Bull. Environm. Contam. Toxicol. 25, 277-282 (1980)

# Mutagenicity Studies Involving Aldrin, Endosulfan, Dimethoate, Phosphamidon, Carbaryl and Ceresan

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Agriculture use of pesticides carries with it potential hazards to man directly by exposure to toxic residues in food and indirectly through its environment. Organchlorine pesticides are persistent and their toxicity varies remarkably. These pesticides are residual in nature, accumulate in the body and cause chronic toxicity (SYED QUDRI 1978). Organophosphorus pesticides are moderately to highly toxic and hazardous to handle. Chromosome damage due to organophosphorus insecticides through accidental and occupational exposure has been reported by TRINH VAN BAO, et al. (1974) and YODER et al. (1973). Further, occupational workers exhibited a higher frequency of chromosomal aberrations in lymphocyte culture during spraying season (YODER et al. (1973), showing genotoxic effects in man. Carbamates are widely used as insecticides, some of them were tested for their mutagenic effects (MARSHALL et al. 1976, DEAN BLEVINS et al. 1972, SINHA et al. 1978). Mutagenic potentialities of mercuric pesticides have also been reported (RAMEL 1969), SKERFVING et al. 1970).

Despite the extensive use of pesticides our information on their possible mutagenicity is inadequate. In the present investigations the genetic and cytogenetic effects of two organochlorine pesticides (aldrin and endosulfan), two organophosphorus pesticides (dimethoate and phosphamidon), a carbamate insecticide (carbaryl) and a mercuric pesticide (ceresan) have been tested to screen their possible mutagenicity using micronucleus test and/or host mediated assay.

## MATERIALS AND METHODS

Effects of 1/3 $LD_{50}$ or $LD_{50}$ doses were tested in Swiss albino male mice weighing 23-25 g.

MICRONUCLEUS TEST: Mice were orally fed either with 146 mg/kg carbaryl, 51.7 mg/kg dimethoate, 5 mg/kg phosphamidon, 13 mg/kg aldrin, 43.3 mg/kg endosulfan, or 10 mg/kg ceresan in sterile distilled water. Each animal received the dose in two equal instalments separated by an interval of 24 h. Control animals were treated in an identical manner with distilled water.

0007-4861/80/0025-0277 $01.20

Six h. after the second dose, animals were killed by cervical dislocation and air-dried smears of the bone marrow were prepared by the method of SCHMID (1975). From each animal 2000 polychromatic erythrocytes and corresponding number of normochromatic erythrocytes were enumerated.

HOST MEDIATED ASSAY: Animals were treated orally with 438 mg/kg carbaryl, 155 mg/kg dimethoate, or 7.5 mg/kg phosphamidon. Doses were administered in three equal instalments for three days. Controls were maintained simultaneously. One day (24 h) following the last treatment, the mice were injected intraperitoneally with Salmonella typhimurium G46 strain of bacteria. Bacteria, 3 h after growth in the peritonium, were collected and serial dilution was performed in saline, and plated on selective agar media plates. Plates were incubated at 37°C for 48 h and scored for revertants (GABRIDGE & LEGATOR 1969).

## RESULTS

Results of the induction of micronuclei in bone marrow cells of mice treated with various pesticides are shown in Table 1.

There is significant increase in the frequency of polychromatic erythrocytes with micronuclei in mice received organophosphorus insecticides ($p < 0.01$). The frequency was 0.28% in the control while the frequencies were 0.85 and 0.90% in dimethoate and phosphamidon treated groups, respectively. The organochlorine insecticides showed no significant increase in the frequency of micronuclei in the treated group ($p > 0.05$). With carbaryl the frequency of micronuclei was almost similar to the control group. Ceresan also showed no significant increase in the frequency of micronuclei.

The data obtained on the mutation rates of S.typhimurium are given in Table 2.

The incidence of reverse mutation frequency was taken as the criterion for assessing the mutagenicity of the test compound. After determination of the total bacterial count, the number of revertants obtained by taking the mean of colony count on minimal agar the mutation rates (revertants/$10^8$ bacteria) for each animal was calculated.

There was significant increase in the reversion frequencies in dimethoate and phosphamidon treated group. The mutation factor was taken as the ratio of the mean mutation rate for treated group ($Mf_t$) to the mean mutation rate for control group ($Mf_c$). The highest mutation factor is 3.4 in dimethoate treated group.

