2,4-D/TOX

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STATUS REPORT ON

THE CHEMISTRY AND TOXICOLOGY

OF 2,4-D

as of May 1, 1970

Releasable

Bureau of Foods, Pesticides and Product Safety
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Status Report of 2,4-D

Introduction

Although various herbicides have had extensive use in this country and abroad for many years, with peak production in 1960, only recently has major attention been focused on the potential health hazard to the general population of certain defoliants such as 2,4,5-T. Chloracne, a persistent andsevere skin disease, has been reported some 20 years ago in USA and Germany as an occupational hazard to workers in plants manufacturing chlorophenols, 2,4,5-T and 2,4-D. Associated with this focus on 2,4,5-T, on the basis of recent biological testing, is a more critical assessment of 2,4-D and its safety to man. For example, recent experiments reported by Bionetics Research Laboratories (Litton Industries, Bethesda, Maryland), while under contract to National Cancer Institute, established that 2,4,5-T was teratogenic to rats and mice and tend to establish that certain esters of 2,4-D should also be so classified.

Subcutaneous injection of high levels of 2,4-D in DMSO into various strains of mice and oral administration to one strain of mice increased the incidence of fetal abnormalities as compared to control group animals. Some esters of 2,4-D produced a statistically significant increase in proportion of litters containing abnormal fetuses and an increased incidence of abnormal fetuses within litters.

Of interest is the fact that finite tolerances are established for 2,4-D on food crops under the Act which sanctions the presence of residues in the diet as contrasted with 2,4,5-T which has no finite tolerances granted on food crops.

In further reference to biological testing, it has been found in FDA laboratories that a "purified" sample of 2,4-D administered orally to pregnant hamsters during organogenesis showed no significant difference in survival of the pups but a significant number of live pups exhibited terata. Very preliminary assay data from FDA laboratories on a sample of 2,4-D, using the chick embryo, indicated that 2,4-D showed embryotoxicity at levels comparable to those obtained with 2,4,5-T and some polychlorophenols. When N-butyl ester of 2,4-D was bioassayed by chick embryo test, chick edema syndrome and terata were observed.

Detailed analyses on 2,4-D for the presence of the various dioxins have not been performed but there is good reason to suspect that 2,4-D may have as a contaminant a series of various dioxins which have been

Registered uses:

No tolerances: blueberries, corn, cranberries, potatoes, rice, raspberries, soybeans, sugarcanes, and pastures.

Tolerances: 5 ppm - apples, citrus fruits, pears, quinces, asparagus
20 ppm - forage (barley, oats, rye, wheat)

Photodecomposition

2,4-D acid in water when exposed to UV light or sunlight will break down into 2,4-dichlorophenol, 4-chlorocatechol, 2-hydroxy-4-chlorophenoxyacetic acid, 1,2,4-benzenetriol, and polymeric humic acid. This is pH dependent.

Bacterial decomposition

Various metabolites have been reported in the breakdown of 2,4-D in soil. In general, their formation involves going through the intermediates of 2,4-dichlorophenol and 4-chlorocatechol. The presence of the reported metabolites in plants has not been investigated.

Nature of the residue

2,4-D esters are rapidly hydrolyzed on leaf surfaces and it is 2,4-D acid which is translocated. In plants, 2,4-D has been found to undergo esterification, decarboxylation, ring hydroxylation, and conjugation with amino acids, proteins, and glucose. Degradation of the side chain to form 2,4-dichlorophenol has been observed in many plants. The anisole structure may be an intermediate in the degradation. Two hydroxyphenoxyacetic acids have been identified: 4-hydroxy-2,5-dichlorophenoxyacetic and 4-hydroxy-23-dichlorophenoxyacetic. Hydroxylation of 2,4-D is made by a shift of the chlorine atom from the 4 to the 3 or 5 position. β-D-glucoside of 4-hydroxy-2-chlorophenoxyacetic acid and β-D-glucose ester of 2,4-D have been reported. The rates at which these reactions will occur will depend upon the plant species being treated and the time of application or use pattern.

