) from a plane which made 20 passes at a velocity of 150 km/h at a height generally less than 1 m above the cotton.

The exposure of a swamper and 2 flagmen was assessed. A pre-exposure blood sample was taken, and blood was taken 4 h after exposure. Blood samples were also taken 3 and 7 days after exposure. All samples were analysed for plasma and erythrocyte ChE. Gauze patches

were attached to each man on the right and left shoulders, legs, thighs and wrists, and in the middle of the chest and back. No protective clothing was worn by any of the subjects. Exposed patches were analysed for monocrotophos residues.

Three days after spraying, 2 field checkers checked the cotton field for a 1-h period. These workers had had ChE levels determined on the day of spraying. They wore no protective clothing, and had similar gauze patches attached as did the swamper and flagmen. Blood samples were taken 3 h after the end of the exposure, and also on the 3rd and 7th days after exposure.

Of the workers, the swamper, one of the flagmen and one of the field checkers reported that they worked with organophosphorous and carbamate insecticides on a daily basis.

The gauze samples analysed showed a range of residues. The swamper samples showed a very high level (1.8 mg) on the left wrist. All other samples from this worker had residues of 6 µg or less. One of the flagmen had residues around 20 µg on the shoulders; in the other flagmen, there was no such increase. The field checkers had more consistently raised residue levels, with between 10 and 40 µg on the wrist, thigh and legs, with <10 µg on the shoulders, chest and back. These residues reflect the activities the workers were involved in; the high levels on the swampers left wrist may be the result of wiping his face.

There was no clear pattern of ChE inhibition resulting from the exposure. Two worker (one flagman and one field checker) showed either plasma or erythrocyte ChE inhibition on day 3 after exposure, but the inhibition was marginal and had resolved by day 7 after exposure. Thus it appear that in this trial, despite the lack of protective clothing, the ChE inhibition resulting from exposure to monocrotophos was minimal.

Blok AC & Mann AH (1977) Organophosphorus insecticide exposure of spraying under field conditions on rice in India. II Azodrin (Monocrotophos) The Hague, Shell International Research Maatschappi, BV Report Series Tox 77-006

The effects of monocrotophos on workers involved in its application to rice fields in India was assessed in field conditions. Five workers were involved in the application of the pesticide on 6 consecutive days, working 7 h/day. A 40% water soluble solution of monocrotophos was diluted to 0.06% and applied from a knapsack sprayer containing 10 L of formulation. On the first day of spraying, the formulation was accidentally made up at 0.12%; all other days used the correct dilution. Workers applied an average of 66 g monocrotophos/day. Workers did not use protective clothing; their normal clothing exposed the arms, legs and feet. Clean clothes were worn for each day's work, and the workers washed their hands before meals. Workers were trained in common sense ways of avoiding contamination, such as avoiding direct contact with formulation and spray mix, giving attention to the containers to ensure they were not leaking, and avoiding spraying against the wind or upwind of other workers. All workers were normal farmhands, aged between 17 and 40 years, and described as healthy and fit, with normal nutritional status. They had not had contact with pesticides for 2 weeks prior to the trial.

Five pre-test blood samples were taken from the workers applying monocrotophos, and also from 5 workers not involved in pesticide applications to act as controls, to determine plasma and erythrocyte ChE levels. Samples were taken regularly throughout the trial, and for two days at the end of the trial. It was recognised that there were practical difficulties with the sampling and testing methods, due to the uncontrolled field conditions.

No clinical signs of exposure were seen during the trial. Plasma ChE appeared to be inhibited on the evening of the first day, and morning of the second day. Control workers also had some depression of ChE, so there may have been a problem with the testing method at these times. No inhibition of plasma or erythrocyte ChE was seen for the rest of the trial. Therefore it appears that there is little effect from applying monocrotophos from a knapsack spray without protective clothing, however the quality of the assay method may obscure any real effects.

Rao RR, Marathe MR & Gangoli SD (1979) Effect of exposure of human volunteers to the aerial spray of monocrotophos. Ecotoxicol environ Safety 3: 326 - 334

Volunteers were exposed to an aerial spray of monocrotophos (40% formulation in water). In the first trial, 12 male and 5 females volunteers (aged 13 - 57) were exposed to a single aerial spray. Volunteers were examined for clinical signs, erythrocyte and leucocyte counts, Hct and ChE activity pre-test, and at 2, 24, 48 and 72 h after exposure. In the second trial, 12 male volunteers were exposed to either 1, 2 or 3 sprays (4 men/group). Hct and ChE activity were determined 2 h after exposure, then once daily for seven days, then 3 times in the next week. In both trials, men removed their shirts and women wore light clothing. They remained in the cotton field during aerial spraying and for 1 h after spraying. The only protective equipment worn was rubber finger gloves on the fingers used to take blood samples. In both trials, no abnormal clinical signs were observed. There were no significant variations in ChE activity or haematological findings, although there was a non-significant decrease in ChE activity in a number of individuals 2 h after exposure, which had completely resolved by 24 h.

Rao RR, Quadros Fmazmudar RM, Marathe MR & Gangoli SD (1980) Toxicological effects of aerial applications of monocrotophos. Arch environ Contam Toxicol 9:473-481

Twelve volunteers were exposed to monocrotophos during aerial spraying of a cotton field and adjoining grazing area with a 40% solution of monocrotophos, further diluted using 400 mL in 9L of water. The dose delivered to the area was not specified. Additionally, 2 cows, 2 bulls, 3 buffaloes and 6 chickens were exposed. Blood samples were taken 2 h and 1, 2, 3, 7 and 15 days after exposure. No abnormal clinical signs were observed. Hct, erythrocyte and leucocyte counts and ChE activity were normal in all exposed humans and animals.

