The Mutagenicity of Dichloroacetaldehyde

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Z. Naturforsch. **33 c**, 783–785 (1978);
received July 12, 1978

Dichloroacetaldehyde, Dichloroethanol, Dichlorvos, Mutagenicity, *Salmonella*/Microsome test

Dichloroacetaldehyde, a presumed metabolite of the insecticides dichlorvos and trichlorphon, is mutagenic in the Salmonella/microsome test. Its mutagenic potency is higher than that of the established mutagen dichlorvos. It is possible that the bacterical mutagenicity test only or mainly detects the effect of methylation by dichlorvos.

2,2-Dichloroacetaldehyde is a presumed metabolite of the insecticides 0,0-dimethyl 1-hydroxy-2,2,2-trichloroethyl phophonate (trichlorphon, dipterex) [1] and 0,0-dimethyl 0-2,2-dichlorovinyl phosphate (dichlorvos, DDVP) [2] in the metabolism leading to dichloroethanol-glucuronide. The mutagenicity of dichlorvos has been detected in a number of test systems [3, 4] and the effect has largely been ascribed to the methylation of nucleophilic targets by dichlorvos [5–7]. It has been inferred that methylation of DNA cannot occur *in vivo* at practical use concentrations of dichlorvos due to its rapid metabolism [8]. It has also been suggested that the genotoxic effects of dichloroacetaldehyde should be investigated [9].

Mutagenicity tests were performed with the Salmonella/microsome test system using the plate incorporation assay [10]. The microsomal preparation (S-9) was from Aroclor 1254 treated male rats and the activation system (S-9 mix) was prepared as described by Ames *et al.* [10]. The *Salmonella typhimurium* strain TA 100 *(hisG46, rfa, ΔuvrB, pKM101)* has been used. Its spontaneous reversion frequency has been in the range 144–189 throughout the study.

Analytical standard dichlorvos was a gift from Shell Chemical Co. Dichloroacetaldehyde was prepared from dichloroacetal (K & K Laboratories, Irvine, CA) as described by Paterno [11]. The chloroacetaldehyde was the same as used by McCann *et al.* [12]. 2,2-Dichloroethanol and 2-chloroethanol were obtained from K & K Laboratories and Matheson Coleman & Bell respectively. For the testing the aldehydes were dissolved in dimethyl sulfoxide (DMSO) and the alcohols in water or used undiluted. Dichlorvos was tested in both DMSO and aqueous solution.

The results of a number of assays are shown in the figures.

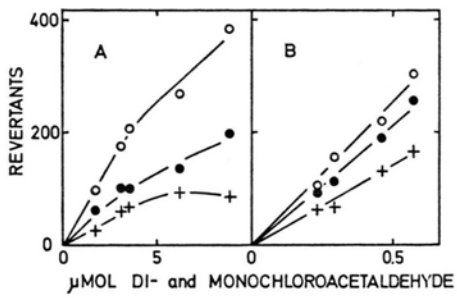

Fig. 1. Reversion of TA 100 with (A) dichloroacetaldehyde and (B) chloroacetaldehyde. ○, without S-9 mix; ●, with S-9 mix lacking NADP and glucose-6-phosphate; +, with complete S-9 mix (20 μl S-9 per plate).

Dichloroacetaldehyde (Fig. 1) causes a reversion frequency of 58 revertants/μmol. This is about one tenth of the mutagenicity of the chloroacetaldehyde sample (Fig. 1) giving 515 revertants/μmol which is in accordance with an earlier study [12] in which 746 revertants/μmol was obtained. The mutagenicity of both chloroacetaldehyde and dichloroacetaldehyde decreases in the presence of the microsomal activation system. A part of this decrease is dependent on the presence of the co-factors NADP and glucose-6-phosphate. Parallel assays have shown that the presence of S-9 mix lacking S-9 does not influence the mutagenicity of the compounds. It is thus clear that part of the inactivation of these aldehyde has also been tested with the complete set of tester strains. It is negative in TA 1537 and TA aldehydes as mutagens is due to a NADP(H)-dependent microsomal/S-9 reaction. Dichloroacet-1538 and positive in TA 1535 and TA 98 showing a mutagenicity spectrum of a type caused by base pair substitutions.

