

PYRAZOPHOS

First draft prepared by S. Caroldi
Istituto di Medicina del Lavoro
Padova, Italy

EXPLANATION

Pyrazophos is an organophosphorus systemic fungicide used on a wide range of crops and cereals in the control of powdery mildew. Pyrazophos was scheduled for evaluation at the 1985 Joint Meeting but the data base available at that time was insufficient for the estimation of an ADI (Annex 1, reference 44).

EVALUATION FOR ACCEPTABLE DAILY INTAKE**BIOLOGICAL DATA****Biochemical aspects****Absorption, distribution, and excretion****Rats**

Eight Wistar rats/sex were intubated with a single 2 mg dose of ^{14}C -pyrazophos (3a- ^{14}C) in 1 ml sesame oil. Radioactivity was measured at different times in blood, plasma, urine, exhaled air, faeces and after 168 hours post-dosing, in several tissues and organs.

Blood and plasma levels of radioactivity peaked between 4 and 6 h post-dosing in both sexes; the calculated half-life was approximately 5 h. Over a period of 24 h, approximately 71% of the original dose was eliminated via urine and 24% via faeces. Radioactivity was completely eliminated within 168 h (73% via urine and 27% via faeces). A negligible amount of radioactivity (less than 0.01%) was found in exhaled air within 24 h and in tissues and organs at 168 h. Metabolites were detected in urine and faeces collected during the first 24 h when approximately 95% of total radioactivity was eliminated. Pyrazophos was not detected in urine but it was the main substance found in the faeces (66% of radioactivity in faeces and 16% of total radioactivity). Four metabolites were identified in urine; the most concentrated was a pyrazophos P-O hydrolysis product present in urine as free compound or sulphate and glucuronide conjugates (Lachmann, 1986).

Eleven Wistar rats/sex were intubated with a single dose of 0.4 mg ^{14}C -pyrazophos (3a- ^{14}C) in 1 ml sesame oil. A further 10 Wistar rats/sex received 14 daily doses of 0.4 mg of pyrazophos followed by a single dose of 0.4 mg ^{14}C -pyrazophos. Radioactivity was measured at different times in blood, plasma, urine, exhaled air, faeces and at 168 h post-test substance in several tissues and organs. Peak blood and plasma radioactivity levels were measured within 4 h post-dosing. The calculated half-life was about 5 h. Over the initial 24 h, 75% of the original dose was excreted via urine and 18% via faeces. No differences between single or repeated doses were detected. Negligible amounts of radioactivity were present in tissues and organs at 168 h from the last pyrazophos administration. Six different metabolites were found in urine. The most important was the product of a double hydrolysis 2-hydroxy-5-methyl-pyrazolo(1,5-a)pyrimidine-6-carboxylic acid. Other metabolites were O-pyrazophos and the products of hydrolysis either at the phosphate or carbonate bond. Two metabolites remained unknown. Pyrazophos was the only radioactive component present in faeces (Lachmann, 1987).

Toxicological studies

Acute toxicity studies

Results of acute toxicity tests with pyrazophos are given in Table 1.

Guinea-pigs

Pyrazophos produced no indications of any allergenic properties in the sensitization test in the guinea-pig according to the Buehler test (Leist & Weigand, 1982).

Rabbits

Undiluted pyrazophos was non-irritant to the rabbit skin. Undiluted pyrazophos and a 10% dilution of pyrazophos in sesame oil resulted in slight irritation to the eye. A 1% dilution of pyrazophos in sesame oil resulted in no irritation to the eye (Hollander & Weigand, 1977c)

Short-term toxicity studies

Mice

Five groups of ten SPF mice/sex were treated with pyrazophos for 28 days at dietary concentrations of 0, 1, 5, 25 and 125 ppm equal to 0, 0.2, 0.9, 4.7, 23.3 mg/kg/day and 0, 0.2, 0.9, 5.0, and 22.2 mg/kg bw/day for males and females, respectively. No signs of toxicity were observed; body-weight gain and food intake were not affected by pyrazophos administration. Dose-related inhibition of both plasma (from 5 ppm) and erythrocyte (from 25 ppm) cholinesterase was measured

Table 1. Acute toxicity of pyrazophos

| Species | Sex | Route | LD ₅₀ (mg/kg bw) | LC ₅₀ (mg/m ³) | Reference | |
|---------|-------------------------|--------|--------------------------------|---|--|-------------------------------|
| Rats | M | oral | 242-778 ^a | | Hollander & Weigand (1978a,b,c) Otaka <i>et al.</i> (1981a,b) Hollander & Weigand (1977f) Scholz & Weigand (1972a) Hollander & Weigand (1977a,b) | |
| | F | | 151-468 | | | |
| | M | i.p. | 280 | | | |
| | F | | 305 | | | |
| | M | s.c. | 172 | | | Otaka <i>et al.</i> (1981e,f) |
| | F | | 135 | | | |
| | M | | 202 | | | Otaka <i>et al.</i> (1981c,d) |
| | F | | 193 | | | |
| | M | | 221 | | | Hollander & Weigand (1977g,h) |
| | F | | 266 | | | |
| F | dermal (24 h exp) | > 2000 | Hollander & Weigand (1981) | | | |
| M&F | inhalation (4 h exp) | | > 1220 | Hollander & Weigand (1982) | | |
| Mice | M | oral | 214 | Hollander & Weigand (1977d,e) Otaka <i>et al.</i> (1981g,h) Scholz & Weigand (1972b) Otaka <i>et al.</i> (1981m,n) Otaka <i>et al.</i> (1981i,l) Hollander & Weigand (1977i,l) | | |
| | F | | 205 | | | |
| | M | i.p. | 413 | | | |
| | F | | 321 | | | |
| | M | | 581 | | | |
| | F | 438 | | | | |
| | M | s.c. | 167 | | | |
| | F | | 146 | | | |
| | M | | 339 | | | |
| | F | | 348 | | | |
| | M | | 181 | | | |
| F | | 199 | | | | |
| Dogs | M&F | oral | > 500 ^b | Hollander & Weigand (1978d) | | |
| | M&F | | > 100 ^c | Hollander & Weigand (1979b) | | |

^a Symptoms were mostly of the cholinergic type. Pathology showed atrophy of spleen and haemorrhage of intestine. Histopathology was negative.

^b Clear cholinergic symptoms were observed from 200 mg/kg bw, no animals died up to the highest tested dose. At 500 mg/kg bw, emesis occurred within 2 h from dosing which likely reduced the absorbed dose.

^c Clear cholinergic symptoms were observed from 50 mg/kg bw, no animal died up to the highest tested dose. At 100 mg/kg bw, emesis occurred within 2 h from dosing which could have reduced the absorbed dose.

in animals of both sexes. Marginal inhibition (approximately 20%) of brain acetylcholinesterase activity was detected after 28 days of treatment at 125 ppm in male mice only. The NOAEL in this study was 25 ppm equal to 4.7 mg/kg bw/day and 5.0 mg/kg bw/day for male and female mice, respectively (Hollander & Weigand, 1978^c).

Four groups of twenty Charles River CD-1 mice/sex were treated with pyrazophos technical at dietary concentrations of 0, 1, 5, or 25 ppm for 28 days. No signs of toxicity were observed; body-weight gain was not affected by pyrazophos administration. Inhibition of both plasma and erythrocyte cholinesterase was observed in animals of both sexes from the 1 ppm dose level. Brain cholinesterase activity was not reduced by pyrazophos administration up to the highest dose. The NOAEL in this study was 25 ppm equivalent to 4 mg/kg bw/day (Estes, 1979).

Rats

Seven groups of ten albino rats/sex from the CIVO-colony (Wistar-derived) were treated with pyrazophos (technical) at dietary concentrations of 0, 1, 2.5, 5, 15, 45 or 150 ppm for 28 days in a range-finding toxicity study. Ten additional rats per sex at 0 and 150 ppm dietary concentrations were discontinued from the feeding of pyrazophos for 21 days to examine recovery of cholinesterase activities. No signs of toxicity were observed; body-weight gain and food and water intake were not affected by pyrazophos administration. Haematology and pathology did not show any adverse effects. Dose-related inhibition of both plasma and erythrocyte cholinesterase was observed at 15 ppm and higher in both sexes. Plasma cholinesterase activity returned to normal levels in a week after discontinuing pyrazophos administration at 150 ppm. Inhibition (approx. 20%) of brain acetylcholinesterase activity was detected after 28 days of treatment at 150 ppm in female rats only. The NOAEL in this study was 45 ppm equivalent to 4.5 mg/kg bw/day in both sexes (Til *et al.*, 1978)

Five groups of ten weanling Wistar-derived rats/sex were fed pyrazophos (technical substance, purity 90%) at dietary concentrations of 0, 5, 8, 10 or 50 ppm for 14 weeks. The diets were prepared every two weeks and stored at room temperature. Data on stability and homogeneity of test substance in diets are not reported. Growth, symptoms, food consumption, haematology, blood chemistry (whole blood cholinesterases included), urinalyses, organ weights and pathology were the parameters checked in this study. No mortality occurred throughout the duration of the study. Food and water intake and body-weight were not affected by pyrazophos administration. Scattered differences of no biological relevance between groups were observed on haematology, biochemical blood values, urinalysis and pathology. Dose-related inhibition of whole blood cholinesterase activity was observed at 10 and 50 ppm pyrazophos in both sexes. The NOAEL

in this study was 8 ppm of pyrazophos equivalent to 0.8 mg/kg bw/day (deKnecht-vanEekelen & Dreef-vanderMeulen, 1978).