Nayak NJ, Shingatgeri MK, Rao RR, Marathe MR & Gangoli SD (1975) Toxicological, residual and biological evaluation of NUVACRON 40 (monocrotophos) by aerial application under Indian field conditions. Ciba-Geigy of India Ltd. Bombay

Monocrotophos (Nuvacron 40, 40% w/v) was diluted in water (400 mL/8.5L) and applied aerially at 8.5L/acre to a 10-acre plot using a helicopter flying at 2 to 3 metres, and spraying approximately a 40 m swath. There was approximately 2 kg of monocrotophos applied overall. Workers, cattle and fowls were exposed, with the domestic animals being tethered along footpaths at the edges of the sprayed plot, while workers continued to work in the plot during spraying. Only light clothing was worn, and men remover their shirts. The only protective clothing used was a rubber finger glove to protect the blood sampling site.

Blood was taken from each volunteer 1 to 2 days before spraying, and then between 1 and 3 h after spraying. Samples were also taken 24, 48 and 72 h after the application of the monocrotophos. No abnormal clinical signs were noted in the volunteers. There were no changes in ChE activity, erythrocyte or leucocyte counts or Hct values, either before or after spraying.

Ullmann L, Phillips J & Sachase K (1979) Cholinesterase surveillance of aerial applicators and allied workers in the Democratic Republic of the Sudan. Arch environ Contam Toxicol 8:703 - 712.

Whole blood cholinesterase activity of all personnel engaged in seasonal aerial spraying with Nuvacron 40 SCW (40% formulation of monocrotophos in water) and Nuvacron Ulvair Combi C500 (monocrotophos and DDT) in the Sudan was monitored. No baseline figures could be obtained for the groups, as they had been working with pesticides previously. Control values from similar populations were used. The Hct of these workers was also determined to rule out anaemias which may have altered the results. In all cases, blood was taken from washed fingertips of the left hand. Four groups were investigated: pilots, aircraft engineers, aircraft, landing strip and ground personnel and entomologists. All checks were done between 8 am and 10 am, with some regions being checked 3 times over the 4 week spraying period, and others being checked twice. In total, 70 600 ha were sprayed, with a total of 148 445 L of 40% monocrotophos in water, and 4795 L of the monocrotophos/DDT mixture.

Protective clothing was supplied, and workers were encouraged to wear it, however it was unpopular as the conditions were very hot. Overalls were supplied to landing strip and ground personnel, and were laundered 2 to 3 times per week, depending on use. Goggles and gas masks were used when handling pesticides, and gloves were used when handling any chemicals or chemical equipment.

Tests on ground personnel revealed significant ChE inhibition. On the first check in the Medani region, 82% of ground personnel had inhibition of >20%, while 41% had >60% inhibition. At the second check, 90% had >20% inhibition, however 25% had >60% inhibition. On the 3rd check, 86% had >20% inhibition, while 11% had >60% inhibition. The techniques to reduce exposure appeared to be effective in decreasing the more significant exposures, but were not effective on lower level exposure. On the first check in the Suki region all workers showed inhibition of >20%, with 67% showing inhibition of >60%. On the second check, 80% of workers had inhibition of >20% while 30% had inhibition of >60%. In the Managil region, no workers had inhibitions of >60%, while approximately 50% had inhibitions greater than 20% in both tests. The ChE inhibition seen in other workers (pilots, entomologists and aircraft engineers) was significantly less, with very few workers showing inhibition of >60%. Among the engineering staff, many had inhibitions of >20% on the first 2 tests.

Therefore, there was significant exposure of many staff involved in aerial spraying in the Sudan. Exposure was reduced, but not eliminated by attempts to limit exposure. Ground personnel were the most exposed group, with pilots, entomologists and engineers showing lower levels of inhibition.

Gaeta R, Puga FR & Mello D. de (1975) Determination of cholinesterase activity in workers exposed to the action of monocrotophos, an organic phosphorus insecticide. O Biologico 41: 73 (translated from Portuguese)

Monocrotophos (60% EC, diluted at application to 0.25% aqueous solution) was applied by 2 groups of workers, 46 working on immature plants, and 26 working on mature plants. All workers were male, aged between 15 and 54 years. No protective clothing was worn, and clothes were not washed after each day's work. Blood samples were taken one day before and one day after each application cycle. ChE activity in whole blood was determined on site; additional samples were collected and the plasma ChE activity determined in a laboratory.

During the study, one individual showed clinical symptoms of intoxication, and a number of workers on the property who were not involved in the study required hospitalisation. There was no difference in the pattern of ChE activity in the workers applying chemicals to mature or immature plants. There were significant decreases in plasma ChE (to approximately 10% of pre-exposure values) and whole blood (to approximately 65% of pre-exposure values) on the first day after spraying. These had not returned to normal after 31 days.