The mutagenicity of dichlorvos (Fig. 2) corresponds to 17 revertants/μmol and is independent of the microsomal activation system over a wide range of S-9 additions. No difference has been found between water and DMSO as solvents for dichlorvos. Other assays with the modification of liquid incubation [10] for 20 min at 37 °C give about the same reversion frequency, 19 revertants/μmol in

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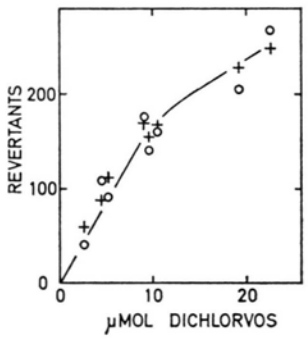

Fig. 2. Reversion of TA 100 with dichlorvos. ○, without S-9 mix; +, with S-9 mix (average of assays containing S-9 in the range 20–150 μl per plate).

the absence of S-9 and the possibility of a decreased mutagenicity to about 15 revertants/μmol in the presence of S-9.

A weak mutagenicity of chloroethanol has previously been reported [12] of which a part is detectable in the absence of S-9 and another part is dependent on the presence of S-9 but independent of NADP. This behavior has been confirmed (Fig. 3). Dichloroethanol gives under similar conditions no detectable mutagenicity.

The present study focuses the attention on the importance of evaluating the mutagenic effects of metabolites of a compound under study. It seems likely that – in the testing of dichlorvos – the Salmonella/mircosome test system only or mainly

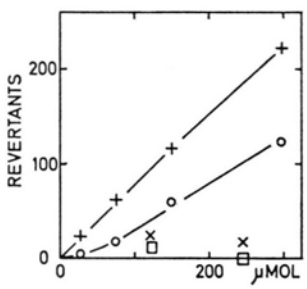

Fig. 3. Reversion of TA 100 with (○, +) chloroethanol and (□, ×) dichloroethanol. ○, □, without S-9 mix; +, ×, with S-9 mix lacking NADP and glucose-6-phosphate (150 μl S-9 per plate).

detects the effects of methylation by dichlorvos. *In vitro* studies with liver fractions have indicated that these mainly metabolize dichlorvos by dealkylation to desmethyldichlorvos [1]. An *in vivo* mutagenicity study of dichlorvos using the host-mediated assay was negative [13], but it has been emphasized that this result is not in conflict with the positive *in vitro* results as the doses had to be kept relatively lower in the *in vivo* test [3].

A direct comparison between chloroacetaldehyde and dichloroacetaldehyde is not possible. The chloroacetaldehyde used in this study is, as pointed out by Elmore *et al.* [14], a mixture of the monomer hydrate and dimer hydrate forms of which the dimer has a lower mutagenicity than the monomer. The actual mutagenicity of chloroacetaldehyde is thus higher than that measured in the present study. Both chloroacetaldehyde and dichloroacetaldehyde are mainly hydrated in aqueous solution [15]. The order of the mutagenic potency, *i. e.* chloroacetaldehyde > dichloroacetaldehyde, is in agreement with the finding by Waskell [16] that trichloroacetaldehyde (chloral) is a very weak mutagen for the TA 100 strain. Changes suggestive of a premalignant condition have been reported in a subacute toxicity test of chloroacetaldehyde [17]. Dichloroacetaldehyde has recently been shown to be mutagenic in mice in the dominant lethal test having a mutagenic activity comparable with that of trichlorphon [1].

Dichlorvos has been tested for carcinogenicity in two major assays. It was concluded from a 2-year inhalation study in rats that there was no dose-related increase in tumor risk [18]. It was concluded in the National Cancer Institute bioassay of dichlorvos that the compound was not demonstrated to be carcinogenic but that the possibility of tumorigenicity is not precluded [19]. Trichlorphon has been reported to be tumorigenic [20].

This work has been supported by the Swedish Natural Science Research Council and by ERDA grant E(04-3)-34-PA156 and carried out in the laboratory of Dr. B. N. Ames, University of California, Berkeley, whom I would like to thank for his support and advice.

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