Method of analysis

The present methods being used in the District laboratories are the one listed in the Pesticide Analytical Manual, Volume I, sections 221 and 222. The methods are suitable for the analysis of acids, salts, and esters of 2,4-D. In principle, the sample is extracted with mixed ethers under acidic conditions. The other phase is, in turn, extracted with a basic

identified in 2,4,5-T and associated with the recent adverse effects reported for that herbicide. The possibility of interaction of these compounds, that is the chlorodioxins with each other, or between the chlorodioxins and chlorophenols and the herbicides, cannot be overlooked. It is to this problem of identification, surveillance, and biological assessment that we must direct our attention as forcefully as we are now doing in the case of 2,4,5-T.

Chemistry

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2,4-dichlorophenoxyacetic acid (2,4-D) is a systemic compound whose activity is attributed to a plant hormone-like effect. Its primary use has been as a selective herbicide against many broad-leaf weeds in cereal and grass crops. Other herbicidal uses involve the elimination of unwanted vegetation on railroad rights-of-way, roadsides, airports and similar sites. Certain aquatic weeds are also controlled, notably water hyacinths in canals. A secondary use has been as a plant growth regulator for the purpose of obtaining higher quality fruit.

The production of 2,4-D continues to increase but at a slower rate. 2,4-D reached its peak in the early 1960's but due to the demands in Southeast Asia, production has been kept high. In 1968, 94 million pounds of 2,4-D acid, esters, and salts were produced. Because of its effectiveness and low price, we do not see any decrease in future production. There appears to be need for use of 2,4-D in brush control and in the use of 2,4-D combinations with EPTC, fenac and dicamba for weed control.

Preparation of 2,4-D

2,4-D is commercially made from 2,4-dichlorophenol and chloroacetic acid. Contaminants may include 2,4-dichlorophenol although it is quite volatile. A possible contaminant which may be of concern is 2,4-dichlorodibenzo-p-dioxin.

Formulations

2,4-D is a white crystalline material, very slightly soluble in water. It may be formulated as the parent acid, as a salt of alkali metals or amine, or as esters of short and long chain alcohols. Each formulation is designed for maximum effectiveness based on the crop, weeds present, and environmental conditions.

solution. Any esters present are retained in the ether phase and after separation of the phases, the ether phase is reserved for further treatment if desired. The basic solution containing 2,4-D is acidified and 2,4-D is extracted with chloroform. This is methylated and the methyl ester is analyzed by microcoulometric gas chromatography.

The sensitivity of the method will depend upon the type of sample to be analyzed. On individual crops or products, the method may be sensitive to 0.01 ppm or less. With the total diet sample, the sensitivity is 0.02 ppm; however, District chemists have encountered some problems with these methods. A number of low recoveries have been reported.

Residue Chemistry Branch is working on the modification of the techniques so as to make the methods more reliable at the lower sensitivities. The modifications are: (1) use of acetonitrile for extraction where practical, (2) modifying the methylation procedure to avoid losses by volatization of the methyl ester, (3) additional cleanup by Florisil chromatography, (4) isothermal gas chromatographic analysis. The modified procedure is being tested in intra-lab study.

The method in the PAM, Volume I, will not detect nor determine any of these conjugated metabolites of 2,4-D. In order to analyze for these complexes, a hydrolysis step must be introduced into the procedure to liberate the 2,4-D.

2,4-dichlorophenol will be analyzed along with the 2,4-D acid but its retention time by GLC is so short that it elutes with the solvent front. Hydroxychlorophenoxyacetic acids have not been tested to determine whether they are measured by the above method.

2,4-D forms a number of different conjugates in various media. At the present time not sufficient work has been done on residues of the conjugates for use to know the quantitative relationship of the conjugated to the free 2,4-D acid. The few samples checked in our lab (grasses and lemons) have shown the amount of residue as a conjugate to be minor compared to the free acid.