Sittert NJ van & Tordoir WF (1981) Exposure and biomedical monitoring study of AZODRIN/DDT hand-held ULV application on cotton in South Africa. The Hague, Shell International research Maatschappij BV Report Series TOX 81-002
and
Sittert NJ van, Tordoir WF & Kummer R (1985) Exposure and biomedical monitoring study of AZODRIN/DDT hand-held ULV application on cotton in South Africa. A re-evaluation after reconsideration of cholinesterase results. The Hague, Shell International research Maatschappij BV Report Series TOX 85-005

Two monocrotophos formulations were tested under field conditions in cotton for their potential to inhibit ChE activity. The first formulation contained 250 g/L monocrotophos and 300 g/L DDT in Emulsogen, cylcohexanone and Shellsol AB. The second formulation contained 250 g/L monocrotophos and 300 g/L DDT in cyclohexanone and ethyl dioxitol, while the third formulation contained only the hexylene glycol, cyclohexanone and Shellsol AB solvents. The formulations were applied by hand-held ULV applicators, with the flow rate set at 1 mL/sec. Sprayers were not involved in mixing, loading or filling activities; therefore their total exposure can be assumed to be related to spraying activities. Most workers wore long-sleeved overalls and boots, while some wore long trousers and long sleeved shirts. Most wore a hat. Face masks, face shields, respirators, goggles or gloves were not worn.

In the initial protocol, spraying was to occur on 5 consecutive days. Spraying with pesticides actually occurred on 2 days, with a 2-day break between spraying days. While spraying, workers wore alfoil strips attached to the chest, back and both forearms. There were removed immediately after spraying and analysed for DDT deposition. The DDT levels in the blood were also measured. The level of dimethyl phosphate in a 24-h urine sample following spraying was analysed to determine exposure to monocrotophos. Medical examinations were done both before and after spraying, including an examination of neurological functions and blood tests including glucose, urea, total bilirubin, total protein, calcium, inorganic phosphorus, uric acid, AP, AST, ALT, LDH, creatinine and cholesterol. Blood for ChE activity determination was taken in capillary tubes from finger-tip punctures. Fingers were well washed prior to sampling, however this method was later called into question.

During the study, no toxic signs were observed. The urinary excretion of dimethyl phosphate was much higher in workers spraying the formulation containing Shellsol AB than workers using the formulation containing ethyl dioxitol. This indicates a greater absorption of monocrotophos in workers using Shellsol AB, probably indicating a greater dermal penetrance. Whole blood and plasma ChE activities were significantly inhibited in both spraying groups, with levels in some cases showing 80% inhibition. This resulted in the second day's spraying being canceled due to concern for workers health. The study recommended that due to the significant inhibition of plasma ChE, the ULV formulations should not be used for hand-held application. Later re-evaluation of the study suggested that skin contamination may have partly contributed to the depressed ChE levels. It also suggested that plasma ChE may not be a suitable indicator, and that erythrocyte ChE inhibition should be used as a preferred measure of anticholinesterase activity. The re-evaluation did not amend the initial recommendations. The

study authors felt that, as there was not data available on the effects of five days consecutive spraying, it was inappropriate to amend these recommendations.

Kummer R & van Sittert NJ (1985) Field study on health effects from the application of a 20% AZODRIN formulation by hand-held ULV to cotton in South-East Celebes. Report no HSE 85.001. Shell Internationale Petroleum Maatschappij.

Monocrotophos (20% ULV formulation in hexylene glycol and ethyloxitol acetate) was handsprayed to cotton, and the exposure and effects assessed. There were five groups of workers with a variety of exposures. Group 1 was a control group of 7 workers. Group 2 consisted of 1 filler and 4 spraymen who sprayed 10 L/person in one day. Group 3 consisted of 1 filler and 4 spraymen who sprayed 10 L/person/day on 2 consecutive days. On their second day of spraying, the filler worked as a sprayman, and all workers filled their own spraytanks. Group 4 had 3 spraymen, spraying 10 L/person, with each person doing their own filling and cleaning. Group 5 had 4 spraymen applying 4 L/person in one day. The recommended protective equipment for workers was long pants and a long sleeved shirts. Workers specifically involved in filling wore overalls, mask, rubbers boots and gloves; this equipment was not worn when sprayers filled their own drums. Not all workers wore the recommended clothing, with some choosing to wear shorts and short sleeved shirts.

The study involved observing spraying, to determine possible times and degrees of exposure, checking workers for signs of intoxication either during or after spraying, determining the ChE activity in whole blood, erythrocytes and plasma, using blood collected either from a finger prick or from the earlobe, and collecting a 24-h urine sample to determine the excretion of dimethyl phosphate, a monocrotophos metabolite.

Exposures were noted in the fillers when they touched contaminated equipment with ungloved hands, and also an occasion where a filler adjusted his mask with contaminated gloved hands. Sprayers were occasionally contaminated following changes in wind velocity or direction. Where they were filling their own containers, there was significant exposure both at filling and cleaning of the containers. All workers washed their hands after filling; this was likely to significantly reduce the absorption of monocrotophos.

No adverse clinical signs were noted during the trial. Urinary dimethyl phosphate concentrations were higher when sprayers also acted as fillers and equipment cleaners. Whole blood ChE activity was inhibited up to 19 and 36% after the first and second applications, respectively. This probably reflected plasma ChE activity, which was inhibited up to 50 and 74% after the first and second applications, respectively. Erythrocyte ChE activity was not altered.

