NATIONAL RESEARCH COUNCIL
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T CENTER ON TOXICOLOGY
ISTRY AND CHEMICAL TECHNOLOGY

13 February 1969
Ref: BUMED-732-DER:rmo

ef, Bureau of Medicine & Surgery
partment of the Navy
shington, D. C. 20390

ar Sir:

On 29 May 1968 you requested information regarding the potential zards associated with the use of herbicides for weed control in Alaskan ilitary antenna fields and transmission lines. We have delayed respon- ng to your request until we could obtain detailed information on several w developments which may affect your decision on this proposal. This cent information is included in the enclosed summaries of the toxicities f the various materials involved, but we would like to call your particular tention to the following aspects:

1. The use of chlorinated aromatic hydrocarbons as pesticides, particularly DDT but not limited to that compound, has been under intensive attack in this country and in Europe. The state of Wisconsin is currently the scene of very bitter and emotional litigation to prohibit the use of such materials. Copies of two pertinent publications are enclosed for your information.

2. We have just recently been granted confidential access to unpublished information developed under a five year contract with the National Cancer Institute in which several pesticides, including one of the compounds included in your proposal, have been shown to be carcinogenic to experimental mice by single subcutaneous injections.

3. In the same study we have been informed that another of the herbicides proposed for your usage has been found to be highly teratogenic.

4. The significance of the findings in items 2 and 3 above is very difficult to assess scientifically without a great deal of additional study. However, it is quite clear that when or if these data are released they will result in a great deal of additional controversy similar to that over the use of defoliants in Viet Nam, and will certainly call into the question the need for modification of the regulations of the U. S. Department of Agriculture Pesticides Regulation Division and the Food and Drug Administration's regulations on food additives.

We are returning herewith your enclosure (1) Land Management Plan as requested. Our comments on this plan are as follows:

1. The procedures suggested are in conformance with the recommendations of the manufacturer and when carried out under the supervision of a qualified pest control operator should result in minimal exposure of personnel involved and are not expected to produce any significant health hazard to the applicators.

2. The recommended weather conditions are in accordance with standard practice for these materials and should result in maximum effectiveness with minimum loss of active ingredients.

We are unable to evaluate whether or not areas within 100 feet of open or running water should be sprayed or controlled by alternate methods of weed removal. It is known that 2-4-D and 2-4-5-T are not highly persistent in the soil. For example, soils treated with 2-4-D at the rate of 10 pounds per acre showed no phytotoxicity after 14 weeks. However, there were some traces of phytotoxicity from similar treatments with 2-4-5-T at 19 weeks. The literature indicates that there is little lateral motion of herbicides after the agents have infiltrated the soil.

There are multiple factors affecting the contamination of surface waters from herbicide spraying operations which preclude an unequivocal statement predicting the actual concentration which might develop. Accordingly it is suggested that the judgment of a qualified pest control operator who is thoroughly familiar with these local factors be given major consideration.

These herbicides are known to impart an unpleasant taste to drinking water at threshold levels as low as 8 µgm per liter. This concentration seems to be well below that which would result in any fish kill since levels ranging from 1 to 500 ppm have been reported as safe for several species of fish.

4. The proposed levels and conditions of treatment when compared with the enclosed toxicity data do not seem to present any acute hazards to local wildlife.

Sincerely yours,

Ralph C. Wands
Director
Advisory Center on Toxicology

RCW/rg

Enclosures:
1. Natura 220:1098-1102, 1968.
2. Science 163:548-551, 1969.
3. Toxicity of Bromacil
4. Toxicity of 2-4-D
5. Toxicity of 2-4-5-T
6. Land Management Plan

SUMMARY OF THE TOXICITY OF BROMACIL

Bromacil is chemically identified as 5-bromo-3-sec-butyl-6 methyluracil. It decomposes slowly in strong acids and is subject to microbial decomposition under moist soil conditions. It is thermally stable up to its melting point and is stable in water, aqueous bases and common organic solvents. It was first introduced under the commercial trade name "duPont Hyvar X Bromacil Weed Killer" for non-crop uses. Its use was subsequently expanded to include the control of weeds in crop lands and is now commonly used for citrus groves and pineapple fields. In March 1967 the Food and Drug Administration established a tolerance of 0.1 ppm Bromacil for residues in or on citrus fruits and pineapples.

Acute Toxicity in Animals

The following data are available on the acute toxicity of Bromacil in animals.