Four groups of thirty-six F344 Charles River rats/sex were dosed with technical pyrazophos (92.8% purity) for 13 weeks at dietary concentrations of 0, 2.5, 50 or 1000 ppm equal to 0, 0.21, 4.2 or 90 mg/kg bw/day and to 0, 0.21, 4.0 or 100 mg/kg bw/day for males and females, respectively. Twelve rats sex/group were killed at the end of the exposure period, the remaining 24 rats/sex/dose were killed, 12 after two weeks and 12 after four weeks of recovery. The diets were prepared every three weeks and stored at 4 °C. Homogeneity and accuracy of the test diets were checked (analytical results not reported). Survival was not affected by pyrazophos administration. Clinical symptoms of the cholinergic type were observed during the first 4 weeks of treatment at the highest dose level in both sexes. Body-weight gain was decreased at the highest dose level during the first 7 weeks of treatment in males and throughout the duration of the study (recovery period included) in females. Scattered differences of no biological relevance in food intake, food efficiency and water intake were observed throughout the study in all groups. Reduced erythrocyte counts, haematocrit and haemoglobin were measured at the highest dose level at the interim examination and at the end of the exposure (also an increased number of reticulocytes was measured at this time) but these parameters recovered to normal level within the 4 weeks of recovery period.

Cholesterol and albumin concentrations in blood were also reduced during pyrazophos administration at 1000 ppm and returned to normal level after cessation of exposure. Dose-related inhibition of plasma and erythrocyte cholinesterase was measured at 50 and 1000 ppm dose levels. At the end of the exposure period at 1000 ppm, brain cholinesterase were inhibited by 64% and 93% in males and females, respectively. Brain cholinesterase was also marginally affected (22% inhibition) at 50 ppm in female rats but not in males. Inhibition of brain cholinesterase was detectable at 1000 ppm 4 weeks after the end of exposure. Several organ weights of the 1000 ppm group differed from control values: the absolute and relative weights of adrenals, spleen and liver (only the relative weight of liver was increased in females) were increased in both sexes, testis and pituitary (females only) weights were decreased. Most of the differences in organ weights disappeared during the 4 weeks of recovery. Proliferation of large mononuclear cells of spleen and fat deposition in fascicular zone of adrenals were considered as dose-related, but they disappeared after the 2 weeks recovery period. The NOAEL in this study was 2.5 ppm, equal to 0.21 mg/kg bw/day, in both sexes, based on marginal brain cholinesterase inhibition measured at the termination of the study in females at 4.0 mg/kg bw/day (Otaka *et al.*, 1981o)

Four groups of forty F344 rats/sex were fed technical pyrazophos (purity 92.9%) for 52 weeks at dietary concentrations of 0, 2, 20 or 200 ppm equal to 0.1, 1.0 or 11 mg/kg bw/day and 0.1, 1.4 or 14 mg/kg bw/day in males and females, respectively. Diets were prepared every three weeks and were stored at 4 °C until

use. Homogeneity and accuracy of the test diets were checked just before starting the study and thereafter every three months. Actual concentrations of pyrazophos (mean values) were within $\pm 10\%$ of nominal concentrations. General conditions, body-weight, food and water intake were recorded throughout the study. Parameters investigated were: haematology, blood biochemistry, PSP and BSP test, in plasma in erythrocytes and brain cholinesterase activity and urinalysis. Pathology (organ weights) and histopathology (incomplete) were performed at the end of the study. Moreover the concentration of unmetabolized pyrazophos was measured in plasma, brain, liver, kidneys and fat at weeks 13, 26 and 52 (detection limit: 0.05 ppm).

No clinical signs of toxicity or excess mortality related to administration of the test compound were detected. No differences in food and water intake and body-weight gain were observed among different groups. Scattered differences of some parameters were observed throughout the duration of the study. Among them, increased erythrocyte counts and haematocrit and decreased cholesterol concentration in blood were observed at the highest pyrazophos level in both sexes. Urinalysis and the excretory function for PSP and BSP did not reveal toxic effects. Plasma and erythrocyte cholinesterase activities were reduced in both sexes at 20 and 200 ppm pyrazophos. A slight inhibition of brain cholinesterase activity (30%) was noted at 200 ppm of pyrazophos in females only. Pathology (organ weights) and histopathology did not show biologically relevant changes. Pyrazophos was measured only in fat tissue of male and female rats fed 200 ppm. Pyrazophos concentration in fat was higher in females than in males and it was higher in both sexes at the end of the study than at 13 week and 26 week determinations. The NOAEL in this study was 20 ppm equal to 1.0 and 1.4 mg/kg bw/day in males and females, respectively. The NOAEL was based on 30% inhibition of brain cholinesterase activity detected in the brain of female rats fed dietary concentrations of 200 ppm. The results of this study were obtained at the interim sacrifice of a carcinogenicity study discontinued because of the high mortality rate occurring in all groups, including controls (Otaka, 1983).

Dogs

Four groups of four pedigree English beagle dogs/sex were fed pyrazophos at dietary concentrations of 0, 0.5, 2.0, 5.0 or 10/125/320 ppm for 92 days. The highest dose level of 10 ppm was increased to 125 ppm on day 60 from the beginning of the study and further increased to 320 ppm on day 75. Two animals/sex/dose (except 0.5 ppm group) were observed for approximately 6 weeks after the end of pyrazophos administration and cholinesterase activities were estimated.

The following parameters were investigated: clinical observations (general conditions, body-weight, behaviour, reflex excitability, eye examinations, visible mucosae, dentition), laboratory examinations (haematology, cholinesterase activity in serum and in erythrocytes, other serum enzymes, urinalyses) and pathology

(organ weights, gross pathology, histopathology). Slight reduction of food intake, impairment of general conditions, tetanic cramps of the cervical muscles, disturbances of motility and slight miosis were observed in the highest dose group when the dietary concentration was increased to 320 ppm. Toxic effects were not observed in animals of the other groups. Slight anaemia was found at the highest dose level after the dose of pyrazophos was raised to 320 ppm; other clinical chemistry tests did not show any adverse effects. A dose-related inhibition of serum cholinesterase activity was observed at 5 ppm and higher in both sexes. At 5 ppm approximately 20% inhibition of erythrocyte cholinesterase activity was measured (range of 6 determinations 15%-25%) in females only. At the highest dose, erythrocyte cholinesterase activity was reduced in both sexes. Increased mean weight of the pancreas was noted in all treated groups which did not correspond to any microscopic abnormalities. The NOAEL in this study was 5 ppm, equivalent to 0.4 mg/kg bw/day based on marginal inhibition of erythrocyte cholinesterase activity measured at the next highest doses (Scholz & Brunk, 1973).

Four groups of eight English beagle dogs/sex, 13 months old at the beginning of the study, were fed pyrazophos (92.9% purity grade) at dietary concentrations of 0, 1.2, 18 or 320 ppm for 6 months. Diets were prepared daily, immediately before feeding. The following parameters were investigated: clinical observations (general conditions, body-weight, behaviour, reflex excitability, ophthalmoscopic examinations, hearing test, visible mucosae, dentition), laboratory examinations (haematology, clinical chemistry, cholinesterase determinations, hepatic and renal function tests, urinalyses) and pathology (organ weights, gross pathology, histopathology). Clinical symptoms of the cholinergic type were observed at 320 ppm as occasional diarrhoea and tetanic spasm. Some dogs of this group developed atrophy of temporal muscles. Decline of general health conditions, reduction of both food intake and body-weight were also detected. One dog in poor nutritional state died on day 107 of treatment, possibly due to respiratory muscle failure resulting from extreme cholinesterase reduction. Animals showed slight anaemia and increase of ASAT, ALAT and alkaline phosphatase in serum. Apart from occasional diarrhoea, no other toxic symptoms were observed at 18 ppm and dogs in the 1.2 ppm group were comparable to controls. Serum and erythrocyte cholinesterase activities were reduced in animals at 18 and 320 ppm throughout the duration of the study. At the end of the study, dose-related inhibition of brain cholinesterase was observed at 18 and 320 ppm (20% and 76%, respectively). The relative weights of several organs differed from control values at the 320 ppm dose level only. Pathology did not reveal compound related organic lesions. The NOAEL in this study was 1.2 ppm equivalent to 0.09 mg/kg bw/day, based on occasional diarrhoea and marginal brain cholinesterase inhibition observed at 18 ppm (Brunk *et al.*, 1982).