Ware GW, Morgan DP, Estesen BJ & Cahill WP (1974) Establishment of reentry intervals for organophosphate-treated cotton fields based on human data: II AZODRIN, ethyl- and methyl parathion. Arch environ Contam Toxicol 2(2):117 - 129

In a re-entry exposure study, 4 men worked in a cotton field in Arizona for a 5-h period 24 h after the field was sprayed with 1.3 kg monocrotophos/hectare (formulation not stated). A number of measurements of contamination were done. These included foliar residue levels of applied pesticide, pesticide contamination of the hands and lower arms of workers (measured by collection of wash samples after exposure), adsorption onto clothing, concentration of pesticide in air samples (collected by 2 workers), and the effect on plasma and erythrocyte ChE levels. The foliar residues were approximately 4.7 mg/m². No clinical signs of intoxication were observed. Residues extracted from hands and clothing were: hands 3 mg; shirts 16 mg; trousers

71 mg. A respiratory dose of 27 µg/5 h was estimated. Therefore, the main exposure was dermal. Plasma ChE activity was not altered, whilst erythrocyte ChE activities were decreased, showing between 15 and 30% inhibition. In 2 subjects, erythrocyte ChE activity was beginning to rise by 24 h after exposure.

Ware GW, Morgan DP, Estesen BJ & Cahill WP (1975) Establishment of reentry intervals for organophosphate-treated cotton fields based on human data: III 12 to 72 hours post-treatment exposure to monocrotophos. Arch environ Contam Toxicol 3:9113 - 9130

Monocrotophos was applied to cotton at approximately 1 kg active/ha. Five volunteers entered the cotton fields 48 or 72 h after application. The air concentration of the pesticide was analysed via collection devices carried by 2 volunteers. Foliar residues were determined immediately after application, and after 24, 48 and 72 h. Clothes and hand rinse samples of the volunteers were analysed for residues, and the plasma and erythrocyte ChE activities of volunteers were determined.

Foliar residues of monocrotophos decreased over the sampling time from 12.8 mg/m^2 immediately after exposure to 4.3 mg/m^2 after 72 h. The air samples did not reveal detectable monocrotophos levels. The monocrotophos residues after 48 h were 2 mg from hand rinses, 13.5 mg on the shirt and 20 mg on trousers. After 72 h, residues were very similar, with 2 mg on hands, 13.4 mg on the shirt and 29 mg on the trousers. No toxic signs were observed in any of the workers. Plasma ChE levels were slightly depressed following exposure at 48 and 72 h, while erythrocyte ChE activity was slightly depressed at 48 h. All decreases in ChE activity were less than 20% in comparison to pre-exposure levels, and were not considered to be of biological significance.

Guthrie M, Domanski JJ, Chasson AL, Bradway DE & Monroe RJ (1976) Human subject experiments to estimate reentry periods for monocrotophos treated tobacco. Arch Environ Contam Toxicol 4:217 - 225.

Monocrotophos was applied at 450 g/ha, using a 300 g/L water miscible formulation. Volunteer workers, wearing long pants and short sleeved shirts entered the are 48, 72 or 96 h after spraying. The volunteers were required to remove the blossom, sucker or top-most leaf of the plant. Pre-exposure blood samples were obtained on the evening before exposure. Post-exposure samples were obtained in the evening of the day of exposure. Pre- and post-exposure urine samples were obtained. Leaves were collected at random from the upper portion of the plant for residue analysis. Mice were also exposed to treated leaves, and the ChE activities determined. The mean ChE inhibition following exposure of workers was 9% for both plasma and erythrocyte activity at 48 h after treatment; inhibition at 72 and 96 h was less than this. Therefore no significant ChE inhibition was seen. Information on the exposure of mice to treated leaves was not supplied.

11.3 Poisoning studies

Simson RE, Simpson GR & Penney DJ (1969) Poisoning with monocrotophos, an organophosphorus pesticide. Med J Aust pp 1013 - 1016, November 15 1969

A 19-year old male splashed approximately 600 mL of an emulsifiable concentrate formulation of monocrotophos onto his bare arms and chest and washed it off with water. Clinical signs including muscle weakness, blurred vision, chest pain and blackouts occurred 28 h after exposure. Signs on admission to hospital included lethargy, dry retching, inability to stand, constricted pupils and increased salivation. After repeated doses of atropine and 2-PAM, recovery from the acute signs occurred after 3 days of treatment. When examined 11 days after

exposure, the patient reported slight numbness in the arms and hands. Whole blood ChE activity decreased to 10% of normal reference values at 1.5 days after exposure and returned to normal after 8 weeks.

Przezdziak, J & Wisniewsa W (1975) (A case of acute organophosphorus poisoning) Wiad Lek 28(12) 1093 - 1095 (In Polish - abstract translated)

A 54-year old man was severely intoxicated after accidentally swallowing 1/2 teaspoon of monocrotophos (Nuvacron). He was administered to hospital with general symptoms of organophosphorus poisoning. Consciousness returned on the fourth day. Recovery was complicated by bilateral bronchopneumonia and extensive thrombophlebitis, but was complete after 33 days.

Gelbke LC & Schlicht HJ (1978) Fatal poisoning with a plant protective containing monocrotophos, dodine and dinocap. Toxicol Eur Res 1(3): 181 - 184

A 22-year old woman, weight 52 kg, was found dead, with 6 empty vials which had each previously containing 0.2 g monocrotophos, 0.06 g dodine and 0.12 g dinocap, beside her. Her blood alcohol level was 0.12%. There were no abnormal macroscopic or microscopic observations at autopsy apart from vascular congestion in the abdominal organs. Monocrotophos was detected in stomach (350 ppm - 52 mg total), liver (1.8 ppm) and other tissues, including blood (11 - 13 ppm). Dodine and dinocap were not detected. Death was thought to be due to monocrotophos poisoning since the ingested dose of 23 mg/kg was similar to the oral LD50 for rats, whereas dodine and dinocap have low acute oral toxicity.

