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DATA EVALUATION RECORD

(1) CHEMICAL: Trichlorfon

(2) TYPE OF FORMULATION: Analytical reagent grade

(3) CITATION: Dedek, W., Loho, K., Fischer, G.W., and Schmidt, R. 1976. Alkylation of guanine in mice in vivo by organophosphorus insecticides: 1. Trichlorphone and butonate. Pestic. Biochem. Physiol. 6:101-110

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(6) TOPIC: This study has information pertinent to discipline toxicology, topic mutagenicity. It relates to the Proposed

Guidelines data requirement 163.84-4 (detection of primary DNA damage).

(7) CONCLUSION: After the intraperitoneal administration of trichlorphone to male mice, small quantities of 7-^{14}C-methylguanine were found in the lung, kidney, testicles, and liver, with approximately 10 times as much of the alkylated purine being present in the liver as in the other three tissues. The 7-methylguanine was excreted rapidly from the body with a half-life of less than 24 hours. The relative extents of alkylation of guanine of nucleic acids in vivo for dichlorvos, butonate, and trichlorphone was 100:10:25, respectively.

CORE CLASSIFICATION: Not applicable

(8) MATERIALS AND METHODS: Methyl-^{14}C-trichlorphone of 99% purity and specific activity of 22 mCi/mmole was prepared by the authors. All of the other chemicals were of analytical grade and were purchased from Fluka AG, Switzerland.

The radioactive compound was dissolved in 0.1 ml of 1,2-propylene glycol and was administered intraperitoneally at a dosage of 160 mg/kg to male mice that had been deprived of food for 24 hours. The animals were killed by chloroform, and the dissected tissues were frozen and stored at -20° until used for isolation of nucleic acids (DNA and RNA). The tissues were extracted repeatedly with chloroform/isoamyl alcohol and phenol. Protein was removed from the extract by incubation with pronase, and the com-

bined DNA and RNA were precipitated with ethanol with subsequent dialysis. After acid hydrolysis of the nucleic acids, the adenine, guanine, and 7-methylguanine were separated and purified by chromatography and rechromatography on ion-exchange resin and assayed for radioactivity. Urinary purines were precipitated as silver salts and purified by ion-exchange chromatography.

In vitro alkylating activity was determined by the reaction of the agent with 4-(4-nitrobenzyl)pyridine in methanol solution at 25°C.

(9) REPORTED RESULTS: ^{14}C was present in adenine, guanine, 7-methylguanine and pyrimidine nucleotides of liver nucleic acids at short intervals after administration of the agent, but no radioactive 7-methylguanine was found by 24 hours after the administration. Small quantities of ^{14}C-7-methylguanine were found in the urine at 3, 6, and 12 hours, and these quantities decreased rapidly with time. During the 24-hour period the fraction of the total dose of ^{14}C that was present in the urine as ^{14}C-7-methylguanine was $1\text{-}1.5 \times 10^{-5}$. Approximately 25% of the administered ^{14}C was excreted in the urine during the first 12 hours and 5% during the 12-24 hour period. The measurement of the quantity of ^{14}C-7-methylguanine in the urine was considered to be an indicator of the extent of alkylation of the nucleic acids throughout the whole animal and could be used for

comparing the extents of alkylation by various agents. On this basis, the relative extents of in vivo alkylation by dichlorvos, butonate, and trichlorphon were 100:10:25, respectively. The relative rates of reaction with 4-(4-nitrobenzyl)pyridine were 100:35:4 for dichlorvos, butonate, and trichlorphone, respectively.

(10) DISCUSSION: The experimental results of the in vivo experiments presented in this report serve to qualitatively show that 7-methylguanine was formed in the liver and that it was present in the urine, and to permit quantitative comparisons of the quantities of 7-methylguanine present in the urine after the administration of various agents.

Although it is stated in the summary that ^{14}C-7-methylguanine was present in the lung, kidney, and testicles, no data or discussion of this was given in the text.

Several defects detract from the significance of this study: No effort was made to separate the DNA and the RNA, so the source of the ^{14}C-7-methylguanine is not known. Since it was shown in the study that ^{14}C was incorporated into the purine rings via the one-carbon pools, it is not possible to quantitate the actual quantity of ^{14}C-7-methylguanine, because it is not known how much of the ^{14}C might be in the purine ring and how much might be in the 7-methyl group. The possibility that some of the ^{14}C-7-methylguanine might be formed by normal transmethylation onto the RNA-guanine

has not been considered. Since the reactions with 4-(4-nitrobenzyl)pyridine were performed in nonaqueous medium, the relevance to the possible alkylating activity under physiological conditions is unknown.

These experiments give only suggestive evidence that alkylation of nucleic acids occurred and do not give quantitative data that would show the absolute extent of the alkylation, if it does indeed occur.

(11) TECHNICAL REVIEW TIME: 3.8 hours