Four groups of four pedigree English beagle dogs/sex (13 months old at the beginning of the study) were fed pyrazophos (purity grade 92%) at dietary concentrations of 0, 2, 5 or 10/125/320 ppm for 2 years. The highest dose level of 10 ppm was increased to 125 ppm on day 14 from the beginning of the study and

further increased to 320 ppm on day 28. The test substance was mixed with food and given daily during the mid-day meal.

The following parameters were investigated: clinical observation (general conditions, body-weight, behaviour, reflex excitability, eye examinations, hearing test, visible mucosae, dentition), laboratory examinations (haematology, cholinesterase activities, serum enzymes, urinalysis), pathology (organ weights, gross pathology, histopathology).

One male dog in the 320 ppm group died of a chronic suppurating pancreatitis and a chronic ascending pericholangitis after 462 administrations of pyrazophos. Several parameters were affected in this animal but since the cause of death was not dependent on pyrazophos administration they were not considered. All remaining dogs survived till the end of the study. Food intake was not affected by administration of the test compound. At the end of the study, body-weight gain of the animals of the 320 ppm group was approximately 50% less than that in the remaining groups. Behaviour, reflex responses, ophthalmic findings, hearing test, dentition and visible mucosae were not affected. Slight anaemia and increased alkaline phosphatase were observed in animals at 320 ppm dose level. Dose-dependent decreases of serum cholinesterase activity were observed at 5 and 320 ppm. Erythrocyte cholinesterase activity was reduced only at 320 ppm. Pathology and organ weights did not show significant difference between groups. Histopathology was comparable to controls in the 2 and 5 ppm groups. At 320 ppm, calcifications in some regions of the basement membranes of the Bowman's capsules of numerous renal glomeruli, in tubular basement membranes and in the interstitium between some renal tubules were observed.

The NOAEL in this study was 5 ppm, equivalent to 0.4 mg/kg bw/day based on erythrocyte cholinesterase inhibition, reduced body-weight gain and histopathological abnormalities in kidneys observed in dogs fed 320 ppm (Brunk *et al.*, 1976)

Long-term toxicity/carcinogenicity studies

Mice

Four groups of 70 Charles River CD-1 mice/sex, 4 weeks of age were treated with pyrazophos technical (92.8% purity grade) at dietary concentrations of 0, 1, 5 or 25 ppm, equal to 0, 0.14, 0.7 or 3.5 mg/kg bw/day for males and 0, 0.16, 0.8 or 4 for females. Ten animals/sex/dose were sacrificed after 12 months of treatment. The remaining animals were sacrificed on week 92 (females) and on week 96 (males). Pyrazophos was stored under refrigeration until used; test substance was offered to mice after being mixed with basal laboratory diet. Due to difficulties in obtaining homogeneous mixture, dietary preparation procedure was changed during the study. Actual content of the test substance in diets was 65% (0-101), 100% (55-241) and 85% (42-100) of nominal (mean of 12 determination,

range in brackets) at 1, 5 and 25 ppm, respectively. Daily observations for overt toxicity signs, abnormalities and masses and weekly observations for feed consumption and body-weights were recorded. Haematology determinations and brain cholinesterase activity on 10 mice/sex/ dose level were performed at 12 months and at termination of the study. Serum and erythrocyte cholinesterase activities were measured on 10 mice/sex/dose at 0, 3, 6, 12, 18 months and at termination of the study. Pathology was performed on all animals found dead or sacrificed at the end of the study.

In both sexes, food intake and body-weight gain were unaffected by pyrazophos administration. The incidence of clinical signs did not differ between groups. The low survival rate noted at 1 ppm (week 92) for the females and at week 96 for the males, prompted the early termination of the study. The mortality rate was 67%, 75%, 65%, 70% (males) and 72%, 80%, 63% and 75% (females) at 0, 1, 5 and 25 ppm pyrazophos, respectively. Pyrazophos did not effects haematological parameters tested. Dose-related inhibition of serum and erythrocyte cholinesterase activities was observed in mice at 5 and 25 ppm (marginal inhibition of erythrocyte cholinesterase at 5 ppm in both sexes). There were no significant changes of brain cholinesterase activity up to the highest dose level.

Trivial differences in organ weights were observed between controls and dosed mice. Both neoplastic and non-neoplastic histopathological observations were spontaneous or naturally occurring lesions of aging mice. Renal amyloidosis was considered to be the most frequent "cause of death" among control and treated animals, the incidence being comparable in all groups. A higher incidence of testicular atrophy and degeneration was observed in treated mice but a dose-effect relationship was lacking. No differences in the incidence of neoplastic lesions were found when treated mice were compared to controls.

Pyrazophos did not cause adverse effects up to the highest nominal dose of 25 ppm, equal to 3.5 and 4.1 mg/kg bw/day in males and females, respectively. However, the poor correspondence of actual and nominal concentrations of pyrazophos in the diets and the lack of toxic effects up to the highest dose level hampered a thorough evaluation of this study (Griggs *et al.*, 1982).

Rats

Five groups of 30 Wistar albino rats/sex were treated with technical grade pyrazophos (purity 90%) at dietary concentrations of 0, 5, 8, 10 and 50 ppm for 104 weeks (except the 8 ppm group which was discarded at week 20). General condition, behaviour, body-weight, food and water intake were recorded throughout the duration of the study. Haematology, blood biochemistry, cholinesterase activity in plasma, erythrocytes and brain and urinalysis were measured. Pathology (organ weights) and histopathology (incomplete) were performed at the end of the study.

Pyrazophos administration did not affect general condition, behaviour, body-weight gain, food and water intake of animals at any dose level. At the end of the study the mortality rate was 40%, 70%, 47% and 60% in males and 27%, 47%, 30% and 33% in females at 0, 5, 10 and 50 ppm, respectively. In the 50 ppm dose group, white blood cell counts significantly decreased in males at week 102 only (differential count normal). Glicemia was increased in the 10 and 50 ppm dose groups in males at week 52. Significant inhibition of plasma and erythrocyte cholinesterase activity was measured in both sexes at the 10 and 50 ppm dietary levels. Brain cholinesterase activity was unaffected at the end of the study. A decrease in the relative spleen weight was observed in females only at all dietary levels. It was not dose-related and was not accompanied by treatment-related histological changes. Both neoplastic and non-neoplastic histopathological observations were those of spontaneous or naturally occurring lesions of aging albino rats. No differences in the incidence of neoplastic or non-neoplastic lesions were found when treated rats were compared to controls. In conclusion scattered differences in blood parameters were not suggestive of a clear toxic effect of pyrazophos. The NOAEL in this study was 50 ppm, equivalent to 2.5 mg/ kg bw/day. (Til *et al.*, 1979a)

Four groups of fifty Wistar rats/sex, 4 weeks of age, were treated via pelleted feed with technical pyrazophos (95.7% purity) at dietary concentrations of 0, 2, 80 or 320 ppm equal to 0, 0.1, 4.0 or 15.9 mg/ kg bw/ day for males and 0, 0.1, 4.8 or 19.3 mg/kg bw/ day for females for 117 weeks. Ten and 20 additional rats/sex/dose were treated with pyrazophos for 52 and 105 weeks, respectively. Pyrazophos was dissolved in acetone, mixed with microgranulated feed and pelleted. This preparation was performed every two weeks. Pyrazophos was stable at room temperature for at least 21 days. Homogeneity (range of variation between -15% and 22% of the mean concentration) and content of the test article in the feed (mean concentrations 98.5%, 95.6% and 94.5% of the nominal concentrations at 2, 80 and 320 ppm, respectively) were determined before the beginning of the study and every two months throughout the duration of the study. Parameters monitored were viability/mortality, clinical signs, food and water consumption, body-weight, ophthalmoscopic examination, hearing test, haematology, clinical biochemistry including cholinesterase activity determination, urinalysis and pathology.