Senanayake N & Karalliedde L (1987) Neurotoxic effects of organophosphorus insecticides. N Engl J Med 316: 761 - 763

"Intermediate Syndrome" experienced after OP poisoning can be distinguished from the characteristic muscarinic, nicotinic and CNS effects observed soon after exposure and from the delayed neurotoxicity effects seen 2-3 weeks later. Intermediate syndrome occurs 24-96 h after exposure and is characterised by muscular weakness affecting neck, proximal limb and respiratory muscles. Since only some OPs are capable of inducing this phenomenon, this study retrospectively (last 3 years) reviewed 10 OP oral poisoning cases (9M & 1F; 9 attempting suicide and 1 after spraying) that exhibited this condition. Although the ingested volumes were unknown, 4 had consumed fenthion, 2 had dimethoate, 2 had monocrotophos, 1 had methamidophos and an unknown OP for the remaining one. The age of most patients ranged between 22-27 with two being substantially older; 55 and 60 respectively. Treatment with pralidoxime (1g, bid) and atropine (40 mg) was initiated immediately upon admission and continued for up to 48 h. ChE activity was not measured, however, nerve conduction velocity measurements (median nerve, motor and sensory; and peroneal nerve, motor) and electromyography (distal and proximal limb muscles) were performed.

Three patients (2 with fenthion and one with dimethoate) died within 3-15 days after exposure despite atropine and oxime therapy. After the classical clinical signs of OP poisoning, namely miosis, salivation, sweating, and fasciculations lasting 24-96 h, the first sign to indicate onset of Intermediate Syndrome was respiratory insufficiency in the absence of any classical signs of OP poisoning. Three of the 7 survivors required assisted ventilation (1 monocrotophos, 1 dimethoate and 1 unknown OP) for up to 18 days. Delayed polyneuropathy, characterised by paralysis of distal muscles of limbs 2-3 weeks after poisoning, was observed in the one patient on methamidophos. Nerve conduction and routine electromyography appeared normal in all cases, however, the tetanic stimulation of the abductor pollicis brevis (thumb or first digit muscle) *via* surface electrodes on the median nerve at the wrist during active Intermediate

Syndrome symptoms revealed marked fade at 20 (20-80%) and 50 Hz (30-70%). There was no post-tetanic facilitation.

Therefore, in one case involving monocrotophos a patient required assisted ventilation for a period beyond that of severe acute signs. It is difficult to determine whether this was a sign of Intermediate Syndrome, as there was no measurement of ChE activity at the time.

Mani A, Thomas MS & Abraham AP (1992) Type II paralysis or intermediate syndrome following organophosphate poisoning. JAPI 40: 542 - 544

This report describes the case histories of 3 patients who ingested Ops. One of the 3 had taken monocrotophos whereas the others took parathion and an unidentified OP (only details referring to monocrotophos will be detailed in this summary). A 19-year old male was admitted to hospital 14 h after ingesting an unknown volume of monocrotophos. Despite gastric lavage and 2-PAM/atropine therapy for 48 h, he had difficulty breathing, thereby necessitating ventilator support. Neurological examination revealed bilateral facial paresis together with neck and proximal leg muscle weakness. Ventilator support was removed on day 9 and after further improvement, he was discharged on day 16 with slight proximal limb muscle weakness. An outpatient visit two weeks later did not reveal any neurological sequelae.

Peiris JB, Fernando R & De Abrew K (1988) Respiratory failure from severe organophosphate toxicity due to absorption through the skin. Forensic Sci Internat 36: 251 - 253

A 32-old man was admitted to hospital with a 50 mm gash above left eyebrow. The injury sustained 3-4 h earlier was the result of a glass bottle, containing 100 mL of 60% monocrotophos, being thrown and breaking on impact. The excess liquid was wiped off but the skin was not washed. After admission, nausea, vomiting, muscle fasciculations, pinpoint pupils, excessive sweating and abdominal pain developed, prompting atropine and pralidoxime therapy 22 h after the initial exposure. Little change occurred until day 4 when respiration became difficult, necessitating assisted ventilation. By day 6, sweating and vomiting had subsided but generalised muscle weakness and respiratory difficulties persisted up until day 15. On day 16, the patient was ambulatory with apparently normal muscle strength, but his blood ChE activity was still between 37.5% and 50% of normal on day 21. Given the low ChE activity at this stage, it is difficult to determine that this was a case of Intermediate Syndrome, but may be more directly related to inhibition of ChE activity.

DISCUSSION

Acute Toxicity

The acute toxicological profile of monocrotophos is typical of organophosphorus anti-ChE pesticides, with clinical symptoms being similar in experimental animals and humans.

Monocrotophos is extremely toxic by the oral route, with a median LD50 in rats of 8.4 mg/kg bw. Dermal toxicity of this compound is related to the solvent used, with significant variability observed. In rats, the dermal toxicity ranges from high to low (LD50 199 - >2000 mg/kg bw), while in rabbits monocrotophos is of moderate to high toxicity (LD50 130 - 709 mg/kg bw). It also has a high inhalation toxicity. The metabolites of monocrotophos are less toxic than the parent compound, although still extremely toxic. The trans-isomer of monocrotophos is significantly less toxic, with an oral LD50 of 207 mg/kg bw in rats.

Cholinesterase Inhibition

As ChE inhibition is a primary target for monocrotophos toxicity, a summary of the NOEL findings for ChE inhibition in a range of repeat-dose studies is shown in the Table below. NOELs are presented for plasma, erythrocyte and brain ChE activity.