Species	Route	Effect	Concentration
Rat	Oral (single dose)	LD_{50}	5,200 mg/kg
Dog	Oral (single dose)	LD_{50}	>5,000 mg/kg
Fish (bluegill, sunfish)	In water	B.S.C.*	10.1 ppm
Mallard duck	Inhalation	LC_{50}	>10,000 ppm
Bobwhite quail	Inhalation	LC_{50}	>10,000 ppm

*B.S.C. refers to the Biologically Safe Concentration which is the maximum concentration in which the fish are expected to survive. In comparison, the B.S.C. for DDT is given as approximately 0.005 ppm.

It should be noted that it was impossible to obtain a lethal oral dose of Bromacil in dogs due to emesis whether the dose was administered singly or in six divided portions 30 minutes apart.

Short term feeding studies of Bromacil to rats at doses of 670 mg/kg per day were carried out for 10 days. Transient histological changes in the liver were noted, consisting of focal cell hypertrophy and hyperplasia.

Chronic Toxicity in Animals

Male and female rats were fed diets containing 0, 50, 250, and 1250 ppm by weight Bromacil for 2 years. There was no nutritional, clinical, hematologic, urinary or biochemical evidence of toxicity in the test groups except among the female rats receiving 1250 ppm. The latter group showed a lower rate of weight gain, a slightly decreased food intake, and a slightly lower food efficiency. All animals receiving the 1250 ppm dietary level had slight hyperplasia of the thyroid.

Male and female beagle dogs were also fed the same levels of Bromacil in their diet for two years and showed no evidence of toxicity.

At the end of these two year studies, tissue analyses showed low levels of Bromacil proportional to the daily diet. There was no evidence of storage or accumulation.

Mutagenic and Teratogenic Studies

In view of the suggestions of hyperplasia noted above, and because of the structural similarities between Bromacil and 5-bromouracil, the mutagenic and teratogenic possibilities of Bromacil were investigated. It is known that 5-bromouracil is incorporated into DNA by a variety of species and thus has found some clinical use for cancer chemotherapy. When Bromacil was evaluated against a thymine-requiring mutant of E. coli, it was not incorporated into the DNA. When mice were given Bromacil they did not incorporate it into the DNA of the liver or the spleen. When tested against a bacteriophage (AP 72), it did not produce mutations even though this is known to be a susceptible strain.

Bromacil was fed to pregnant New Zealand white rabbits at dietary levels of 50 and 250 ppm during the 8th through the 16th day of gestation and produced no teratogenic results. Bromacil was incorporated in the diet at 250 ppm for three generations of rats including six litters. There were no adverse effects upon reproduction or lactation and there were no pathological changes in the weanlings of the F_{3B} generation.

SUMMARY OF TOXICITY OF 2-4-D

(2, 4-Dichlorophenoxyacetic acid)

The hormonal effect of 2-4-D in stimulating plant growth and its subsequent effect as an herbicide was first reported in 1942. Since that time an extensive literature has collected on its effectiveness and upon its toxicity. This summary will only include those papers which appear most germane to the proposal for weed control in antenna and transmission yards.

2-4-D is a stable, nonhygroscopic compound which is a fairly strong acid and as a result it is corrosive and reactive with bases to form the corresponding salts.

Acute Toxicity in Animals

It has been shown that the acute toxicity of the free acid and of the sodium salt are essentially the same. The following Table I summarizes the acute LD_{50} values for several species by a variety of routes of administration. Table II presents a comparison of the LD_{50} values for some of these species with the maximum non-lethal oral dose of the sodium salt of 2-4-D. According to the data in these tables, 2-4-D would be classed as moderately to very toxic. In the most susceptible species, dogs, initial symptoms were frequently noted within six hours after administration of 2-4-D. The symptoms were depression and a slight ataxia which increased in severity especially in the hind legs. Death was delayed for two to nine days after administration and was preceded by a decrease in body weight accompanying anorexia. Histological studies showed some necrosis and inflammation of the intestinal mucosa, hepatic congestion, and pneumonia. In rats lethal doses of 2-4-D produce demyelinization of the dorsal portion of the brain.

Subacute feeding studies in rats and guinea pigs were rather inconclusive, however, it appears that rats can tolerate as much as 0.1% 2-4-D in the diet for two weeks without effect.

Chronic Toxicity in Animals

Dogs were fed 0, 2, 5, 10, and 20 mg/kg daily for 90 days. The 20 mg dose was also given as a divided dose twice a day. The two dogs receiving the 20 mg single doses died at 18 and 25 days, and one of the dogs receiving the 20 mg divided dose died at 49 days. The fourth dog at the 20 mg divided dose level survived. All surviving dogs showed normal body weight changes and were generally free of any symptoms. Those animals which did not survive gradually lost weight and developed weakness in the hind legs and slight ataxia. Immediately prior to death there was difficulty in chewing or swallowing and

eventually food was refused. There were no hematologic changes in any of the animals in this feeding study except those that died showed a decrease in lymphocytes immediately prior to death. There were no outstanding changes in organ weights in any of the dogs and there were no significant microscopic changes upon autopsy.