A slight but statistically significant increase of body-weight was detected in both sexes at 320 ppm during the second year of the study which corresponded to a slight increase of food intake. Absolute water intake was not affected in any group resulting in a slight decrease of relative water intake at the highest dose level. The incidence of clinical signs and palpable masses during life and the results of ophthalmoscopic examinations and hearing tests were comparable between groups. At the end of the 117 weeks treatment, mortality rate was 60%, 40%, 54% and 52% (male) and 60%, 56%, 38% and 48% (female) at 0, 2, 80 and 320 ppm, respectively.

Slight decrease of total lipid content in blood which corresponded to lower cholesterol and triglyceride concentrations was measured in both sexes at 80 and 320 ppm but was not seen consistently throughout the duration of the study. Other haemato-logical and biochemical parameters and urinalysis measured at 26, 52, 78 and 105 weeks were unaffected by pyrazophos administration. Dose-related inhibition of plasma and erythrocyte cholinesterase was detected at 80 and 320 ppm throughout the duration of the study. Absent or marginal (between 13% and 23%) inhibition of brain acetylcholinesterase was measured at 320 ppm in both sexes. No biologically significant differences in organ weights were detected at 52, 105 or 117 weeks. Histopathology showed a slight increase of gastric lesions (ulcers and erosions) in males at 320 ppm; several other non-neoplastic lesions were considered unrelated to test substance administration. An increased incidence of hemangiomas in mesenteric lymph nodes was observed at 105 and 117 week necrosopies. Hemangiomas occurred at incidences of 12%, 13%, 30% and 33% (males) and 6%, 4%, 26% and 16% (females) at 0, 2, 80 and 320 ppm, respectively. This type of neoplasm was practically absent at 52 weeks necrosopies. A statistically significant positive trend was present for males only. Hemangiosarcomas were not reported in mesenteric lymph nodes. The incidence of this type of neoplasm in historical controls is 8% (0-24) and 2% (0-8) in males and females, respectively (mean, range in brackets). Increased incidence of skin fibroma (still within the range of historical controls) was detected in males at the highest dose level (10%) in comparison with controls (0%). The NOAEL was 2 ppm, equal to 0.1 mg/kg bw/day in both sexes based on a higher incidence of hemangiomas in mesenteric lymph nodes detected in males at the higher doses (Tennekes *et al.*, 1991).

In a supplementary study, two groups of 20 Wistar rats/sex were treated via pelleted feed with technical pyrazophos (95.7% purity) at dietary concentrations of 0 or 1 ppm equal to 0 or 0.05 mg/kg bw/day for males and 0 or 0.06 mg/kg bw/day for females for 104 weeks. Monitored parameters were observation for viability/mortality, clinical signs, food and water consumptions, body-weight, ophthalmoscopic examination, hearing test, cholinesterase determination in plasma, erythrocytes and brain and pathology (organ weights and macroscopic examinations). Mortality rate at 104 weeks was 25% in both groups for males and 25% and 15% at 0 and 1 ppm, respectively for females. All tested parameters were unaffected by pyrazophos treatment. The NOAEL was 1 ppm of pyrazophos, equal to 0.05 mg/kg bw/day and 0.06 mg/kg bw/day for male and female rats, respectively (Tennekes *et al.*, 1991)

Reproduction studies

Rats

In a 3-generation, 2-litter/generation study, four groups of 10 male and 20 female rats (CIVO-colony) approximately 3-4 weeks old at the beginning of the study were treated with pyrazophos (90% purity grade) at dietary concentrations

of 0, 5, 10 or 50 ppm. The diets were freshly prepared every two weeks and stored at room temperature (homogeneity and stability of test substance in diets not reported). Rats of the F_0 generation were maintained on their respective diets for 13 weeks prior to mating and then up to weaning of the F_{1b} generation. Litters were culled to 8 pups on day 1. Rats from F_{1b} litters (10 males and 20 females selected at weaning) were mated at weeks 14 and 23 post-weaning. Ten male and 20 females of F_{2b} litters were selected at weaning and mated at weeks 13 and 22 after weaning. F_{1a} , F_{2a} and F_{3a} litters were killed post-weaning. Rats from the F_{3b} generation (10 males and 10 females) were selected and continued on the same diet for a 90-day feeding study.

The following parameters were evaluated in the present study:

- Reproduction phase: litter size, pup survival and litter weight at day 1, 10 and 20.
- 90-day study: signs of toxicity, food intake, body-weight, haematology, blood chemistry (included cholinesterase activity, brain cholinesterase activity, urinalysis and pathology).

No relevant changes in reproductive parameters were observed. An increased percent mortality of pups both at day 10 and 20 was detected at the highest dose level after the second mating of the F_0 generation only. Signs of toxicity were not detected during the 90-day study. Dose-related inhibition of whole blood cholinesterase activity was measured at 10 and 50 ppm but brain cholinesterase activity was normal up to the highest dose level. Blood chemistry and urinalysis did not show changes related to the administration of the test substance. Total leucocyte counts were increased at 50 ppm in both sexes (likely because of lymphocyte increase). The absolute and relative weights of the thymus were increased at the two highest dose groups in both sexes. This change did not correspond to any histological abnormality. Pathology did not show changes attributable to pyrazophos administration. The overall NOAEL in this combined study (reproduction study and 90-day study) was 5 ppm equal to 0.45 and 0.42 mg/kg bw/day for males and females, respectively, based on increased weight of thymus observed at 10 and 50 ppm and increased lymphocyte count observed at 50 ppm in both sexes (Til *et al.*, 1979b)

In a two-generation one-litter study/generation, groups of 25 male and 25 female rats (Wistar/HAN) approximately 6 weeks old at the beginning of the study were treated with pyrazophos technical (95.7% purity) at dietary concentrations of 0, 2, 20 or 200 ppm. Homogeneity and stability of pyrazophos in the diet were checked and found acceptable. Parental rats (F_1 -generation) were maintained on their respective diets for 70 days prior to mating. The F_0 generation received the test compound in the diet for 126 days prior to mating. Both F_0 and F_1 generations received the test substance during the pairing, gestation and lactation periods.

Trivial differences in food intake and body-weight gain were observed throughout the duration of the study in F₀ and F₁ generations. Fertility index, gestation index and viability index were unaffected by pyrazophos administration in both generations. Lactation index was not affected in litters of the F₀ generation but it was significantly reduced in litters of the F₁ generation at 200 ppm. In both the F₁ and F₂ pups, no treatment-related abnormal findings were observed at external examination and the sex ratio of the pups was unaffected by treatment. At 200 ppm, reduced body-weight gain of the pups during lactation was evident in both the F₁ and F₂ pups; additionally, in the F₂ pups, the initial body-weight was significantly reduced. Trivial differences in body-weight gain were also observed at 20 ppm. Dose-related inhibition of both plasma and erythrocyte cholinesterase activity was measured in both sexes at 20 and 200 ppm. The inhibition was evident in F₀ and F₁ generations and also in pups (plasma cholinesterase only was measured in pups). The extent of cholinesterase activity inhibition was higher in erythrocyte than in plasma. Slight inhibition of brain cholinesterase activity was measured only in females, at the highest dose level in both generations. Statistically significant inhibition of brain cholinesterase of no biological relevance was measured in F₂ pups of both sexes at 200 ppm. Several statistical differences of organ weights between groups were judged of no biological relevance. Pathology did not show treatment-related abnormal findings either in parent animals or in pups. The NOAEL in this study was 20 ppm, equivalent to 1 mg/kg bw/day, based on retardation of body-weight gain of pups and brain cholinesterase inhibition measured in F₀ and F₁ generations (females) and in pups of the F₂ generation. However, neither thymus weight nor lymphocyte counts (affected parameters in the previously performed reproduction study) were checked in this study (Suter *et al.*, 1991).

Special studies on delayed neuropathy

Hens

Six white Leghorn hens were treated orally with 150 mg/kg of pyrazophos diluted (10%) in sesame oil on two separate occasions 21 days apart. Another six hens received a single dose of atropine/toxogonin i.v. before being similarly dosed with pyrazophos. In preliminary studies of acute toxicity in hens, the LD₅₀ was set between 150 and 400 mg pyrazophos/kg bw. A third group of 6 hens received a single dose of 500 mg tri-o-cresylphosphate/kg bw (positive controls) and a fourth group of 6 animals received sesame oil (negative controls). All hens were sacrificed on day 42 after first dosing and histological examination of cerebrum, cerebellum, spinal cord and the nerves of the brachial and lumbosacral plexus was performed.

