Available online at www.sciencedirect.com
ScienceDirect
Journal of Environmental Sciences 21(2009) 696–699
JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Potential neoplastic effects of parathion-methyl on rat liver

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Received 06 May 2008; revised 10 September 2008; accepted 23 September 2008

Abstract

The mutagenic and carcinogenic effects of parathion-methyl were examined by bacterial reverse mutation assay and a long-term experiment with wistar rats. The potential mutagenic effect of parathion-methyl in *Salmonella typhimurium* TA100 bacterial cells was observed without rat liver S9 metabolic activation. Parathion-methyl was further investigated for pathological changes in rat pancreas and liver. The long-term rat experiments showed that parathion-methyl exposure for 3 months can cause pathological changes in rat pancreases acinar cells and pancreatic hepatocytes. Atypical acinar cell focuses (AACF) were determined in the liver and pancreas of the rats. The results from short-term Ames test and long-term rat experiments suggested that parathion-methyl would be potential carcinogenic.

Key words: atypical acinar cell focuses; Ames test; carcinogenesis; parathion-methyl
DOI: 10.1016/S1001-0742(08)62326-8

Introduction

Parathion-methyl is an organophosphates insecticide and acaricide. The World Health Organisation classifies methyl parathion as an extremely hazardous pesticide (WHO, 1996). It is highly toxic by inhalation and ingestion, and moderately toxic by dermal adsorption. All the organophosphates are derived from one of phosphorus acids, and are generally the most toxic of all pesticides to vertebrates. It is a non-systemic pesticide that kills pests by acting as a stomach poison. It is used to control chewing and sucking insects in a wide range of crops, including cereals, fruit, vines, vegetables, ornamentals, cotton and field crops (Tomlin, 1994).

Pesticides represent a major source of global contamination: annually approximately 2.9×10^9 kg of active ingredients is consumed world-wide (World Sources, 1999). It has been claimed that excessive pesticide use causes ground and surface water contamination, and unacceptable levels of pesticide residues in foods (Kumuk and Akgüngör, 1995). Kuts *et al*. (1992) reported that approximately 7.5% of the general population had urinary residues consistent with recent organophosphorus ester exposure. In a recent study, children exhibited higher metabolite levels of organophosphates insecticides than in previous studies measuring adult levels (Adgate *et al*., 2001).

The poison effects of parathion-methyl has been known for a long time. In 1992, a massive bird kill occurred in Costa Rica after applying the pesticide by plane in a cotton field. Also, parathion-methyl has been implicated in the deaths of waterfowl in Spain and the acute poisoning of fish, birds, cattle and wild animals in the Sudan (Dinham, 1993). For this reason, using parathion-methyl as a pesticide is banned in Indonesia, Sri Lanka and Tanzania, and is severely restricted in Colombia, Korea, China and Japan. So far it is one of five pesticides identified for inclusion in the Prior Informed Consent Procedures of the Food and Agriculture Organization on the grounds of causing problems under conditions of using in developing countries.

Statistically, it has been shown that there is a gradually increase pesticide usage in Turkey year by year. It has been shown that total active ingredient use in pesticides increased from 8 ton in 1980 to 11.5 ton in 1991 in Turkey (DSE, 1993). Although parathion-methyl has been banned in many countries, its consumption in 2002 was 246828 kg in Turkey. Parathion-methyl is one of the most five insecticides used in Turkey between 1999 and 2002 (Delen *et al*., 2005).

In animal studies, many pesticides are carcinogenic, (e.g., organochlorines, creosote, and sulfallate), while others (notably, the organochlorines DDT, chlordane, and lindane) are tumor promoters (Dich *et al*., 1997; Meyer

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et al., 1993; Williams, 1983; Moser and Smart, 1989). No sign of mutagenicity of parathion-methyl was observed in mice at given doses of 5 to 100 mg/kg, nor in mice fed methyl parathion for 7 weeks (Gallo and Lawryk, 1991). So far neoplastic effects of parathion-methyl pesticide on the liver and pancreas have not been completely understood, although possible carcinogenesis has been suggested (Hallenbeck and Cunningham-Burns, 1985). Therefore, the mutagenic and carcinogenic effects of parathion-methyl were investigated.