Female rats were given 2-4-D five times a week for a four week period at doses of 0, 3, 10, 30, 100, and 300 mg/kg. The doses of 30 mg/kg and less showed no adverse effects as judged by gross appearance and behavior, mortality, growth, hematological values, blood urea nitrogen concentrations, organ weights, and gross and microscopic examination of the tissues. Those rats receiving 100 mg/kg showed gastrointestinal irritation, slight cloudy swelling in the liver, and a depressed growth rate. The 300 mg/kg dose caused a rapid decline and death of all animals with severe gastrointestinal irritation being the principle effect observed. Another set of female rats was given diets containing up to 10,000 ppm of 2-4-D. Diets as high as 300 ppm for 113 days produced no effect. 1000 ppm in the diet for 113 days caused a decreased growth rate, increased mortality and increased liver weights with slight cloudy swelling of the liver. Dietary levels of 3,000 and 10,000 ppm of 2-4-D caused a very rapid decline and the animals were sacrificed after 12 days.

Central Nervous System Effects

Electroencephalograms were recorded on rats, cats and dogs responding to electrical stimulation and sound stimulation following the intraperitoneal injection of single or repeated daily doses of 2-4-D at the level of 200 mg/kg. The repeated daily doses lasted as much as six days before the death of the animals. In all experiments, the 2-4-D produced a definite depression of the electroencephalographic activity with changes in amplitude and frequency and loss of the desynchronization response. In those animals receiving repeated doses, the cardiac output was decreased by as much as 35% and there was a weight loss of 23% during the six days. Histology showed no changes in the grey or white matter of the brains of these animals. However, there was a demyelinization of the dorsal portion of the spinal cord.

Myotonia is readily produced in several species by injection of approximately 1/2 to 2/3 of the LD_{50}. Within 30 to 45 minutes after administration, the animal has lost most spontaneous activity and when startled into a sudden movement loses all muscle coordination. The animals slowly recover without permanent effects.

Excretion and Metabolism

Rats were given single oral doses of 3 to 300 mg/kg of radioactive-labeled 2-4-D. The rate of elimination was found to be dose dependent with no radioactivity in the expired air. Doses up to about 30 mg/kg were completely

excreted in the urine and feces within 48 hours. At higher dose levels, the per cent excretion decreased linearly with increased dosage. At the highest dose levels, the body still retained some radioactivity after six days and the activity was found in all twelve tissues which were examined.

Dermal Effects

2-4-D salts and esters were applied to the skin of rabbits in water and in oil and produced a local inflammatory reaction in all animals. These effects appeared to be more severe when oil was used as the vehicle.

Human Exposure to 2-4-D

From a series of accidental exposures, it appears that the oral dose required to produce symptoms in man is approximately 3 to 4 grams. One man is reported to have consumed 500 mg per day for 21 days without ill effects. The symptoms observed in accidental exposures and suicides resemble those observed in animals. In a suicide case the oral dose was at least 6500 mg. A patient suffering from coccidiomycosis was given progressively increasing intravenous doses of 2-4-D acid during several weeks of treatment without ill effects. A final dose of 3600 mg produced coma, fibrillary twitching of some muscles, hyporeflexia, and urinary incontinence. Within 48 hours he had completely recovered from the effects of the 2-4-D. 2-4-D has produced cases of contact dermatitis and chloracne, but these are not as severe nor as frequent as might be expected from the animal experiments. The Food and Drug Administration has established tolerance limits for residues of 2-4-D at 5 ppm on four crops which make up about 6% of the total diet, so that a level of 0.3 ppm is considered acceptable in the human diet.

Carcinogenicity

The iso-octyl ester of 2-4-D was injected subcutaneously at a single dose of 21.5 mg/kg dissolved in corn oil into two strains of carcinogenic-susceptible mice. Eighteen males and 18 females of each strain were injected once and then held for observation for 18 months, at which time all but five animals survived. All but one of the 72 animals was necropsied and 15 were found to have tumors. Half of the 16 tumors were reticulum cell sarcoma. Using a Chi square statistical analysis, the probability of this incidence of tumors occurring by chance in the experimental animals is less than 1% for both total tumors and for the sarcoma. Similar oral feedings at a maximum tolerated dose of 46.4 mg/kg administered in the diet in the 18 month experiment failed to produce a significant number of tumors. Closely related compounds including the 2-4-D acid, its isopropyl ester, and its butyl ester, were similarly evaluated and did not produce any significant number of tumors.