Summary of NOELs (mg/kg bw/d) for Cholinesterase Activity Inhibition Following Monocrotophos Administration

Species	Duration	Plasma ChE	Erythrocyte ChE	Brain ChE
Mice	5 week	<0.015	<0.015	1.5
Mice	2 year	<0.15	<0.15	<0.15
Rat	5 week	0.005	0.005	0.005
Rat	13 week	<0.005	0.0125	0.025
Rat (dermal)	28 day	1	1	1
Rat	12 week	0.03	0.03	0.03
Rat	2 year	<0.05	0.05	<0.05
Rat	2 year	0.005	0.005	0.005
Dog	13 week	0.038	0.038	0.038
Dog	2 year	0.04	0.04	0.004
Human	28 day	0.0036	0.0059	Not tested

There appears to be no clear difference in binding affinity with plasma (a pseudo- or butyryl-ChE), erythrocyte or brain ChE (acetyl- or true ChE). There is considerable variation between studies, with brain ChE on occasions shown as either the most sensitive (particularly in the 2-year dog study, or the least sensitive (in short term mice and rat studies). Other studies show no difference in sensitivity between the different ChE activities.

Neurotoxicity

The anticipated clinical signs associated with OPs and attributable to an excessive interaction of ACh with the muscarinic and nicotinic cholinergic receptors were common to all animal studies using monocrotophos. Measurements of plasma, erythrocyte and brain ChE activity in a variety of studies did not reveal a clear hierarchy of inhibition.

There was no evidence for delayed neurotoxicity effects in a range of studies using hens, varying from single oral administration to a 78-day study. A 2-year rat study investigated histopathological changes in peripheral and central nerves, and found no evidence for a dose-related increase in abnormalities. Progressive examinations through the 2-year period did not provide evidence for any acceleration of normal age-related changes.
In reports of human poisonings, there were a number of cases of 'intermediate syndrome', involving muscular weakness and paralysis. In many of these reports, ChE activity had not been measured. Overall, the weight of evidence would suggest that monocrotophos does not produce chronic neuropathological changes.

Genotoxicity

Monocrotophos was positive for gene mutation in a number of the *in vitro* assays, including tests in bacteria, fungi and cultured mammalian cells. There was also evidence of chromosomal damage both *in vitro* and *in vivo*, as well as other nuclear damage. Metabolic activation was not required for the genotoxic effects to become apparent. In many of these studies, the dose of monocrotophos required to produce effects was quite high, and cell survival in some trials was low. In *in vivo* trials, monocrotophos was a weak mutagen at relatively high doses. Monocrotophos would appear to be a weak mutagen, however mutagenic effects *in vivo* are only observed at doses significantly greater than those producing ChE inhibition in mammals.

Reproduction and Development

A development study using Sprague Dawley rats showed a dose-related decrease in the percentage of male foetuses. However, this effect was not seen in a developmental study using Charles River rats, or in a number of multi-generation reproduction studies in Wistar or Long-Evans rats.

In New Zealand rabbits, there was an increase in the incidence of premature deliveries seen at 3 mg/kg bw/d. It was not possible to assess whether this was dose related, as the survival rate at the highest dose level (6 mg/kg bw/d)was very low. The effect was not seen in a second study using another strain of rabbits.

Overall, development signs were seen only at doses at or near maternotoxic doses, and there were no significant teratogenic findings.

Carcinogenicity

There were no carcinogenic effects seen over 2 years with monocrotophos at the highest dose tested in CD mice (approximately 1.5 mg/kg bw/d)), in Charles River rats (approximately 5 mg/kg bw/d), in Wistar rats (approximately 0.5 mg/kg bw/d) or Beagle dogs (approximately 0.4 mg/kg bw/day).

Human Studies

Monocrotophos mainly produced classical signs of OP toxicity, however there were a few reports of intermediate syndrome. An absorption study indicated that 22% of monocrotophos applied to forearm skin was absorbed. A field trial involving the use of hand-held ULV spray devices resulted in significant ChE inhibition in applicators. Ground crew involving in loading and cleaning aircraft involved in aerial spraying had significant plasma ChE inhibition. Repeated testing, combined with increased use of protective clothing, decreased the severity of the inhibition, but did not eliminate it during a trial period.

NOEL considerations

A summary of the NOELs determined for monocrotophos are shown in the Table below.