1 Materials and methods

1.1 Confirming genotypes of tester strains

Salmonella typhimurium TA98 and *Salmonella typhimurium* TA100 were employed in this study. The tester strain genotypes were confirmed after receiving the cultures. Freshly thawed frozen strains of 10 μL were inoculated in 10 mL of growth medium and the cultures were grown overnight (12–17 h) at 37°C and 250 r/min. Fresh broth cultures were used for these tests. *Histidine* requirement, *rfa* mutation, *uvrB* mutation *R*-factor and spontaneous reversion tests were performed according to the instructions by Maron and Ames (1983). The His- character of the tester strains was confirmed by demonstrating the histidine requirement for growth on MGA (Minimal Glucose Agar) plates. Strains having the deep rough (*rfa*) character were tested for crystal violet sensitivity. The *uvrB* mutation was confirmed by indicating UV sensitivity in strains. The *R*-factor strains were tested for the presence of the ampicillin resistance factor. *Spontaneous* reversion of the tester strains to histidine was measured and is expressed as the number of spontaneous revertants per plate. All tests were performed with three plates in repetition.

In each experiment we routinely include positive mutagenesis controls using diagnostic mutagens to confirm the reversion properties. For this purpose, sodium azide (NaN_3) (1 μg/100 μL) for TA100 and 4-nitro-*o*-fenilendiamin ($O_2NC_6H_3(NH_3)_2$) (200 μg/100 μL) for TA98 were used.

1.2 Reverse mutations assay with *Salmonella* TA strains

The *Salmonella*/microsome plate incorporation assay was employed according to Ames *et al*. (1975) as revised by Maron and Ames (1983). The *S. typhimurium* TA98 was used for the detection of frame-shift mutations and the TA100 strain for the detection of base-pair substitutions. The cultures were grown in nutrient broth (Difco) for 10 h at 37°C with shaking. The test was performed without S9 fraction. Revertant colonies were counted 48 h after incubation. A mutagenic test was positive if the compound induced in the number of the revertants per plate as compared to the number of revertants per control plates, and the increase had to be at least twice the control rate. All the experiments were repeated thrice and the average values are presented.

The tests were performed with parathion methyl in concentrations of 0.1, 0.25, 0.5, 0.7, 0.9, 1.08 mg/mL, plus a negative control and a positive control. The tester strains were inhibited at the concentrations over 1.08 and 0.9 mg/mL for TA 98 and TA 100, respectively.

1.3 Rat exposure

The experiments were performed with 20 male wistar rats for 4 weeks. Rats were divided into two groups; each included 10 rats and kept separately in cages. Control groups (UnCt-Group 1) were fed with a standard diet. Experimental groups (PMet-Group 2) were fed with a standard diet containing 4 mg/(kg·d) parathion-methyl.

To ensure homogeny distribution of parathion-methyl in diet, parathion-methyl was mixed with standard diet at least one hour. With a period of 15 d, control and experimental group diets were refreshed and kept sealed containers in a dark place.

1.4 Histological procedure

After three month exposure, rats were decapitated. An abdominal dissection for each rat was applied and whole pancreases and liver of rats were taken out. One part of tissues were fixed immediately for 24 h in 10% formaldehyde and then embedded in paraffin. A *Leica* 2125 *RT* microtome has been used to take a section thickness in 5 μm for light microscopy examination. Samples were stained with hematoxilen-eosin (three sections from each rat pancreas and liver) and examined with an Olympus microscopy (BX 50) for pathological changes. Atypical acinar cell focuses (AACF) and control tissues were observed and then photographed.

2 Results and discussion

2.1 Mutagenic of parathion-methyl

Mutagenic and carcinogenic effects of parathion-methyl pesticide were tested by bacterial reverse mutation assay and a long term experiment with Wistar rats.

The results of bacterial mutation tests without the supplementation of S9 rat liver are summarized in Table 1. Parathion-methyl was detectably mutagenic at 0.9 mg/mL for TA100.

Several experimental publications have shown that many pesticides elicit mutagenic effects on living beings

Table 1 Reversion of Ames tester strains with positive control substances and parathion-methyl

Substance	Number of revertant per plate	
	S. typhimurium TA98	*S. typhimurium* TA100
Control	14 ± 2	70 ± 3
4-Nitro-o-fenilendiamin (200 μg/100 μL)	360 ± 43	–
Na-azid (1 μg/100 μL)	–	470 ± 57
Parathion-methyl		
0.1 mg/mL	14 ± 4	80 ± 5
0.25 mg/mL	16 ± 3	75 ± 8
0.5 mg/mL	12 ± 3	103 ± 12
0.7 mg/mL	13 ± 3	172 ± 19
0.9 mg/mL	15 ± 3	189 ± 16
1.08 mg/mL	14 ± 1	–

(Alavanja *et al*., 1994; Coats, 1990; Herrera *et al*, 1992; Woodruff *et al*., 1983; Mathew *et al*., 1990). In this study, Ames plate incorporation assay with the parathion-methyl indicated a mutagenic response with the TA100 tester strain. However, Wagner *et al*. (2003) reported that parathion-methyl without S9 activation was not mutagenic but it increased the mutagenic potency of 2-acetoxyacetylaminofluorene (2AAAF), 2-amino-3-methylimidazo (4,5-f) quinoline (IQ) and 2-amino-1-methyl-6-phenylimidazo-(4,5-b) pyridine (PhIP). The data obtained from our experiments suggest that parathion-methyl is a potent mutagen and thus it is also likely to have a genotoxic effect in human.

