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.