UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
Caswell # 603

DATE: January 15, 1980

SUBJECT: EPA File Symbol 40510-E. PARANITROPHENOL. U.S. Army Natick Research & Development Command, Natick, Massachusetts.

FROM: Roland A. Gessert, D.V.M.; Toxicology Branch Roland A. Gessert

TO: Mr. A. E. Castillo, Product Manager # 34

THRU: Dr. Adrian Gross, Toxicology Branch Chief

The Army requests conditional registration of paranitrophenol as a leather fungicide. Toxicity studies are summarized below. Unless otherwise specified all studies were done in laboratories of the U.S. Army Environmental Hygiene Agency, Aberdeen Proving Ground, Maryland 21010.

CONCLUSION: Conditional registration of Paranitrophenol is toxicologically sound.

ACUTE ORAL TOXICITY: 6 rats per dose per sex. Doses of 79, 100, 126, 200, 316, 501, and 794 mg/kg (female) and 126, 200, 316, 501, and 794 mg/kg (male); administered in propylene glycol. Toxic signs: convulsions, ataxia, & diarrhea were evident at doses of 200 mg/kg or higher.

LD_{50} = 191 mg/kg (male); 170 mg/kg (female). CORE GUIDELINES STUDY. Tox Cat. II.

ACUTE DERMAL TOXICITY: 4 male rabbits per dose. Doses of 1.0, 3.2, and 10 grams per kilogram applied as dried crystals and in propylene glycol.

Dry Crystals: LD_{50} greater than 10 g/kg. All skin application areas showed moderate degree of erythema, reverting to slightly scaling condition by day 7; clear day 14. All survivors at 14 days showed no gross organ lesions at necropsy. Since only male rabbits were used, study is CORE MINIMUM. Tox Category IV.

In Propylene Glycol: LD_{50} = 3.69 g/kg. All skin application areas showed primary irritation effects (strong erythema & edema). At necropsy, animals that died and survivors showed no gross lesions. Since analytical slope is insignificant, study is CORE SUPPLEMENTARY. Toxicity Category III.

PRIMARY EYE IRRITATION: Six rabbits were treated by placing 100 mg of crystals into right eyes of 6 rabbits. All eyes so treated demonstrated severe corneal injury persisting for more than 7 days. CORE MINIMUM, since eyes of 3 additional rabbits were not washed following application. Toxicity Category I.

INTRADERMAL GUINEA PIG SENSITIZATION TEST: 10 Guinea pigs were weighed and closely clipped on the scapular and lumbosacral areas. 0.05 ml of a 0.1% dilution was injected intradermally into the upper right scapular area of each test pig and 0.05 ml of the vehicle into the upper left scapular area. 24 and 48 hours later the skin reactions caused by the 2 injections were measured and scored. The sensitizing doses of 0.1 ml of freshly prepared non-irritating dilution were then injected into the clipped dorsal lumbosacral areas of the guinea pigs the succeeding Wednesdays, Fridays, and Mondays for 3 weeks until nine 0.1 ml doses had been administered, using different spots for each repeated sensitizing dose.

The guinea pigs were clipped over the scapular and lumbosacral areas every week. Following the ninth sensitization dose (0.1 ml), the animals were rested for 2 weeks. On the 14th day they were weighed, clipped closely on the scapular area, and given a challenge dose of 0.05 ml of the 0.1% (or non irritating) dilution. The challenge dose was administered intradermally in the lower right scapular area. A control injection of 0.05 ml of the vehicle was also administered into the lower left scapular area. The reactions were read and scored after 24 and 48 hours and recorded.

Negative and positive control groups also were used. The positive control group received dinitrochlorobenzene.

Paranitrophenol is not a sensitizer. Study is CORE Guidelines.

ENZYME INDUCTION TEST to determine the potential of paranitrophenol for enhancing or inhibiting liver microsomal enzyme activity in the rat, using groups of 10 male rats. Three groups each were pretreated for 4 consecutive days with intraperitoneal injections of a positive control (sodium pentobarbital, 100 mg/kg); paranitrophenol (22 mg/kg) in propylene glycol, solvent control (2 mg/kg), plus untreated cage control group. On the fifth test day each rat received an intraperitoneal injection of hexobarbital (220 mg/kg). The sleeping times were started when a rat failed to right itself and continued until the rat was able to right itself in two consecutive attempts.

The sleeping times of the paranitrophenol treated rats were not significantly different than the propylene glycol or untreated controls. The phenobarbital positive control times were significantly shorter than the other treated groups and indicated that, under test conditions, paranitrophenol was not an inducer of hepatic microsomal enzymes. Study is CORE Minimum.

TERATOLOGY OF PARANITROPHENOL IN RATS: One hundred pre-impregnated female Sprague-Dawley rats shipped for arrival on days 2-4 of gestation were purchased. Arrival weight was to have been between 180 and 200 grams each, but some were as small as 164 g. They were randomly placed into five groups of 20 rats each; were housed two to a cage (same dosage group) in hanging cages and received Purina Lab Blox and tap water ad libitum. The room temperature was kept at 24°C ± 1°C; relative humidity 40-50%, and the lighting period was 12 continuous hours daily. Each rat was given a single daily oral dose starting on day 6 of gestation and continuing through day 16 of gestation. Propylene glycol solutions containing either 125 mg/ml of aspirin, (positive control), 0.[illegible] mg/ml paranitrophenol, 6.9 mg/ml paranitrophenol, 13.8 mg/ml paranitrophenol, or propylene glycol only (vehicle) were made and administered orally via stainless steel stomach tubes (each rat received 2 mg/kg of the appropriate solution daily).

