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Mutagenicity Testing of Paraquat

Inveresk Research International Edinburgh, Scotland
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Notes taken from microfiche, received from Mr. Robert Taylor (PM; RD) on 3/13/81

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SUMMARY

" Paraquat dichloride was accepted from ICI Ltd. for mutagenicity testing with Salmonella typhimurium strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100. The tests were conducted on agar plates both in the presence and absence of a preparation from the livers of male rats treated with Aroclor 1254 and the cofactors required for mixed function oxidase reactions.

Doses of paraquat dichloride used were between 1 µg and 1 mg/plate.

Clear toxic effects were observed at 1 mg/plate, while at 333 µg/plate there appeared to be inhibition of mutant colony growth but normal growth of nonmutant microcolonies. At lower dose levels, no mutagenic effect was observed.

It was concluded that paraquat dichloride was without mutagenic potential detectable by these tests."

① * requires metabolic activation by oxidative reactions.

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MATERIALS AND METHODS

Test mat.: paraquat dichloride from ICI, 99.9% pure; white powder was dissolved in 0.05 M phosphate buffer; pH 7.4.

2-Aminoanthracene (carcinogen ≡ known mutagen) was dissolved in dimethylsulphoxide.

Animals Male rats, 250-300 g, were injected i.p. with Arocior 1254 in corn oil, 500 mg Arocior 1254 / kg body wt., 5 days before they were killed. Food was withdrawn 15 hrs before they were killed.

Bacteria

All 5 strains had mutations in the histidine operon (had to have histidine for growth). The 3 mutations were: his G 46 in TA 1535, TA 100; his C 3076 in TA 1537; his D 3052 in TA 1538 & TA 98. The first type of mutation is reversed by a variety of mutagens that cause bax-pair substitutions. The second type mutation is reversed by 9-aminoacridine, ICR-191 & epoxides of polycyclic hydrocarbons. The 3rd type is reverted by aromatic amines & derivatives.

9000 g supernatant

Homogenized livers in 3 volumes their wt of 0.15 M KCl (cold). Spun at 9000 g for 10 min at 0° to get supernatant + pellet (mostly whole cells, nuclei & mito.)

Testing

Bacteria are grown in nutrient broth. A cofactor solution (0.05 M PO_4 buffer, pH 7.4 + NADP - Na salt + glucose-6-PO_4 - di K-salt, + $MgCl_2 \cdot 6H_2O$ + KCl) is mixed with 9000 g supernatant in the ratio of 9:1 (S-9 mix). Then S-9 mix, bacteria, solvent or test solution & dilutes agar were mixed (agar had histidine+ biotin added to it). This mixture was poured on agar plates containing 2% glucose. The plates were incubated for 2 days at 37° & the colonies were counted. The plates

were also examined for precipitates & (microscopically) for microcolony growth.

RESULTS

(Positive control) System was highly sensitive to 2-Aminoanthracene (0.5 μg/plate)

(Paraquat dichloride) No indications of mutagenic activity either with or without the mixed function oxidase system. Bacteria were almost completely killed at 1mg par. dichloride/plate. At 333 μg/plate and, to a lesser extent, 100 μg/plate, there was inhibition of mutant colony growth.

(Conclusion) "Paraquat dichloride was not found to be mutagenic in these tests, which achieved a satisfactory sensitivity. The substance was, however, toxic at a dose of 1mg/plate." Levels used: 10, 33, 100, 333, 1000 μg/plate of par. dichloride.

(Apparently, number of mutant colonies/plate is counted & compared with control containing buffer in place of the test material. 2-Aminoanthracene greatly increased colony growth). "At least a doubling of these control values was looked for at some concentration of the test substance if a mutagenic effect was to be suspected".

NOEL: 33 μg/plate (paraquat dichloride)

LEL: 100 μg/plate. (paraquat dichloride)

Core-Minimum