TABLE 1. Results showing the incidence of micronuclei in bone marrow erythrocytes of mice treated with pesticides.

| Treatment | No. of animals | % of polychromatic erythrocytes (PCE) with micronuclei | % of normochromatic erythrocytes (NCE) with micronuclei | PCE/NCE Ratio |
|---|---|---|---|---|
| Control | 6 | 0.28 | 0.12 | 0.86 |
| Carbaryl | 6 | 0.27 | 0.15 | 0.85 |
| Dimethoate | 6 | 0.85* | 0.21 | 0.80 |
| Phosphamidon | 6 | 0.90* | 0.21 | 0.81 |
| Aldrin | 6 | 0.48 | 0.13 | 0.83 |
| Endosulfan | 4 | 0.52 | 0.17 | 0.72 |
| Ceresan | 4 | 0.43 | 0.15 | 0.80 |

*$p < 0.01$

TABLE 2. Host mediated assay with pesticides in the mouse with S. typhimurium his $G_{46}$ as indicator organism

| Treatment | Mean No. of surviving cells (0.1 mL of dilution $10^{-7}$) | Mean no. of revertants (0.1 mL of undiluted exudate) | Mean no. of reversions/ $10^8$ survivors | Mutation factor $Mf_t/Mf_c$ |
|---|---|---|---|---|
| Control | 49.6 | 5.6 | 1.13 | - |
| Carbaryl | 34.8 | 8.1 | 2.33 | 2.06 |
| Dimetnoate | 39.7 | 15.5 | 3.89 | 3.44* |
| Phosphamidon | 42.4 | 14.5 | 3.42 | 3.02* |

$Mf_t$ = Mutation frequency in treated group

$Mf_c$ = Mutation frequency in control group

*$p < 0.05$

## DISCUSSION

The bone marrow cells of mammals which have been under the influence of a clastogenic agent, for at least one mitotic cycle, show the presence of micronuclei. Micronuclei originate from chromatin lagging behind anaphase in some of the affected cells. The test is an efficient in-vivo cytogenetic test for screening chemicals for their clastogenic ability (SCHMID 1973).

The results on micronucleus test clearly indicate that organophosphorus insecticides dimethoate and phosphamidon were found to induce the formation of micronuclei. The clastogenic effects as revealed in the present study lend support to the results obtained by others. VAIDYA et al. (1978) and GERSTENGARBE & WISS (1975) reported the clastogenic and genetic effects of dimethoate, respectively. The clastogenic effects of phosphamidon are in accordance with earlier studies of LILIANA GEORGIAN (1975) who reported the chromosome aberrations in human lymphocyte cultures and mouse bone marrow cells. Carbaryl did not induce the formation of micronuclei. This is in agreement with the results of SEILER (1976), who reported negative results with carbaryl. The results are also in accordance with that of REGAN et al. (1976) who reported the absence of any strand breakages in the human DNA treated with carbaryl.

Aldrin and endosulfan which are organochlorines also did not show significant increase in the frequency of micronuclei. Earlier in human lymphocyte cultures, aldrin induced chromosomal aberrations at higher doses were shown by LILIANA GEORGIAN (1975). However, at lower does no chromosomal lesions were observed.

Methyl mercuric fungicides were shown to be highly toxic and cause deaths and teratogenic effects which were attributed to the intake of the mercury containing foods (MILLER 1967). Thus the mercuric fungicide ceresan was tested for its clastogenic ability and no chromosomal damage was observed. But it was reported to be mutagenic in Drosophila (MATHEW & ALDOORI 1976). RAMEL (1969) showed methyl mercury induced polyploidy in roots of Allium cepa.

The host mediated assay has been devised by GABRIDGE & LEGATOR (1969), to establish the direct correlation between microbial test system and mammalian metabolism. A wide variety of carcinogens, drugs, pesticides are mutagenic to the histidine auxotropic strains of S. typhimurium (JOYCE MCCANN et al. 1975).

In the present experiment specific strain of S. typhi murium G46 which is a nonsense mutant due to base substitution was utilized and mose as the host to determine the mutagenic

potency of the insecticides on the metabolism in the host. The results showed no significant increase in the frequency of revertants in the carbaryl treated animals. The results thus support the observations of MARSHALL et al. (1976) and DEAN BLEVINS et al. (1972) who reported no genetic effects in S. typhimurium. The data are also in accordance with that obtained in E. Coli (ASHWOOD SMITH et al. 1972) Sacchromyces cerevisiae (SIEBERT & EISENBRAND 1974) and Bacillus subtilis (SHIRASU et al. 1976).

The results of organophosphorus insecticides showed a significant increase in frequency of revertants in the treated groups ($p < 0.05$). This shows that metabolites of organophosphorus insecticides are mutagenic to micro-organisms and mammalian metabolism has no capacity to detoxify the insecticides. The results are in agreement with that of MOHN (1973) who reported that dimethoate has mutagenic properties in E. coli. It was also found that dimethoate brings about gene conversions in the diploid D4 strain of S. cerevisiae (FAHRING 1973).

The results thus indicate that organophosphorus insecticides are highly mutagenic when compared to the other pesticides tested.

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