Pyrazophos-treated hens (both groups) showed symptoms of the type expected for cholinergic toxicity. Positive controls showed symptoms suggestive of delayed neuropathy. Negative controls did not develop toxic symptoms. Histopathology did not reveal significant changes in pyrazophos treated hens and in negative controls. In positive controls demyelination and glial cell proliferation

were observed in the optic tract, in the medulla and in the cervical, thoracic and lumbar segments of the spinal cord. Peripheral nerves were unaffected in all animals. According to the results of this study, pyrazophos did not cause delayed neuropathy (Hollander & Weigand, 1979a)

Special studies on embryotoxicity and teratogenicity

Rats

Four groups of twenty mated female Wistar rats were dosed with pyrazophos at concentrations of 0, 0.5, 1.6 or 5.0 mg/kg bw/day orally by gavage (vehicle, starch mucilage) on days 7, 10 and 16 of gestation. On day 21 of gestation, animals were killed and fetuses were examined for developmental disorders. Neither toxic signs nor mortality were observed in pyrazophos-treated or control pregnant rats. There were no significant differences in body-weight or feed consumption between the groups. Pathology did not reveal induced changes. There were no significant differences in the number of implantations per litter, incidence of live, dead or resorbed fetuses per litter or in the average weight of live fetuses between groups. The fetuses developed normally and showed no pyrazophos related external abnormalities, anomalies of the internal organs, or skeleton. The NOAEL in the present study was 5.0 mg pyrazophos/kg bw/day for both maternal toxicity and teratogenicity. Cholinesterase activity was not measured (Baeder *et al.*, 1978).

Rabbits

Groups of fifteen pregnant Himalayan rabbits were treated by gavage with pyrazophos (purity 95.6%) during gestation days 6-18 at doses of 0, 10, 32 or 100 mg/kg bw/day (vehicle, starch mucilage). On day 29 of gestation, the dams were killed. Internal, external and skeletal examination were performed on the fetuses. A slight reduction of food intake was observed in the pregnant rabbits at the highest dose level which corresponded to some reduction of body-weight gain (not statistically significant). Intra-uterine development of the embryos and the viability of fetuses were not affected at any dose level of pyrazophos. The morphological examination of the fetuses for development, external anomalies, alterations of internal organs and skeleton yielded no evidence of a teratogenic effect of pyrazophos. The NOAEL was 100 mg/kg bw/ day for both maternal toxicity and terato-genicity. Cholinesterase activity was not measured (Baeder & Kramer, 1985; Baeder *et al.*, 1986).

Special studies on genotoxicity

Table 2. Results of genotoxicity assays on pyrazophos

| Test system | Test object | Concentration of pyrazophos | Purity | Results | Reference |
|------------------------------|--|--|------------------|--------------------------|-------------------------------|
| Ames test (1) | <i>S. typhimurium</i> TA98, TA100, TA1535, TA1537 | 0.2-5000 µg/plate dissolved in DMSO | ? | Negative (2) | Gericke (1977) |
| Reversion assay (1) | <i>E. coli</i> WP2 <i>hcr</i> <i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538 | 10-5000 µg/plate dissolved in DMSO | 92.8% | Negative (2) | Shirasu <i>et al.</i> (1979) |
| Rec-assay | <i>B. subtilis</i> H17 Rec + M45 Rec- | 20-2000 µg/disc dissolved in DMSO | 92.8% | Negative (3) | Shirasu <i>et al.</i> (1979) |
| Mitotic gene-conversion | <i>S. cerevisiae</i> D4 (1) <i>A. nidulans</i> (5) | 800-1600 µg/ml 200-690 µg/ml | Afugan Afugan | Negative (4) Negative | Bertoldi <i>et al.</i> (1980) |
| CHO/HGPRT mutation assay (1) | Chinese hamster lung fibroblasts V79 | 6.25-75 µg/ml (-act.) 25-150 µg/ml (+ act.) | 97.3% | Negative (6) | Muller (1988) |
| Chromosome aberrations | Human lymphocytes | 1-50 µg/ml x 47 h (- act.) 10-175 µg/ml x 1 h (+ act.) dissolved in DMSO | 95.6% | Negative (7) | Taalman <i>et al.</i> (1985) |
| Micronucleus test | Mouse (NMRI strain) | 50, 75, 100 mg/kg p.o. treated twice 24 h apart | 95.6% | Negative (8) | Mayer <i>et al.</i> (1985) |

- (1) Both with and without metabolic activation.
- (2) Positive controls yielded expected positive responses.
- (3) Kanamycin (10 µg/disc) and Mitomycin C (0.1 µg/disc) gave expected negative and positive response, respectively.
- (4) Positive control (methylmethanesulphonate) gave expected positive response at 500 µg/ml.
- (5) Also using germinating conidia.
- (6) Positive control (EMS 1 mg/ml (- act.); DMBA 7.7 µg/ml (+ act.)) gave expected positive responses.
- (7) Positive controls (EMS 0.2 µl/ml (- act.); cyclophosphamide 18.7 µg/ml (+ act.)) gave expected positive responses.
- (8) Positive control (cyclophosphamide) gave expected positive response at 50 mg/kg bw (single dose).

Special study on antidote effect

Rats

Female SPF-Wistar rats were dosed orally with a single pyrazophos (94% pure) dose of 800 mg/kg bw (4 x LD₅₀). A single dose (10 minutes after pyrazophos) of either atropine methyl nitrate, atropine sulphate or obidoxime or combinations of these antidotes given i.p. delayed the time of death without modifying survival rate. The best treatment was a combination of atropine sulphate (3.75 mg/kg bw) and obidoxime (4 mg/kg bw) repeated several times starting 10 min after intoxication up to 57 h later, which increased survival rate from 0% to 90% (Sholz & Weigand, 1971).

Male and female Wistar rats were dosed orally with a single pyrazophos dose (93.6% pure), approximately 2 x LD₅₀. Where no therapeutic measures were taken, all animals with the exception of one female, died within three days. Prolonged treatment with atropine sulphate only did not increase survival rate. The combinations of both atropine sulphate + 2-PAM methiodide or atropine sulphate plus obidoxime proved to be efficient antidotes by reducing mortality to zero (Leist & Weigand, 1983).

Observations in humans

Five healthy humans received 0.15 mg/kg bw of pyrazophos daily for 10 days. Other 6 subjects received 0.15 mg/kg bw of pyrazophos for 3 days, followed by 7 daily doses of 0.07 mg/kg bw. Another 11 subjects received 0.07 mg/kg bw of pyrazophos for 10 days. Pyrazophos was always administered orally, in orange juice, at breakfast. Males and females were similarly represented in the groups. Controls received orange juice only. The great majority of controls were the same subjects who had previously taken pyrazophos.

All subjects were checked for appearance of clinical symptoms, alterations of cardiovascular parameters (blood pressure, pulse rate, ECG). Moreover, haematology, blood biochemistry, urinalysis and quantitative assay of cholinesterase in plasma, whole blood and erythrocytes were performed on several occasions before, during and up to 7 days after the last administration of pyrazophos. At the highest dose, only two subjects completed the study as two withdrew because of upper respiratory infection and dosing was discontinued in one subject because of low baseline cholinesterase level after 3 doses (sic). Three out of six subjects in the 0.15/0.07 mg/kg bw group reported adverse reactions of the type: mild headache, "feeling faint" and slight abdominal discomfort. Two subjects in the lowest dose group referred: dizziness and lightheadedness. The other two in the same group referred symptoms which could be related to upper tract respiratory infection. No alterations of cardiovascular parameters were found. Blood and urine analysis were within the normal range except for a sharp increase of serum CPK measured in one subject of the lowest-dose level. CPK was increased from the first

up to the last day of pyrazophos administration (a pre-dosing normal level was reported) showing a maximum on day 4 (CPK was not measured during the 7 day recovery period). This subject showed similar inhibitions of plasma and whole blood cholinesterase activities of about 25% during the last 3 days of dosing (average inhibition calculated by comparison of the mean of cholinesterase activity on days 9-10-11 of the study with the mean of the three pre-dosing values). Cholinesterase activities in plasma and erythrocytes were normal on the day when CPK was at its maximum. Other biochemical parameters were all in the normal range. The possible explanation for increased CPK offered by authors was running activity performed by the subject during the study.

Cholinesterase values within each group showed that, at 0.15 mg/kg bw inhibition of plasma cholinesterase was observed starting on day 6 (approximately 20-50%) and persisting up to 4 days after the last dose of pyrazophos. In this group marginal inhibition of whole blood cholinesterase was noted but cholinesterase activity was not affected in erythrocytes. Cholinesterase activity was not affected in the other groups.

Evaluation of this study is hampered by the presence of several methodological problems and lack of details. Controls were mainly the same subjects who had previously taken pyrazophos. Upper respiratory disorders are not substantiated by alterations of haematological parameters like ESR or increased WBC and cholinergic toxicity was not ruled out. Cholinesterase activities show a great intra-individual variability (greater than 10-20% which is the normal intra-individual variation in day-by-day analysis). The NOAEL for erythrocyte cholinesterase inhibition in this study is 0.15 mg of pyrazophos/kg bw/day for 10 days, however 3/5, 3/6 and 5/11 subjects in the three groups reported symptoms of the type expected for cholinergic toxicity (Dinsdale & Protheroe, 1983).

