DATA EVALUATION RECORD

TRICHLORFON

Metabolism, in vitro

CITATION: Hassan A, Zayed SMAD, Abdel-Hamid FM. 1965. Metabolism of organophosphorus insecticides. II. Metabolism of 0,0-dimethyl- 2,2,2-trichloro-1-hydroxyethyl phosphonate (Dipterex) in mammalian nervous tissue and kinetics involved in its reaction with acetylcholine esterase. Can. J. Biochem. 43:1263-1269.

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DATA EVALUATION RECORD

STUDY TYPE: Metabolism, in vitro.

CITATION: Hassan A, Zayed SMAD, Abdel-Hamid FM. 1965. Metabolism of organophosphorus insecticides. II. Metabolism of O,O-dimethyl- 2,2,2-trichloro-1-hydroxyethyl phosphonate (Dipterex) in mammalian nervous tissue and kinetics involved in its reaction with acetylcholine esterase. Can. J. Biochem. 43:1263-1269.

ACCESSION NUMBER: Not available.

MRID NUMBER: 00091990.

LABORATORY: Department of Biology, Atomic Energy Establishment and National Research Centre, Cairo, Egypt.

TEST MATERIAL: [^{32}P] Trichlorfon, 98 percent pure.

PROTOCOL:

1. ^{32}P-Dipterex (trichlorfon) with specific activity 0.6mCi/g and 98 percent pure was used in the assay.

2. ^{32}P-Dipterex (1.9×10^{6} cpm) was incubated at a final concentration of 1.4×10^{-2}M with 5.0 ml of 20 percent rat brain homogenate in distilled water for 5 hr at 37 C. The source, age, and sex of the rats used was not specified.

3. The mixture was extracted with CCl_3, carried through column chromatography with Dowex 1-X8, Cl^-, 100-200 mesh, and all acidic fractions were analyzed for total phosphorus. The trichloroethyl grouping was identified using the procedure of Glang et al. (J. Agr. Food Chem. 2:1281, 1954).

4. The metabolites present in the CCl_3 extract and aqueous layer were analyzed by paper chromatography. Following ascending paper chromatography and spot identification, fractions were assayed for radioactivity to quantify the metabolites.

5. Rat brain acetylcholine esterase was assayed according to the method of Hestrin (J. Biol. Chem. 190:249, 1949). Non-radioactive Dipterex (98 percent pure) was used in these studies.

RESULTS:

Following incubation about 17.5 percent of the insecticide was metabolized. Hydrolytic products of Dipterex (acidic metabolites) contributed 10.4 percent of the total radioactivity recovered and the water soluble, nonacidic metabolites accounted for 7.1 percent. Among the latter, the major product had an Rf value of 0.48 vs. 0.95 for Dipterex in an n-butanol-pyridine-water (12:8:6) system. Another major metabolite, with an Rf value of 0.48 vs. 0.80 for Dipterex in a 2-propanol-NH_4OH-water (75:24:1) system was also seen. These 2 metabolites accounted for 70 percent of the total metabolites resolved by chromatography. The data indicated that the main metabolic pathway of Dipterex in the nervous tissue involved hydrolysis of the methyl ester linkage(s) which would result in the production of O-methyl-2,2,2-trichloro-1-hydroxyethyl phosphoric acid, a product identified in this study by the Fujiwara test (G. Fricke and W. Georgi, J. Prakt. Chem. 20:250, 1963) and 2,2,2-trichloro-1-hydroxyethyl-phosphoric acid. Acetylcholine esterase was irreversibly inhibited by Dipterex, suggesting that it was not at all involved in the detoxifying metabolism.

CONCLUSIONS:

The authors concluded that the formation of monodemethylated Dipterex and monomethylphosphate indicated that a phosphomonoesterase and an esterase responsible for cleavage of the phosphonate bond are involved in the metabolism of Dipterex in the nervous tissue. Studies involving acetylcholine esterase suggest that Dipterex and acetylcholine compete for the substrate-binding group in the active center of this enzyme. Because this latter enzyme is irreversibly inhibited, it is implied that this inhibition is responsible for the poisoning effect of this organophosphate.

CORE CLASSIFICATION: Supplemental. The study provides information on the in vitro metabolism of trichlorfon in the brain tissues and the major metabolites formed.