All females on test were sacrificed on their 20th day of gestation by means of an intracardiac injection of Barb-Euthol. Examination of fetuses for malformations was conducted according to the method of Wilson and Warkany. Necropsy for each dam consisted of counting the conceptuses: number, location, living, dead, and resorbed. All fetuses were weighed, measured, and examined for externally visible defects. Approximately 1/3 of the fetuses were fixed in Bouin's fluid and examined by the Wilson technique for neural and visceral defects. The remaining 2/3 of the fetuses were placed in 95% ethyl alcohol, cleared, and stained with Alizarin Red. They then were examined for skeletal abnormalities.

All rats were received in good condition, although in a lower weight range than desired; Immaturity may account for lowered fertility indices.

Maternal body weight gains were slightly reduced in animals receiving 27.6 mg/kg paranitrophenol and reduced to a lesser degree in the positive control (aspirin) rats. All other groups showed consistent weight gains.

All rats which, at necropsy, were shown to be pregnant during the study had viable litters. The exception to this is the positive control group in which 29% of the dams had resorbed their entire litters.

None of the dams sacrificed on the 20th day of pregnancy showed any sign of gross pathology at necropsy. Regarding index of dead and live-born fetuses, no difference of biological relevance was found between the groups other than the positive control where the number of live fetuses per dam was significantly decreased. All treatment groups showed a number of resprptions, although the aspirin group showed a significantly higher number of resorptions per dam.

No abnormalities were seen in the negative controls or the paranitrophenol groups. The group given the known teratogen, aspirin, showed a very high incidence of malformation, especially defects of the axial skeleton. However, a few anomalies were noted in all treatment and solvent control groups.

The positive control group showed a significant decrease in the absolute number of fetuses and the mean number of fetuses per dam. The average fetal weight of the positive control group was significantly lower than the averages of the negative control and the paranitrophenol-treated groups.

CONCLUSION: Paranitrophenol did not demonstrate a potential for causing terata in rats under the test conditions. Study is CORE Minimum.

AMES SALMONELLA/MICROSOME PLATE TEST FOR MUTAGENICITY: Paranitrophenol did not demonstrate genetic activity in any of the assays conducted in the evaluation and was considered not mutagenic under the test conditions. CORE Minimum Study.

CLINICAL WEAR TEST: In a wear test of PNP-treated combat boots, 26 male military volunteers wore the boots for 4 consecutive 5-day weeks at Army Chemical Center, Maryland, during mid-summer 1958. Test subjects served as their own controls by wearing untreated boots during the initial week. At the beginning of the 3d week each subject stood in water while wearing the boots until the inside of the boots and the feet were completely soaked. The boots were then worn in the regular manner for the remainder of the week.

There were no cases of intoxication, nor were there any signs, either subjective or objective, which suggested any adverse systemic effects. Blood pressures, pulse rates, and respiratory rates of all subjects were within normal limits throughout the course of the test. Furthermore, their values at the beginning and end of the control week and at the end of the three week test weeks were comparable. Hemoglobin values were, without exception, within normal limits in all subjects. The mean values of the initial and weekly determinations were without significant difference. Similarly, mean methemoglobin values on initial examination and at the end of the test weeks were not significantly different and no elevation was recorded which was considered to have any pathologic significance.

With respect to the results of urine analyses, mean concentrations of nitrophenol found at the end of the control and test weeks were, respectively, 8.3, 9.6, 9.6, and 9.3 micrograms per 100 milliliters. For aminophenol the values were 6.2, 7.4, 6.7, and 5.9 micrograms, respectively. (It should be noted that the presence of nitrophenol and aminophenol in the urine do not constitute conclusive evidence of paranitrophenol absorption. This is true because in using the highly sensitive analytical method which was employed in the analyses, small amounts of certain substances conceivably present in such a complex mixture as urine are considered capable of producing "positive" values for nitrophenol and aminophenol. The fact that the mean values found at the end of the control week were comparable to those found at the end of the test weeks gives strength to this explanation for the analytical results.)

Local skin effects attributed to wearing the boots were essentially negative.

CONCLUSION: On the basis of evidence obtained from this test, and evidence previously developed, it is concluded that no undue health hazards are likely to result from use of paranitrophenol in shoe leather. CORE Minimum Study.

CARCINOGENICITY IN MICE: The applicant has arranged for carcinogenicity to be studied by NIH (contract to Microbiological Associates, 5221 River Road, Bethesda, Maryland 20016) by pulmonary tumor response in Strain A mice. Exposure will be by tri-weekly I.P. injection, with urethane as a positive control.

Also in progress is a Dermal Penetration Kinetics Study (using ^{14}C-labeled paranitrophenol).

RECOMMENDATION: From the standpoint of Toxicology Branch, paranitrophenol may be conditionally registered pending completion of the carcinogenicity test.

LABEL is satisfactory.