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The Effects of Fenitrothion Emulsion (Organic Phosphorous Pesticide) and Its Degraded Solution on Mice

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ABSTRACT

Experiments were carried out to examine the effects of degradation products on mice. Fenitrothion (MEP) emulsion, a kind of an organic phosphorous insecticide, adjusted to pH 8, pH 10, and pH 14 were degraded by exposure to natural sunlight. Physiological saline, an untreated MEP emulsion, and the three degraded solutions were prepared as experimental chemicals and were administered subcutaneously at a dosage of 0.1 ml/10 g body weight (BW) into the backs of pregnant mice once a day, from the 3rd to the 15th fetal day. Each dose included 20, 40, or 90 mg/kgBW of MEP and several degradation products of MEP. LD50 was determined according to Behrens' method.

The mean fetal body weights of 18th day of pregnancy in 40 and 90 mg/kgBW of MEP of the pH 8 degraded solution were 1.09 and 1.16 g, respectively, significantly lower than that of untreated MEP emulsion (1.27 g). Further, LD50 values of the three kinds of degraded solutions were 60—120 mg/kgBW, much lower than that of the untreated MEP emulsion (410 mg/kgBW).

These results indicate that the degradation products of MEP emulsion degraded by the exposure of sunlight have an influence on both adult and fetal mice.

Key words: *Degraded and undegraded fenitrothion emulsion, Mouse, Embryo, Toxicity*

When a safety assessment of agricultural control chemicals is conducted, the influence of those chemicals on organisms is examined in detail from the toxicological, a physiological, an ecological, and a biochemical standpoint. However, an examination of their degradation products is never carried out. The chemical applied starts to degrade immediately after application and the duplicate effects of the original chemical and its degradation products influence organisms. It is very important, therefore, to find the toxicity of the degraded solution, including both the original chemical and its degradation products on organisms since it influences the ecosystem and human health by way of intake of vegetables, fruits, and so on.

The effects of such degradation products on organisms have received very little study. Miyamoto et al[6] have been the only researchers to examine the acute toxicity of each degradation product of fenitrothion (MEP) and report the results. In nature, degradation products do not affect organisms individually. Thus, adopting a more realistic standpoint, in the present study, it was considered that the collective toxicity of the degradation products should be examined. However, it has been reported that the kind of degradation products and their component ratio in the degraded solution differ very markedly depending on degrading conditions, for instance, pH value[1], oxidation[1], and ultraviolet rays[7]. These factors being considered in the present study, some experiments were conducted to obtain fundamental information on the effects of degraded solution on organisms.

We have already reported the effect of the degraded solution of MEP on medaka[2]. In the present study the effects of MEP and its degraded solution on mice were examined.

MATERIALS AND METHODS

1. Degradation of fenitrothion: A fenitrothion (MEP) emulsion includes 50.0% of dimethyl-(3-methyl-4-nitrophenol) thiophosphate and 50.0% of organic solvent, emulsifier, and so on. The maker does not mention all the components except the main chemicals. This organic phosphorous insecticide is used quite frequently in Japan and served as the material for the present study.

Fifty ml of undiluted MEP emulsion and 50 ml of pH 8 buffer solution prepared accoding to Mikami et al[5] were mixed. The diluted MEP emulsion (2.5×10^5 ppm MEP) was transferred to a petriplate (15 cm in diameter) and exposed to natural sunlight at 30 ± 3 °C for 8 hrs per day in Hiroshima, Japan. The MEP concentration in the

Table 1. Degradation of MEP Emulsion Adjusted to pH 8, pH 10, and pH 14

	pH 8 degraded solution	pH 10 degraded solution	pH 14 degraded solution
Date of start of degradation	16 Dec 1986	27 Feb 1987	19 Dec 1986
Date of end of degradation	21 Jan 1987	27 Mar 1987	27 Dec 1986
pH value at beginning of degradation	7.5	9.8	13.6
MEP concentration at beginning of degradation (ppm)	2.5×10^5	2.5×10^5	2.5×10^5
MEP concentration at end of degradation (ppm)	3.2×10^4	2.4×10^4	1.7×10^4
Residual rate of MEP (%)	13	9.6	6.8
pH value at end of degradation	1.8	1.8	9.2
Days required for degradation	36	28	8

Table 2-A. Effects of Fenitrothion (MEP) Emulsion and Its Degradation on Pregnant Mice