Toxicology

A 2-year feeding study in Osborn-Mendel rats using 0, 5, 25, 125, 625 or 1250 ppm of 2,4-D. No effect was noted on growth rate, survival rate, organ weights, hematologic values or incidence of tumors at any level teste

A 2-year feeding study in 30 beagle dogs using 0, 10, 50, 100 or 500 pp of 2,4-D. No compound related effect was noted in 28 dogs. The microscopi pathology in the two dogs which died was not considered to be due to the 2,4-D feeding.

A 3-generation, 6-litter Osborn-Mendel rat reproduction study using 0, 100, 500 and 1500 ppm. At the highest level there was a decrease in the percent of pups born that were weaned and in the weight of the weanlings. The study covered the period 1964-1966.

Studies 1, 2 and 3 have been incorporated into a manuscript which has b submitted to Toxicology and Applied Pharmacology.

Livers from Osborn-Mendel rats about 90 days old which were the second litter of a second generation reproduction study and had been fed 2,4-D at levels of 0, 100 and 500 ppm were tested for activity of aliesterase and acylamidase. The liver enzyme activities in the control rats were the same as in treated rats. Study was completed in 1966.

Male and female Osborn-Mendel rats fed 2,4-D from the weaning period at concentrations of 100 and 500 ppm in a diet mix were tested for alterations in normal physiological responses at 4 months of age. There was no statistical difference between corresponding control and experimental rats in hexobarbital sleep time, heart rate, blood pressure, spontaneous motor activity, and electroshock threshold. This study was completed in September 1965.

Measurement of 2,4-D levels in workers in a herbicide manufacturing plant is being done by Atlanta Toxicology Branch.

Groups of Sherman female rats were fed 0, 1000 and 2000 ppm of 2,4-D in the diet for 95 days and bred to untreated males. No overt signs of toxicity were noted, but the pups of the treated rats were abnormally small at birth and had a lower survival rate than controls. This study was done at Atlanta in 1969.

Dr. Verrett of the Division of Toxicology, OFNS, tested 2,4-D both technical and recrystallized sample, in the chick embryo. At 10 ppm she found terata and chick edema. Lower levels are now being tested.

One preliminary test for teratogenicity of 2,4-D in hamsters was made. The sample had been purified by recrystallization and was administered at 100 mg/kg to pregnant hamsters on day 6 through 10 of organogenesis. Mortality of pups was similar in control and treated hamsters, but terata was noted in 15 out of 62 live pups. The terata: 12 lacked complete head fusion, one had a short tail, 2 poor mandible and maxilla development.

Research performed by Bionetics Laboratories on 2,4-D involved the subcutaneous injection of the agent in DMSO at dosages of about 100 mg/kg to C3H, BL6, AKR, and B6AK mice. The agent was administered orally to BL6 mice at 100 mg/kg. Increased incidence of fetal abnormalities was seen in four of the six adequately sized groups used for subcutaneous injections and in the one group administered the agent orally. (Bionetics Research Laboratories, Inc., Volume III, Evaluation of the Teratogenic Activity of Selected Pesticides and Industrial Chemicals in Mice and Rats, p. 21)

Not from DPCT

2,4-D isooctyl ester, 2,4-D butyl ester, 2,4-D isopropyl ester each produced a statistically significant increase in the proportion of litters containing abnormal fetuses and in the increased incidence of abnormal fetuses within litters. The latter two esters were statistically signific at the 0.01 level for one or more tests. The isooctyl ester was significa at the 0.05 level. (Report of the Secretary's Commission on Pesticides, Dec. 1969, p. 666).

Finite tolerances established for 2,4-D on food crops under the Act sanction the presence of residues in the diet as opposed to those from 2,4,5-T. (Sec. 120.142)

Widespread use in home yard care of 2,4-D (and 2,4,5-T) must be recognized as a source of exposure in the U.S., the magnitude of which cannot be accurately estimated at this time.

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