2.2 Long-term toxicity and carcinogenicity

Parathion-methyl was investigated for pathological changes in rat pancreas and liver. During experimental process, rats have been seen healthy. After killing it has been observed that there were not any nodules being able to see with naked eyes in pancreases and livers of rats. But light microscopy examination results showed that experimental group (PMet-Group 2) had atypical acinar cells in different size, whereas control rats (UnCt-Group 1) showed no change. It is possible to suggest that the span of experimental procedure was not long enough to produce acinar cell nodules and adenomas.

Atypical acinar cell focuses were characteristic in both pancreas and liver of the rat (PMet-Group 2). The histological examination of liver and pancreas showed that atypical acinar cells appear polygonal shaped and no stroma was seen around focuses. The cells of focuses were characterized with small nuclei (Fig. 1). Also Kupffer cells were dominant in some part of focuses.

Similarly atypical acinar focuses were determined in pancreases of rat (PMet-Group 2). This focuses were characterized with an elaborated nucleus and more acidophilic staining pattern. The rounded capsules appearances of focuses were common. An increase in the number of zymogene granules and acidophilic staining pattern were observed in focuses (Fig. 2).

Morphologic changes in the acinar cells of pancreases and hepatocytes of rat liver showed that parathion-methyl can affect general structural and metabolic pattern of these cells. It is possible to say that a gradual changes acidophilic and basophilic staining property of the same part of tissues

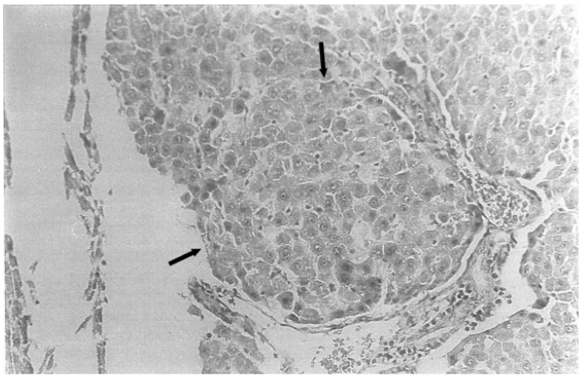

Fig. 1 Atypical acinar cell focus in rat liver (×300).

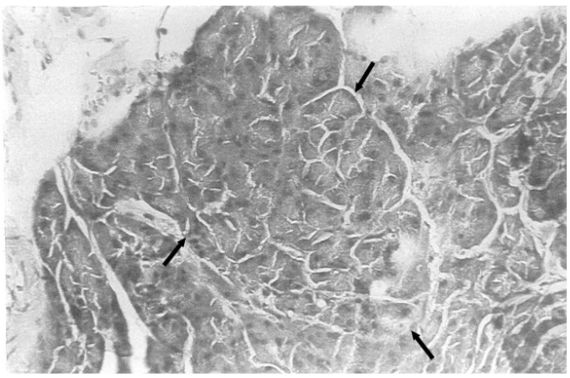

Fig. 2 Atypical acinar cell focus in rat pancreas (×400).

can reflect neoplastic changes.

In this study, zymogene granule-rich cytoplasma, pleomorphism in nuclei and different stained cells focuses were observed in AACF of pancreatic acinar cells, whereas similar morphological changes were not found in control rats (UnCt-Group 2). Bannash *et al*. (1985) claimed that the differences depend on the glycogen amount, amount of endoplasmic reticulum, ribosome and peroxisome in hepatocyt. Glikoz-6-phosphate dehyrogenase is usually accepted as an indicator of acidophicilic pattern in hepatocyte and acinar cell of pancreas. The differences in the staining property of these focuses cells may reflect a neoplastic property. It is well known that the size and acidophilic property of atypical acinar cell focuses changed because of exposing chemical substance (Roebuck *et al*., 1984).

3 Conclusions

The potential mutagenic effect of parathion-methyl was observed in the experiments with *Salmonella typhimurium* TA100 bacterial cells. This result had confirmed by long-term (three months) rat experiments. Our results from short term Ames test and long-term rat experiments showed that parathion-methyl has a mutagenic and neoplastic effect.

Acknowledgments

This study was supported by Mustafa Kemal University, Natural and Applied Sciences Institute.

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