	Control	Untreated MEP Emulsion			pH 8 Degraded Solution		
Dosage of MEP (mg/kgBW)	0	20	40	90	20	40	90
No. of tested mice with plug	25	5	15	15	5	5	5
No. of live mice (A)	25	5	15	15	4	4	4
No. of mice with live fetuses (B)	23	4	12	13	4	3	3
Successful pregnancy rate (B/A)	92	80	80	87	100	75	75
Total No. of implants	300	53	152	190	53	41	37
Total No. of live fetuses	276	45	138	172	49	39	37
No. of live fetuses per litter	12.0	11.3	11.5	13.2	12.3	13.0	12.3
No. (%) of resorbed or dead fetuses	24 (8.0)	8 (15.1)	14 (9.2)	18 (9.5)	4 (7.5)	2 (4.9)	2 (5.4)
Mean fetal body weight (g; M±SD)	1.34±0.14	1.27±0.15	1.29±0.13	1.22±0.12	1.24±0.13	1.09±0.08**	1.16±0.14**
No. (%) of live fetuses with external abnormalities	0	0	0	0	0	0	0
No. (%) of live fetuses with skeletal abnormalities	13 (4.7)	0 (0.0)	7 (5.1)	14 (8.1)	3 (6.1)	2 (5.1)	4 (11.0)

**: $p<0.01$, compared to the untreated MEP emulsion. Statistical analysis was by students' t test.

MEP degradation was checked regularly by gas chromatography with a flame photometric detector (GC-FPD)[2]. When around 90% of the MEP had degraded, the exposure was discontinued. This degraded solution was preserved as a stock solution (Table 1). We designated this test solution pH 8 degraded solution.

Fifty ml of undiluted MEP emulsion and 50 ml of pH 10 buffer solution prepared according to Mikami et al[5] were mixed and the above treatment was repeated. This degraded solution was preserved as a stock solution (Table 1). We designated this test solution pH 10 degraded solution.

Likewise, 50 ml of undiluted MEP emulsion and 50 ml of 4 N NaOH were mixed and the above treatment was repeated. This degraged solution was preserved as a stock solution (Table 1). We designated this test solution pH 14 degraded solution.

2. The effects of untreated MEP emulsion and the degraded solution on pregnant mice and their fetuses: Specific pathogen-free male and female mice of ICR strain were received at 8 weeks of age and reared for more than 1 week in our laboratory. Food and water were given *ad libitum*. The above-mentioned chemical was given to the pregnant mice and their fetuses were observed since the early stage of development are sensitive to toxic compounds.

The 1st day of pregnancy was considered to start 9 A.M. on the following morning when vaginal plugs were found and the mice were randomly divided into groups. Physiological saline (control), untreated MEP emulsion, and the three kinds of degraded solution were administered subcutaneously at a dosage of 0.1 ml/10g body weight (BW) into the backs of pregnant mice, once daily, from the 3rd to 15th day of pregnancy. The pH 8 and pH 10 degraded solutions were adjusted to approximately pH 5 by adding 2 N NaOH before dosing since their acidity was extremely low. In diluting untreated MEP emulsion and the degraded solution, physiological saline was used. In each dose, relative MEP concentration represents 20, 40, or 90 mg/kgBW. Several unknown degradation products of unknown concentration in the case of the degraded solution, and organic solvent, emulsifier, and so on in the case of untreated MEP emulsion,

Table 2-B. Effects of Degradation of Fenitrothion (MEP) Emulsion on Pregnant Mice

	pH 10 Degraded Solution			pH 14 Degraded solution		
Dosage of MEP (mg/kgBW)	20	40	90	20	40	90
No. of tested mice with plug	5	5	5	5	5	5
No. of live mice (A)	5	5	5	5	5	5
No. of mice with live fetuses (B)	5	5	5	4	4	2
Successful pregnancy rate (B/A)	100	100	100	80	80	40
Total No. of implants	67	74	68	54	48	29
Total No. of live fetuses	63	67	63	52	48	29
No. of live fetuses per litter	12.6	13.4	12.6	13.0	12.0	14.5
No. (%) of resorbed or dead fetuses	4 (6.0)	7 (9.5)	5 (7.4)	2 (3.7)	0 (0.0)	0 (0.0)
Mean fetal body weight (g: M ± SD)	1.27±0.15	1.34±0.14*	1.21±0.09	1.26±0.11	1.31±0.07	1.25±0.07
No. (%) of live fetuses with external abnormalities	0	0	0	0	0	0
No. (%) of live fetuses with skeletal abnormalities	0 (0.0)	3 (4.5)	3 (4.8)	1 (1.9)	2 (4.2)	6 (9.2)

*: $p<0.05$, compared to the untreated MEP emulsion. Statistical analysis was by students' t test.