An unknown quantity of Afugan 30 EC squirted out of a damaged metal container into the face of a 36-year-old male worker, mainly in the orbital region. The worker was soon hospitalized. Both eyes developed conjunctivitis. Mild systemic clinical effects of the cholinergic type developed within 2 hours, namely miosis, tremors, muscle twitchings, isolated spasms, psychic agitation, bronchial spasticity and bradycardia. The patient received a total of 4 mg atropinum sulfuricum i.v. and as soon as systemic symptoms disappeared on the same day of poisoning, he was transferred to the out-patients department. Corrosion of the conjunctivae of both eyes and corrosion of the cornea of the right eye were diagnosed. Cholinesterase activity measured both in the serum and in erythrocytes at approximately 3 and 7 h after intoxication, in the presence of clinical symptoms, resulted in the normal range (Stasik, 1987).

COMMENTS

In rats after a single or 14 daily (only the last dose radiolabelled) oral administrations of ¹⁴C-pyrazophos, blood radioactivity peaked within six hours after

administration. The half-life was approximately five hours. Radioactivity was eliminated mainly via urine (71-78%) and faeces (16-24%). The parent compound accounted for most of the radioactivity detected in faeces, indicating incomplete absorption from the gastrointestinal tract. Intact pyrazophos was not found in urine, where the radioactivity mostly corresponded to its hydrolysis products.

Pyrazophos was moderately toxic after single oral doses to mice, rats and dogs. No significant differences between sexes or routes of administration were detected. WHO has classified pyrazophos as moderately hazardous (WHO, 1992).

In two 28-day studies in mice at dietary concentrations of 0, 1, 5, 25 or 125 ppm (the highest dose in only one study), the NOAEL was 25 ppm equal to 4.7 and 5.0 mg/kg bw/day for males and females, respectively. The NOAEL was based on 20% inhibition of brain cholinesterase observed at 125 ppm.

In a 13-week study in rats at dietary concentrations of 0, 2.5, 50 or 1000 ppm the NOAEL was 2.5 ppm, equal to 0.21 mg/kg bw/day, in both sexes, based on brain cholinesterase inhibition at the end of the study at 50 ppm.

In a 52-week study in rats at dietary concentrations of 0, 2, 20 or 200 ppm the NOAEL was 20 ppm, equal to 1.0 and 1.4 mg/kg bw/day in males and females, respectively. The NOAEL was based on 30% inhibition of brain cholinesterase activity in females at 200 ppm.

In a 92-day (moist semi-solid diet) study in dogs at dietary concentrations of 0, 0.5, 2.0, 5.0 or 10/125/320 ppm the NOAEL was 5 ppm, equivalent to 0.4 mg/kg bw/day, based on inhibition of erythrocyte cholinesterase activity at the next highest dose (brain acetylcholinesterase activity was not determined). Clinical signs of the cholinergic type were observed when the dietary concentration was raised to 320 ppm.

In a 6-month study in dogs at dietary levels of 0, 1.2, 18 or 320 ppm pyrazophos (moist semi-solid diet), the NOAEL was 1.2 ppm, equivalent to 0.09 mg/kg bw/day, based on marginal brain acetylcholinesterase inhibition observed at 18 ppm.

In a two-year study in dogs at dietary concentrations of 0, 2, 5 or 320 pm (moist semi-solid diet), the NOAEL was 5 ppm, equivalent to 0.4 mg/kg bw/day based on erythrocyte cholinesterase inhibition, reduced body-weight gain and histopathological abnormalities observed in the kidneys of dogs fed 320 ppm.

In a 92/96 (female/male)-week study in mice at dietary concentrations of 0, 1, 5 or 25 ppm, pyrazophos did not cause adverse effects up to the highest nominal concentration of 25 ppm, equal to 3.5 and 4.1 mg/kg bw/day in males and females, respectively. Inhibition of serum and erythrocyte cholinesterase activities, but not of brain acetylcholinesterase activity, was observed at 5 ppm and above.

The poor correspondence between actual and nominal concentrations of pyrazophos in diets hampered definitive evaluation of this study.

In a two-year study in rats at dietary levels of 0, 2, 80 or 320 ppm, the NOAEL was 2 ppm equal to 0.1 mg/kg bw/day, based on a higher incidence of hemangiomas in mesenteric lymph nodes detected in males at the higher doses. Marginal brain acetyl cholinesterase inhibition was noted at 320 ppm only.

In a two-year study in rats at dietary concentrations of 0, 5, 8, 10 or 50 ppm, the NOAEL was 50 ppm, equivalent to 2.5 mg/kg bw/day, based on the absence of adverse effects including brain acetyl cholinesterase inhibition at this dose level. No compound-related abnormalities were detected in mesenteric lymph nodes.

In a two-litter, three-generation study in rats (a 90-day toxicity study was also conducted on the F_{3b} generation) at dietary levels of 0, 5, 10 or 50 ppm, the NOAEL was 5 ppm, equal to 0.45 and 0.42 mg/kg bw/day for males and females, respectively, based on increased thymus weight observed at 10 and 50 ppm and increased lymphocyte counts observed at 50 ppm in both sexes in the 90-day toxicity study.

In a two-generation reproduction study in rats at dietary concentrations of 0, 2, 20 or 200 ppm of pyrazophos, the NOAEL was 20 ppm, equivalent to 1 mg/kg bw/day, based on retardation of body-weight gain of pups of both generations, reduced lactation index of the F₁ generation and slight inhibition of brain acetyl cholinesterase activity in parental females and in pups of the F₂ generation.

Pyrazophos did not cause delayed neuropathy in hens.

Pyrazophos was not teratogenic in rats or rabbits. The NOAELs for both maternal and embryofetal toxicity in rats was 5 mg/kg bw/day and in rabbits 100 mg/kg bw/day, the highest doses tested. Maternotoxicity was not observed. However, cholinesterase activity was not measured.

Male and female human volunteers received pyrazophos orally at 0.07, 0.07/0.15 or 0.15 mg/kg bw/day for 10 days. At the highest dose level only plasma cholinesterase activity was inhibited (20-40%), with marginal inhibition of erythrocyte cholinesterase activity. Symptoms which could be attributed to cholinergic toxicity were observed in all groups. The study was considered inadequate because of deficiencies in its design and conduct.

After reviewing the available genotoxicity data, it was concluded that pyrazophos was not genotoxic.

The Meeting concluded, after consideration of the long-term studies and the genotoxicity data that pyrazophos was unlikely to pose a carcinogenic hazard for humans.

An ADI was allocated on the basis of NOAELs in the two-year study in dogs and the three-generation study in rats, using a 100-fold safety factor.

TOXICOLOGY EVALUATION

Level causing no toxicological effect

| | |
|--------|--|
| Mouse: | 25 ppm, equal to 4.7 mg/kg bw/day (28-day study) |
| Rat: | 5 ppm, equal to 0.4 mg/kg bw/day (three-generation reproduction study) |
| Dog: | 5 ppm, equivalent to 0.4 mg/kg bw/day (two-year study) |

Estimate of acceptable daily intake for humans

0-0.004 mg/kg bw

Studies which will provide information valuable in the continued evaluation of the compound

Further observations in humans.

REFERENCES

Baeder, C.H., Weigand, W., & Kramer, M. (1978) Hoe 02873 O F AS201. Embryotoxicity testing in Wistar rats after oral administration. Unpublished report No. 544/78 from Hoechst Pharma Fo. To., Germany. Submitted to WHO by Hoechst.

Baeder, C.H. & Kramer, M. (1985) Hoe 002873- Active ingredient technical (Code: Hoe 002873 OF ZD94 0001). Testing for embryotoxicity in Himalayan rabbits following oral administration. Unpublished report No. GT85.1105 by Hoechst Pharma Fo.To., Germany. Submitted to WHO by Hoechst.

Baeder, C.H., Mayer, D., & Langer, K.H. (1986) Hoe 002873 - Active ingredient technical (Code: Hoe 002873 OF ZD94 0001). Testing for embryotoxicity in Himalayan rabbits following oral administration. Supplement to report No. 85.1105. Unpublished report No. 86.0589 by Hoechst Pharma Fo.To., Germany. Submitted to WHO by Hoechst.

Bertoldi M., Griselli M., Giovannetti M., & Barale R. (1980) Mutagenicity of pesticides evaluated by means of gene-conversion in *Saccharomyces cerevisiae* and in *Aspergillus nidulans*. *Environmental mutagenesis*, 2: 359-370.