Effects on Fish

Four species of fish (goldfish, minnows, sunfish, and catfish) were exposed for seven days to concentrations of 2-4-D ranging from 10 ppm to 10,000 ppm and total mortality was observed. The data for the goldfish were erratic and the unexposed controls had a high mortality rate. The results with the other three species led to the conclusions that the maximum non-lethal dose of 2-4-D is 1,500 ppm for minnows, 500 ppm for sunfish, and 500 ppm for catfish. It was further concluded that since a spray concentration of 1000 ppm was adequate for herbicidal purposes, there would probably be no significant fish kill in surface waters contaminated by runoff when spraying operations were properly conducted, since there would be at least a 50% dilution during runoff.

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SUMMARY OF THE TOXICITY OF 2-4-5 Trichlorophenoxyacetic acid

We have no information on the stability of this compound or its chemical properties, except that the salts are soluble in water and insoluble in hydrocarbon solvents whereas its esters are insoluble in water and soluble in hydrocarbons.

Acute Toxicity in Animals

The acute oral LD_{50} for 2-4-5-T acid in a variety of species is given in the following table:

Species	LD_{50} (mg/kg)
Rat	300 to 500
Mouse	390
Guinea Pig	380
Chick	310
Dog	100

As was seen with 2-4-D, the dog is the most susceptible species to 2-4-5-T acid. It produces effects very similar to those of 2-4-D and the significant findings in those animals receiving lethal doses were irritation and necrosis of the intestinal mucosa, pneumonia, focal necrosis of the liver and congestion and anorexia accompanied by weight loss and myotonia.

Chronic Toxicity in Animals

Dogs were fed 2-4-5-T acid in capsules five days a week for 13 weeks at dose levels of 0, 2, 5, 10, and 20 mg/kg. All animals survived the doses as high as 10 mg/kg without symptoms, but those receiving 20 mg/kg either as a single daily dose or a divided daily dose, died at 11 through 59 days out of the 90 day test. The symptoms which were observed in these animals prior to their death included stiffness in the hindquarters and slight ataxia, with difficulty in swallowing food and some oozing of blood from the gums. There were no hematological changes or significant changes in organ weights except a slight increase in heart and kidney weights. The autopsy findings on the dogs at the 20 mg/kg level showed irritation of the gastrointestinal tract. One animal showed generalized icterus.

A variety of livestock were permitted to graze on alfalfa sprayed with 2-4-5-T at two to four times the recommended herbicidal dosage with no ill effects or indications of grazing preference between the treated and untreated forage.

2-4-5-T was incorporated in the feed of turkeys at a dose rate of 60 mg/kg per day without adversely affecting growth rate or feed consumption. At this level there was minimal and reversible cumulative action. However, neither the symptoms nor duration of feeding are reported.

Teratogenicity

Discussions with the staff of a private laboratory working under an NIH contract have produced information on current research findings that 2-4-5-T appears to be highly teratogenic. Since this work is not yet completed, the significance of these observations cannot now be determined.

Human Exposure

As usual, the conditions of exposure of humans are not well documented and are complicated by simultaneous exposure to other materials. There is one report of hallucinations following inhalation of 2-4-5-T, probably in the form of a mist or dust. There are also a few reports of chloracne among professional applicators. The American Conference of Governmental Industrial Hygienists recommends an atmospheric concentration of 10 mg/M^3 for repeated daily 8 hour exposures, with a warning that skin absorption may be significant. It has been reported that 2-4-5-T has an odor resembling that of iodoform which is just detectable at a concentration of 2.9 ppm in water. It is also reported that 2.3 ppm in water is toxic to fish.

Combination Exposures

There are a few reports in the literature of exposure of both men and animals to combinations of 2-4-5-T and 2-4-D. A commercial brush killer consisting of 30% 2-4-D esters and 34% 2-4-5-T esters was given orally to steers at 1000 mg/kg. A single dose produced no effect, however, repeated daily doses were fatal in three days. Symptoms prior to death were general depression, decreased food and water intake and decreased rumen motility. At necropsy the rumen was dry and smelled strongly of the herbicide. The abomasum was impacted and the intestinal contents were fluid. The mesenteric vessels were congested and the spleen was dark and shrunken. Steers receiving 500 mg/kg on each of three successive days showed no effects. One steer was fed 100 mg/kg daily for 15 days with no symptoms and at autopsy there was only slight irritation of the gastrointestinal tract with small areas of focal hemorrhagic necrosis in areas of fatty degeneration of the liver. There was also very slight interstitial edema and congestion in the kidneys.

In a suicide, an adult male ingested one cupful of weed killer containing approximately 20% 2-4-D and 40% 2-4-5-T. Within an hour he became nauseated and vomited, shortly after which he was found conscious but dazed.