Study Type	NOEL (mg/kg bw/day)	LOEL and Toxic Effect
CD mouse: 5 week dietary	<0.015	Plasma and erythrocyte ChE inhibition in males at 0.015 mg/kg bw/day
CFE rats: 5 day dietary	< 1.68	Slight cholinergic signs seen at 1.68 mg/kg bw/day
Wistar rat: 13 week dietary	< 0.005	Plasma ChE inhibition in females at 0.005 mg/kg bw/day
Wistar rat: 5 week dietary	0.005	Plasma and erythrocyte ChE inhibition at 0.025 mg/kg bw/day
TifRAIf rat: 28 day dermal	1	Plasma, erythrocyte and brain ChE inhibition at 10 mg/kg bw/day
NZW rabbit: 3 week dermal	< 42	Clinical signs at 42 mg/kg bw/day
Albino rabbit: 3 week dermal	22	Skin irritation and kidney and splenic enlargement at 44 mg/kg bw/day
Long Evans rats: 12 week dietary	0.03	Blood and brain ChE inhibition at 0.15 mg/kg bw/day
Beagle dogs: 13 week dietary	0.038	Plasma, erythrocyte and brain ChE inhibition at 0.38 mg/kg bw/day
Beagle dogs: 12 week dietary	1.2	Tremors at 3.6 mg/kg bw/day
CD mice: 2 year dietary	< 0.15	Plasma, erythrocyte and brain ChE inhibition at 0.15 mg/kg bw/day
Charles River rats: 2 year dietary	<0.05	Plasma and brain ChE inhibition at 0.05 mg/kg bw/day
Wistar rats: 2 year dietary	0.005	Plasma, erythrocyte and brain ChE inhibition at 0.05 mg/kg bw/day
Beagle dogs: 2 year dietary	0.004	Brain ChE inhibition in females only at 0.04 mg/kg bw/day
Wistar rats: 2 generation	0.05	Impaired teat development, decreased pup survival at 0.15 mg/kg bw/day
Long-Evans rats; 3 generation	maternal effects: 0.25 reproductive effects: 0.1	Body weight decreases in adults at 0.6 mg/kg bw/day; decreased pup survival at 0.25 mg/kg bw/day

Long Evans rats: 3 generation	Maternal effects: 0.25 reproductive effects: 0.1	Body weight decreases in adults at 0.6 mg/kg bw/day; decreased litter size at 0.25 mg/kg bw/day
Sprague Dawley rats: gavage teratology	maternotoxicity: 0.3 foetotoxicity: 0.1	Clinical signs in dams at 1 mg/kg bw/day; delayed ossification at 2 mg/kg bw/day
Charles River rats; Gavage teratology	1	Clinical signs in dams at 2 mg/kg bw/day; decreased mean foetal bodyweight and crown-rump length at 2 mg/kg bw/day
New Zealand White rabbits: gavage teratology	1	Maternal deaths and clinical signs at 3 mg/kg bw/day; agenesis of lung lobe at 3 mg/kg bw/day
Dutch banded rabbits: Gavage teratology	0.8	No abnormalities observed at 0.8 mg/kg bw/day (highest dose tested)
Male humans:28 day oral gelatin capsule	0.0036	Plasma ChE inhibition at 0.0057 mg/kg bw/day
Male humans:28 day oral gelatin capsule	0.0036	Plasma ChE inhibition at 0.0059 mg/kg bw/day

A NOEL of 0.0036 mg/kg bw/day was observed in 2 studies using human volunteers in 28-day oral dosing trials. This is comparable to the lowest NOEL found in long term animal studies, with a NOEL of 0.004 mg/kg bw/day in a 2-year dog study, and 0.005 mg/kg bw/day in a 2-year rat study. The similarity in NOELs indicates that there may be limited interspecies variation in the potency of monocrotophos inhibition of cholinesterases.

Determination of Public Health Standards

Acceptable Daily Intake

The current acceptable daily intake (ADI) is 0.0003 mg/kg bw/d. This ADI was derived from a NOEL of 0.0036 mg/kg bw/day, based on plasma ChE inhibition in human oral dosing experiments.

No change is recommended to the current ADI. The NOELs found in a short-term human study are similar to those found in long term animal studies.

Public exposure

In Australia, monocrotophos does not have any registered domestic uses, and the greatest potential for public exposure is via ingestion of monocrotophos residues in food. Monocrotophos has MRLs established in a wide range of foods, including fruits and vegetables and cereal grains; the current Australian MRL list is outlined below.

Australian Maximum Residue Limits for Monocrotophos

Commodity	MRL (mg/kg)
Apple	0.5
Banana	0.5

Beans, except broad beans and soya been	0.2
Broad bean (green pods and immature seeds)	0.2
Cereal grains	*0.02
Cotton seed	0.1
Edible offal (mammalian)	*0.02
Eggs	*0.02
Meat (mammalian)	*0.02
Milks	*0.002
Pear	0.5
Potato	0.1
Poultry, edible offal off	*0.02
Poultry meat	*0.02
Sweet corn (corn on the cob)	*0.01
Tomato	0.5
Vegetable oils, edible	*0.05

* denotes the MRL has been set at or about the limit of analytical determination.

Dietary Exposure Considerations

In estimating dietary exposures, the "Guidelines for Predicting Dietary Intake of Pesticide Residues (Revised)" circulated by the Codex Alimentarius Commission in November 1996 recommends the use of National Theoretical Maximum Daily Intakes (NTMDI) as an initial estimate, while admitting that these can produce a gross overestimation of the exposure for a number of reasons. The calculation involves the use of the MRL as an estimate of the amount of pesticide in the food, and national estimates of consumption for the quantity of food consumed.

When this procedure is followed for monocrotophos, using the 1983 survey of average food consumption in Australia, it was calculated that a 75 kg adult male (weight as used by the Australian Market Basket Survey) could possibly consume 0.0012 mg/kg bw/day, as would a 60 kg adult female. This is approximately four times the current ADI (0.0003 mg/kg bw/d).

A more reliable estimate of monocrotophos intake may be derived from the Australian Market Basket Survey, a procedure which uses the measure of monocrotophos residues found in that year, rather than assuming that the pesticide is present at the MRL. The estimated intake in the group with the highest consumption of monocrotophos residues (toddlers aged two), based on the average energy intake in 1994, was 0.0000072 mg/kg bw/day. This makes up less than 3% of the ADI. Estimated intake of all groups in 1992 was approximately 10 times lower than the intake in 1994.