Brunk, R., Weigand, W., & Kramer, M. (1976) Hoe 02873 O F AS202. Chronic (2-year) feeding study with Beagle dogs. Unpublished report No. 279/76 from Hoechst Pharma Fo. To., Germany. Submitted to WHO by Hoechst.

Brunk, R., Weigand, W., & Kramer, M. (1982) Toxicological testing of pyrazophos active ingredient technical (Hoe 02873 OF AS207) by repeated oral administration for six months in beagles. Unpublished report No. 711/82 from Hoechst Pharma Fo. To., Germany. Submitted to WHO by Hoechst.

DeKnecht-van Eekelen, A., & Dreef-van der Meulen, H.C. (1978) Sub-chronic (90 day) toxicity study with Hoe 2873 in Albino Rats. Unpublished report No. R5905. from TNO, NDL. Submitted to WHO by Hoechst.

Dindsdale, J., Protheroe, D.A. (1983) Final report on a repeated dose tolerance study of the organophosphorus fungicide (Hoe 02873OF) in normal adult healthy subjects. Unpublished report No. RD71/11603/12460 83/0191. From Simbec Res. Ltd., GBR. Submitted to WHO by Hoechst.

Estes, F. (1979) Pyrazophos technical. Four week pilot study in mice. Unpublished report No. 439-001. from IRDC, USA. Submitted to WHO by Hoechst.

Gericke, D. (1977) Test for mutagenicity in bacteria strains in the absence and presence of liver preparation. Unpublished report No. 8/77 from Hoechst Krebsfo. lab., Germany. Submitted to WHO by Hoechst.

Griggs, L. M. P., Jefferson, N., & Blair, M. (1982) Life-time carcinogenicity study in mice. Unpublished report No. 439-002 from IRDC, USA. Submitted to WHO by Hoechst.

Hollander, H. & Weigand, W. (1977a) Acute intraperitoneal toxicity of O,O-diethyl-O-(5-methyl-6-ethoxycarbonyl -pyrazolo -(1.5-a) -pyrimid-2-yl)thionophosphate (Hoe 02873 OF AS201) to the male SPF-Wistar-rat (vehicle:sesame oil). Unpublished report No. 55/77 from Hoechst Pharma Fo. To. Germany. Submitted to WHO by Hoechst.

Hollander, H. & Weigand, W. (1977b) Acute intraperitoneal toxicity of O,O-diethyl-O-(5-methyl-6-ethoxycarbonyl -pyrazolo- (1.5-a)-pyrimid-2-yl)thionophosphate (Hoe 02873 OF AS201) to the female SPF-Wistar-rat (vehicle:sesame oil). Unpublished report No. 56/77 from Hoechst Pharma Fo. To. Germany. Submitted to WHO by Hoechst.

Hollander, H. & Weigand, W. (1977c) Hoe 02873 O F AS201. Irritation to the rabbit skin and eye mucose Unpublished report No. 252/77 from Hoechst Pharma Fo. To. Germany. Submitted to WHO by Hoechst.

Hollander, H. & Weigand, W. (1977d) Hoe 02873 O F AS201. Acute oral toxicity to the male SPF-NMRI-mouse. Unpublished report No. 351/77 from Hoechst Pharma Fo. To. Germany. Submitted to WHO by Hoechst.

Hollander, H. & Weigand, W. (1977e) Hoe 02873 OF AS201. Acute oral toxicity to the female SPF-NMRI-mouse. Unpublished report No. 351/77 from Hoechst Pharma Fo. To. Germany. Submitted to WHO by Hoechst.

Hollander, H. & Weigand, W. (1977f) Acute oral toxicity of Hoe 02873 OF AS201 to the male SPF-Wistar-rat (vehicle: sesame oil). Unpublished report No. 353/77 from Hoechst Pharma Fo. To. Germany. Submitted to WHO by Hoechst.

Hollander, H. & Weigand, W. (1977g) Acute subcutaneous toxicity of Hoe 02873 O F AS201 to the male SPF-Wistar-rat (vehicle:sesame oil). Unpublished report No. 354/77 from Hoechst Pharma Fo. To. Germany. Submitted to WHO by Hoechst.

Hollander, H. & Weigand, W. (1977h). Acute subcutaneous toxicity of Hoe 02873 O F AS201 to the female SPF-Wistar-rat (vehicle:sesame oil). Unpublished report No. 355/77 from Hoechst Pharma Fo. To. Germany. Submitted to WHO by Hoechst.

Hollander, H. & Weigand, W. (1977i). Acute subcutaneous toxicity of Hoe 02873 O F AS201 to the male SPF-NMRI-mouse (vehicle: sesame oil). Unpublished report No. 356/77 from Hoechst Pharma Fo. To. Germany. Submitted to WHO by Hoechst.

Hollander, H. & Weigand, W. (1977l) Acute subcutaneous toxicity of Hoe 02873 O F AS201 to the female SPF-NMRI-mouse (vehicle: sesame oil). Unpublished report No. 357/77 from Hoechst Pharma Fo. To. Germany. Submitted to WHO by Hoechst.

Hollander, H. & Weigand, W. (1978a) Acute oral toxicity of Hoe 02873 O F AS201 to the female SPF-Wistar-rat (vehicle: sesame oil). Unpublished report No. 1024/77 from Hoechst Pharma Fo. To. Germany. Submitted to WHO by Hoechst.

Hollander, H. & Weigand, W. (1978b) Hoe 02873 OF AS 201: Acute oral toxicity to the male SPF-Wistar-rat. Unpublished report No. 293/78 from Hoechst Pharma Fo. To. Germany. Submitted to WHO by Hoechst.

Hollander, H. & Weigand, W. (1978c) Acute oral toxicity to the female SPF-Wistar-rat. Unpublished report No. 294/78 from Hoechst Pharma Fo. To. Germany. Submitted to WHO by Hoechst.

Hollander, H. & Weigand, W. (1978d) Hoe 02873 OF AS201 Acute oral toxicity to the male and female Beagle dog (vehicle: starch suspension). Unpublished report No. 295/78 from Hoechst Pharma Fo. To. Germany. Submitted to WHO by Hoechst.

Hollander, H. & Weigand, W. (1978e) Range finding test with Hoe 02873 OF AS201 in a 28-day study with SPF-mice. Unpublished report No. 604/78 from Hoechst Pharma Fo. To. Germany. Submitted to WHO by Hoechst.

Hollander, H. & Weigand, W. (1979a) Hoe 02873 OF AS201. Neurotoxicity study in hens. Unpublished report No. 16/79 from Hoechst Pharma Fo. To. Germany. Submitted to WHO by Hoechst.

Hollander, H. & Weigand, W. (1979b) Hoe 02873 OF AS201 (active ingredient techn.). Acute oral toxicity to the male and female Beagle dog. Unpublished report No. 461/79 from Hoechst Pharma Fo. To. Germany. Submitted to WHO by Hoechst.

Hollander, H. & Weigand, W. (1981) Acute percutaneous toxicity of Hoe 02873-Active ingredient (Code: Hoe 02873 OF AS207) to the female rat. Unpublished report No. 573/81 from Hoechst Pharma Fo. To. Germany. Submitted to WHO by Hoechst.

Hollander, H. & Weigand, W. (1982) Aerosol inhalation of Hoe 02873- Active ingredient (Code: Hoe 02873 OF AS207) to the male and female SPF-Wistar-rat. 4 hr- LC 50. Unpublished report No. 152/82 from Hoechst Pharma Fo. To. Germany. Submitted to WHO by Hoechst.

Lachmann G. (1986). Metabolism of ¹⁴C-pyrazophos in male and female rats after a single oral administration of 2 mg per animal. Unpublished report No. BIeV-V-66.588 from Battelle-Institute Toxikologie und pharmakologie Am Romerhof 35, 6000 Frankfurt am Main 90, FRG. Submitted to WHO by Hoechst.

Lachmann G. (1987). Metabolism of ¹⁴C-pyrazophos in male and female rats after a oral administration of 0.4 mg per animal (single and repeated dosing). Unpublished report No. BIeV-V-66.736 from Battelle-Institute Toxikologie und pharmakologie Am Romerhof 35, 6000 Frankfurt am Main 90, FRG. Submitted to WHO by Hoechst.

Leist, K. H. & Weigand, W. (1982) Test for sensitizing properties of Hoe 02873-Active ingredient (Code: Hoe 02873 OF AS207) in the guinea-pig according to Buehler. Unpublished report No. 9/82 from Hoechst Pharma Fo. To. Germany. Submitted to WHO by Hoechst.

