

DATA EVALUATION RECORD

- (1) CHEMICAL: Trichlorfon
- (2) TYPE OF FORMULATION: Unspecified
- (3) CITATION: Hassan, A., and Zayed, S.M.A.D. 1965. Metabolism of organophosphorus insecticides: III. Fate of the methyl groups of dipterex in vivo. Can. J. Biochem. 43:1271-1275

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- (6) TOPIC: This study has information pertinent to discipline toxicology, topic metabolism. It relates to none of the Proposed Guidelines data requirements.

(7) CONCLUSION: A single dose of 200 mg/kg of trichlorfon, in which the two methyl groups were ^{14}C -labeled (O,O- ^{14}C -dimethyl-2,2,2-trichloro-1-hydroxyethyl phosphonate), was injected intraperitoneally into rats. $^{14}\text{CO}_2$ was detected in the expired air at 1 hour after administration; by 24 hours, about 24% of the injected dose was recovered as $^{14}\text{CO}_2$ in the expired air, and about 32% of the dose was recovered in the urine. Dimethylphosphate and formate were detected in the urine. No other routes of excretion were examined. The findings indicate that metabolism of trichlorfon involves hydrolysis of the P-OCH₃ ester bonds.

CORE CLASSIFICATION: Not applicable

(8) MATERIALS AND METHODS:

Test Substance: O,O- ^{14}C -dimethyl-2,2,2-trichloro-1-hydroxyethyl phosphonate was used in this experiment. The material was determined to be 98% pure, with a specific activity of 0.5 mcurie/g.

Test Organism: Albino rats weighing 100-120 g were used. No further information on test animals was provided.

Test Procedure: ^{14}C -trichlorfon was injected intraperitoneally in rats in sublethal doses of 200 mg/kg. Metabolic cages were used for the collection of respiratory $^{14}\text{CO}_2$; the ^{14}C -activity was trapped by sodium hydroxide solutions and determined as Ba $^{14}\text{CO}_3$.

Urine was dried over P_2O_5 and ^{14}C -activity determined according to the procedure of Aronoff (1957. Techniques of Radiobiochemistry. The Iowa State College Press) using Van Slyke-Folch reagent. Radio-paper chromatography was used for analysis of ^{14}C -activity in the urine, using a Frieske and Hoepfner radioscanner.

An inverse isotope dilution technique was used for determination of ^{14}C -formate in the urine. Inactive sodium formate was added to a urine sample, pH was adjusted to 1.0, and the solution was steam-distilled. The distillate was rendered alkaline and evaporated under vacuum. The residue was extracted and crystallized in ethanol-water mixtures. ^{14}C -activity was determined (according to Aronoff 1957) and the specific activity of ^{14}C -formate was calculated. The formate was also characterized by paper chromatography.

^{14}C -metabolites of trichlofon were identified by paper chromatography and analyzed radiometrically.

All radioactivity measurements were carried out in an end-window Geiger counter, and corrected for background and self-absorption.

- (9) REPORTED RESULTS: $^{14}CO_2$ measurements in the expired air were reported for hourly intervals for the first 8 hours after administration, and at 10, 20, and 24 hours. The formation of labeled CO_2 , from the hydrolysis of $P-O^{14}CH_3$

began "at once." By 10 hours, about 24% of the total administered dose, or 85% of the total expired dose, was eliminated. It was reported that in four similar experiments, 19.2, 22.2, 26.8, and 20.6% of the radioactivity was eliminated as $^{14}\text{CO}_2$ in the expired air within 10 hours. Investigators calculated a mean value of 1.75 $\mu\text{moles/hour}$ per 100 g rat for the rate of hydrolysis of trichlorfon at the o-methyl ester bonds. Based on the relationship of percentage of dose excreted in expired air over time, it was hypothesized that the rate of hydrolysis could be resolved into three phases: the first 3-4 hours involving factors such as absorption, distribution, and enzyme saturation; the next 6 hours characterized by a steady state in which turnover proceeds at a maximum, constant rate of hydrolysis; and after 10 hours, where a decrease in the rate of hydrolysis was observed.

About 32% of the administered dose of ^{14}C was recovered in the urine after 24 hours. The only metabolite identified by radio-paper chromatography was dimethylphosphate, at 16 hours and at 24 hours (comprising 65-75% of the urine ^{14}C -activity at 24 hours). Monodemethylated trichlorfon could not be detected in vivo. Radioactive formate was found to contribute 7-15% of the total urine ^{14}C -activity. It was stated that formate activity was not easily detectable, probably as a result of the acidic nature of the

urine, which would release the readily volatile formic acid.

In their discussion, the investigators pointed out that earlier studies found hydrolysis of trichlorfon to occur at the phosphate (C-P) bond (Arthur and Casida, 1958. J. Agric. Food Chem. 6:360). In light of the present findings, the investigators stated that a second metabolic pathway also exists involving cleavage at the P-OCH₃ bond.

It was proposed that CO₂ in the expired air is a result of the following chain reaction: trichlorfon to methanol to formaldehyde to formate to CO₂. They suggested, based on evidence in the literature, that the rates of elimination of the intermediates in this chain when administered to rats are relatively rapid, that the rate determining reaction in this reaction sequence is hydrolysis of trichlorfon at the P-OCH₃ bond(s) to give methanol.

The investigators proposed that hydrolysis of o-methyl ester linkages may take place in the liver and kidney. Based on the work of other researchers, it was stated that methanol is oxidized almost exclusively by the action of catalase-H₂O₂ complex, and formaldehyde further oxidized to formate by aldehyde oxidase and possibly the action of catalase-H₂O₂ complex. The possibility of formaldehyde being back cyclized to methanol through the alcohol dehydrogenase system was also noted.

- (10) DISCUSSION: This paper provides some preliminary information concerning the metabolism and excretion of trichlorfon. However, a number of the conclusions drawn by the investigators can only be considered hypothetical because of the limited excretion data reported.

Based on their detection of formate in the urine and CO_2 in the expired air, it is reasonable to suggest that metabolism of trichlorfon includes hydrolysis of the P-OCH_3 ester bond.

From the curve of the elimination of $^{14}\text{CO}_2$ in expired air (as a percentage of administered dose) versus time, an estimate for the mean rate of hydrolysis of trichlorfon at the ester bonds can be obtained. The data also indicate that hydrolysis begins at once following administration of 200 mg/kg of labeled trichlorfon, with the rate of hydrolysis decreasing after about 10 hours. Any further conclusions drawn by the investigators from this elimination curve can only be considered speculative.

The results presented on ^{14}C -activity in the urine are extremely limited and are not supported by any raw data. The authors did not give the collection times for urine samples and report only results after 24 hours. Since only about 56% of the total administered radioactivity was recovered in the urine and expired air, it would be valuable to know whether this is a function of a slow rate of excretion in the urine, excretion in the feces

that was not measured, bioaccumulation in the tissue or low recovery due to the methods of detection used. Measurement of radioactivity was carried out in an end window Geiger counter. Although an appropriate method for determination of ^{14}C -activity at the time of the study, 1965, more sensitive methods of detection (i.e., scintillation counting) would be used today. Because of these uncertainties in reliability of excretion data for ^{14}C -labeled compounds, this study has little quantitative value.

The investigators also suggested a reaction sequence in the metabolism of trichlorfon to CO_2 , with the hydrolysis at the P-OCH_3 ester bond as the rate-determining step. While this is a plausible hypothesis, the investigators present no support for this pathway. Again, such conclusions can only be considered speculative.

(11) TECHNICAL REVIEW TIME: 6.0 hours