Market Basket Survey

The 1994 Market Basket Survey found detectable levels of monocrotophos in a small range of products. Residues were found at a maximum level of 0.01 mg/kg in white bread, 0.02 mg/kg in wholemeal bread and 0.12 mg/kg in capsicum. The residue in capsicum is a violation, as there is no registered use on this vegetable. The residues in bread are lower than the MRL for cereal grains.

Acute Reference Dose

To reflect safe/acceptable exposure from a single or short exposure to monocrotophos, an acute reference dose (acute RfD) may be derived using appropriate data. No human studies using a single oral dose are available for monocrotophos; the only human trials were the 28-day oral dosing trials used to set the ADI, with a NOEL of 0.0036 mg/kg bw/d. The only short term animal studies have NOEL which would result in an acute RfD lower than the ADI. An acute RfD for monocrotophos may be set at 0.0003 mg/kg bw, based on a 28-day human oral gelatin capsule study, ie. the same as the ADI.

Safety Directions

The current safety directions are as follows:

Monocrotophos

AC 400 g/L or less	
Very dangerous, particularly the concentrate	100, 101
Product and spray are poisonous if absorbed by skin contact, inhaled or swallowed	120, 121, 130, 131, 132, 133
Repeated minor exposure may have a cumulative poisoning effect.	190
Avoid contact with eyes and skin	210,211
Do not inhale spray mist	220, 223
Obtain an emergency supply of atropine tablets 0.6 mg	373
When preparing spray and using the prepared spray,	279, 281, 282
wear protective waterproof clothing, cotton overalls buttoned to the neck and wrist and a washable hat,.	291, 292
elbow-length PVC gloves, goggles and half facepiece respirator with combined dust and gas cartridge	294, 297, 300, 303
If product or spray on skin, immediately wash area with soap and water.	340, 341, 342
After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water.	350
After each day's use, wash gloves, goggles, respirator and if rubber wash with detergent and warm water and contaminated clothing.	360, 361, 363, 364, 366

EC 400 g/L or less in ethylene glycol, monomethyl ether	
Very dangerous, particularly the concentrate	100, 101
Product and spray are poisonous if absorbed by skin contact, inhaled or swallowed	120, 121, 130, 131, 132, 133
May irritate the eyes and skin.	160, 162, 164

Repeated minor exposure may have a cumulative poisoning effect.	190
Avoid contact with eyes and skin	210,211
Do not inhale spray mist	220, 223
Obtain an emergency supply of atropine tablets 0.6 mg	373
When opening the container, preparing spray and using the prepared spray or product	279, 281, 282, 283
Wear cotton overalls buttoned to the neck and wrist and a washable hat,	290, 292
elbow-length PVC gloves, goggles and half facepiece respirator with combined dust and gas cartridge	294, 297, 300, 303
If clothing becomes contaminated with product or wet with spray, remove clothing immediately	330, 331, 332
If product or spray on skin, immediately wash area with soap and water.	340, 341, 342
After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water.	350
After each day's use, wash gloves, goggles, respirator and if rubber wash with detergent and warm water and contaminated clothing.	360, 361, 363, 364, 366

These Safety Directions are considered to be acceptable for public health considerations.

First Aid Instructions

Currently a, h. No changes to the first aid directions are recommended.

The T-value is currently 0.8. No change is recommended.

DRAFT RECOMMENDATIONS FOR PUBLIC HEALTH STANDARDS

1. Acceptable Daily Intake

The current acceptable daily intake (ADI) for monocrotophos is 0.0003 mg/kg bw/day. This ADI was derived from a NOEL of 0.0036 mg/kg bw/day, based on plasma ChE inhibition seen in a 28-day human oral dosing study.

No change to the current ADI of 0.0003 mg/kg bw/day is recommended.

2. Acute Reference Dose

An acute RfD for monocrotophos may be set as 0.0003 mg/kg bw, based on plasma ChE inhibition seen in a 28-day human oral dosing study.

3. Poisons Scheduling

No change to the current Schedule 7 of the SUSDP is proposed for monocrotophos.

4. First Aid and Safety Directions

No changes to the current safety directions are recommended.

Note: Safety Directions recommendations relating to the use of personal protective equipment are to be provided by National Occupation Health and Safety Commission.

No changes to the current first aid directions (monocrotophos: a, h) and T-value (currently 0.8) are recommended.

5. Clearance Status

No change is recommended to the clearance status of monocrotophos.

SUMMARY OF ACUTE TOXICOLOGY HAZARD

Date of Preparation:	November, 1997
Chemical name:	Monocrotophos
Worst oral LD50 in rats:	8.4 mg/kg bw
Worst oral LD 50 in other species:	10 mg/kg bw, in mice
Worst dermal LD50, rat:	123 mg/kg bw
Worst inhalation LC50, rat:	80 mg/m^3
Skin irritation:	Slight
Eye irritation:	Slight
Skin sensitisation:	Negative
T-value:	0.8
NOEL:	0.0036 mg/kg bw/day (28-day human)

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APPENDIX I

MONOCROTOPHOS TOXICOLOGY DATA SUBMISSION DETAILS

Sponsor/Provider	Submission Number	Data Details
Shell Chemical Co.	3308	22 volumes (153 studies)
Ciba-Geigy Ltd	00035	3 volumes (24 studies)
Ciba-Geigy Ltd	10662	6 volumes (46 studies)
United Phosphorus Ltd	11544	1 volume (10 studies)
Novartis Crop Protection Australasia Ltd	11523	1 volume (4 studies)