Leist, K. H. & Weigand, W. (1983) Pyrazophos-active ingredient technical. Effects of antidotes tested on male and female Wistar rats. Unpublished report No. 83.0241 from Hoechst Pharma Fo. To. Germany. Submitted to WHO by Hoechst.

Mayer, D. & Leist, K.H. (1985) Hoe 002873 OF ZD94 0001. Micronucleus test in male and female NMRI mice after oral administration. Unpublished report No. GT85.0492 from Hoechst Pharma Fo. To. Germany. Submitted to WHO by Hoechst.

Muller, W. (1988). Pyrazophos substance technical (Code: Hoe 002873 OF ZD96 0001) detection of gene mutations in somatic cells in culture. HGPRT-test with V79 cells. Unpublished report No. 88.0333 from Hoechst Toxicology, Germany. Submitted to WHO by Hoechst.

Otaka, T., Nakayoshi, H. & Abe, M. (1981a) Acute toxicity study on pyrazophos. Oral administration in male rats. Unpublished report No. NRI79-2843 726/81a. Nomura Res.Inst., JPN. Submitted to WHO by Hoechst.

Otaka, T., Nakayoshi, H., & Abe, M. (1981b) Acute toxicity study on pyrazophos. Oral administration in female rats. Unpublished report No. NRI79-2843 727/81a. Nomura Res.Inst., JPN. Submitted to WHO by Hoechst.

Otaka, T., Nakayoshi, H., & Abe, M. (1981c) Acute toxicity study on pyrazophos (Hoe 02873 O F AS201). Subcutaneous administration in male rats. Unpublished report No. NRI79-2843 728/81a. Nomura Res.Inst., JPN. Submitted to WHO by Hoechst.

Otaka, T., Nakayoshi, H., & Abe, M. (1981d) Acute toxicity study on pyrazophos (Hoe 02873 OF AS201). Subcutaneous administration in female rats. Unpublished report No. NRI79-2843 729/81a. Nomura Res.Inst., JPN. Submitted to WHO by Hoechst.

Otaka, T., Nakayoshi, H., & Abe, M. (1981e) Acute toxicity study on pyrazophos (Hoe 02873 O F AS201). Intraperitoneal administration in male rats. Unpublished report No. NRI79-2843 730/81a. Nomura Res.Inst., JPN. Submitted to WHO by Hoechst.

Otaka, T., Nakayoshi, H., & Abe, M. (1981f) Acute toxicity study on pyrazophos (Hoe 02873 OF AS201). Intraperitoneal administration in female rats. Unpublished report No. NRI79-2843 731/81a. Nomura Res.Inst., JPN. Submitted to WHO by Hoechst.

Otaka, T., Nakayoshi, H., & Abe, M. (1981g) Acute toxicity study on pyrazophos. Oral administration in male mice. Unpublished report No. NRI79-2843 732/81a. Nomura Res.Inst., JPN. Submitted to WHO by Hoechst.

Otaka, T., Nakayoshi, H., & Abe, M. (1981h). Acute toxicity study on pyrazophos. Oral administration in female mice. Unpublished report No. NRI79-2843 733/81a. Nomura Res.Inst., JPN. Submitted to WHO by Hoechst.

Otaka, T., Nakayoshi, H., & Abe, M. (1981i) Acute toxicity study on pyrazophos (Hoe 02873 O F AS201). Subcutaneous administration in male mice. Unpublished report No. NRI79-2843 734/81a. Nomura Res.Inst., JPN. Submitted to WHO by Hoechst.

Otaka, T., Nakayoshi, H., & Abe, M. (1981l) Acute toxicity study on pyrazophos (Hoe 02873 O F AS201). Subcutaneous administration in female mice. Unpublished report No. NRI79-2843 735/81a. Nomura Res.Inst., JPN. Submitted to WHO by Hoechst.

Otaka, T., Nakayoshi, H., & Abe, M. (1981m) Acute toxicity study on pyrazophos (Hoe 02873 O F AS201). Intraperitoneal administration in male mice. Unpublished report No. NRI79-2843 736/81a. Nomura Res.Inst., JPN. Submitted to WHO by Hoechst.

Otaka, T., Nakayoshi, H., & Abe, M. (1981n) Acute toxicity study on pyrazophos (Hoe 02873 O F AS201). Intraperitoneal administration in female mice. Unpublished report No. NRI79-2843 737/81a. Nomura Res.Inst., JPN. Submitted to WHO by Hoechst.

Otaka, T., Nakayoshi, H., & Abe, M. (1981o) Subacute toxicity study on pyrazophos (Hoe 02873 OF AS20). Unpublished report No. NRI79-2532 738/81a. Nomura Res.Inst., JPN. Submitted to WHO by Hoechst.

Otaka, T. (1983) Chronic (52 weeks) toxicity study of pyrazophos (Hoe 02873 OF AS 206) in rats. Unpublished report No. NRI82-7581, gt84/0336. Nomura Res.Inst., JPN. Submitted to WHO by Hoechst.

Shirasu, Y., Moriya, M., & Koyashiki, R. (1979) Microbial mutagenicity testing on pyrazophos. From Inst. Environ. Toxicol., JPN. Submitted to WHO by Hoechst.

Sholz, J. & Brunk, R. (1973) W11099 = Hoe 2873. Toxicological examination. 92 day feeding trial in beagles.

Sholz, J. & Brunk, R. (1973) Pyrazophos, active ingredient. 90 day oral toxicity study (feeding) in beagle-dogs. Supplement. Individual data. Analysis. Unpublished report No. 363/73 from Hoechst Pharma, Germany. Submitted to WHO by Hoechst.

Scholz, J. & Weigand, W. (1971) Determination of the antidote effect in case of poisoning by Hoe 2873 = W11 099. By Hoechst Pharma Fo. To. Germany. Submitted to WHO by Hoechst.

Scholz, J. & Weigand, W. (1972^a) Acute oral toxicity determined with male and female rats. Hoechst Pharma Fo. To. Germany. Submitted to WHO by Hoechst.

Scholz, J. & Weigand, W. (1972^b) Acute intraperitoneal toxicity determined with male and female rats. Hoechst Pharma Fo. To. Germany. Submitted to WHO by Hoechst.

Stasik, M.J. (1987) Acute human poisoning with the organophosphate pyrazophos (Afugan(R) 30EC). Doc. No. A44268 from Hoechst Werksaerztl. Abt., Germany. Submitted to WHO by Hoechst.

Suter, P., Vogel, W., & Luetkemeier, H. (1991) Pyrazophos substance technical (Code: Hoe 002873 OF ZD96 0001) two-generation reproduction study in the rat. Unpublished report No. 071583 from RCC, Swiss. Submitted to WHO by Hoechst.

Taalman, R. & Horn, A. (1985). Mutagenicity evaluation of pirazophos. Substance technical grade (Code: Hoe 002873 OF ZD94 0001) in an *in vitro* cytogenic assay measuring chromosome aberration frequencies in human lymphocytes. Final report. Unpublished report No. GT85.1217 from Litton Bionetics, NLD. Submitted to WHO by Hoechst.

Tennekes, H., Janiak, T., Probst, D., Luetkemeier, H., Vogel, O., Schlotke, B., Biedermann, K., & Heusner, W. (1991) Pyrazophos substance technical. Chronic toxicity/oncogenicity feeding study in rats. Unpublished report No. 071526 from RCC, Research and Consulting Company AG, Itingen/Switzerland. Submitted to WHO by Hoechst.

Tennekes, H., Janiak, T., Probst, D., Luetkemeier, H., Vogel, O., Schlotke, B., Biedermann, K., & Heusner, W. (1991) Pyrazophos substance technical. Chronic toxicity addendum to RCC project 071526 satellite feeding study in rats with a supplementary test concentration. Unpublished report No. 209226 from RCC, Research and Consulting Company AG, Itingen/Switzerland. Submitted to WHO by Hoechst.

Til, H.P., Leegwater, D.C., & Feron, V.J. (1978) Range-finding (28 days) toxicity study in albino rats. Unpublished report No. R 5786. CIVO/TNO Netherlands. Submitted to WHO by Hoechst.

Til, H.P., Dreef-van der Meulen, H.C., Leegwater, D.C., & Huismans, J.W. (1979a) Chronic (two-year) toxicity study with Hoe 2873 in rats. Unpublished report No. R6027. TNO, NDL. Submitted to WHO by Hoechst.

Til, H.P., Spanjers, M. T., Dreef-van der Meulen, H.C., & Leegwater, D.C. (1979b) Multi-generation study with Hoe 2873 in rats. Unpublished report No. R5948 from TNO/NDL. Submitted to WHO by Hoechst.

WHO (1992) The WHO recommended classification of pesticides by hazard and guidelines to classification 1992-1993 (WHO/PCS/92.14). Available from the International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland.