Table 3. 24 h LD50 Values of Mice exposed to Fenitrothion (MEP) Emulsion and Its Degraded Solution

	Female				Male			
	Untreated MEP emulsion	pH 8 degraded solution	pH 10 degraded solution	pH 14 degraded solution	Untreated MEP emulsion	pH 8 degraded solution	pH 10 degraded solution	pH 14 degraded solution
Dosage of MEP (mg/kgBW)	410	120	120	70	410	60	120	60

were contained in addition to fixed MEP. The mice were sacrificed on the 18th day of pregnancy and the number of fetuses and implantations, their weight, and externals were observed visually. The bone and cartilage of fetuses were stained by Inouye's method[4] and observed by stereoscopic microscopy.

3. Acute to mice toxicity of untreated MEP emulsion and the degraded solution: LD50 was determined by Behrens' method[9]. Namely, untreated MEP emulsion and the three kinds of degraded solutions were intraperitoneally given to male and female ICR strain mice at 7-8 weeks of age and mortality within 24 hrs was observed. Ten mice were used at each experimental group and a dosage of 0.1 ml/kgBW was administered to each mouse. Though several unknown degradation products at unknown concentrations existed in the case of the degraded solutions, dosage was determined only by on the basis of MEP concentration.

RESULTS

1. The effects of untreated MEP emulsion and the degraded solution on pregnant mice and their fetuses (Table 2-A, 2-B): The mean fetal body weights in the untreated MEP emulsion and the three kinds of degraded solutions were low as compared with that in the controls. The mean fetal body weights in 40 and 90 mg/kgBW of MEP of the pH 8 degraded solutions were 1.09 and 1.16, respectively and significantly low as compared with that of untreated MEP emulsion ($p<0.01$).

There were few differences in maternal mortality, successful pregnancy rate, the rate of resorbed or dead fetuses, or the number of live fetuses per litter among either the contol, the untreated MEP emulsion, and the three kinds of degraded solutions.

No fetuses with external abnormalities were observed. There was a slight increase in the tendency towards skeletal abnormalities in direct proportion to the increase in dosage, although the incidence was low. In the case of skeletal abnormalities, a small and round cavity in the lower sternum was observed most frequently. However, it is not yet clear whether this affects the mice physiologically or in behavioral aspects.

2. Acute toxicity of untreated MEP emulsion and the degraded solution on mice (Table 3): All LD50 values in both male and female of the three kinds of degraded solutions were markedly low as compared with that of the untreated MEP emulsion. Comparing male and female, the LD50 value of males was smaller than that of famales in the pH 8 degraded solution. However, there was little

diference between LD50 values of males and females in the untreated MEP emulsion and the pH 10 and pH 14 degraded solutions.

DISCUSSION

The degraded solution given to mice in this experiment included both a fixed ocncentration of MEP and its several degradation products, whose chemical names and concentrations were unknown. The differences in the results between undegraded and degraded solutions appear to be due to the toxicity of the degradation products. This is because the MEP concentration in the present experiments was almost the same in the corresponding experimental groups subjected to the degraded solution and the untreated MEP emulsion.

From the results obtained in these experiments, it was found that the mean fetal body weight of the pH 8 degraded solution was the smallest and that the acute toxicity of the three kinds of degraded solutions was stronger than that of the untreated MEP emulsion. It is reasonable to suppose that these results were due to the toxicity of the degradation products in addition to the toxicity of the MEP. In comparing the mean fetal body weight among pH 8, pH 10, and pH 14 degraded solutions, that of pH 8 degraded solution was the lowest in all cases, that is at a dosage of 20, 40, and 90 mg/kgBW. It is said that the degradation products vary according to the degrading conditions of MEP. It is inferred that, among these three kinds of degraded solutions, the degradation products of pH 8 affect the growth of fetuses the most.

The MEP emulsion on the market, used in the present study, is commonly diluted to 1000—2000 times in actual application, in which case, the MEP concentration is 0.3—0.5 mg/ml. Compared with this, the dosage in the experiments was extremely high at 20—90 mg/kgBW. Thus, the results obtained from the experiments cannot be realistically applied. However, the finding that the MEP emulsion, frequently used in Japan, is degraded by exposure to sunlight, and that degraded products exert noxious effects on mice, is of great interest.

It has already been reported that the MEP given to pregnant mice passed into their fetuses[8], but there has been no report as to whether the degradation products of MEP also do so. From the results that mean fetal body weights in the groups of degraded solutions were lower than those in the groups of untreated MEP emulsion, it is inferred that the degradation products might also pass into the fetus. The possibility that this result may be due to the poor nutritional condition of the pregnant mice is to be also considered.

Hiraoka[2,3] have reported that abnormalities were frequently observed in the medaka fry hatched from eggs exposed to the degraded solution, which was degraded in the same way for 5 days from the morula stage of development. Thus, it is clear that the degradation products also have a toxic effect on fish.

Thus, since the degradation products possess the toxic capability detailed above, hereafter, greater consideration should be given to the degradation products when the safety assessment of agricultural control chemicals is examined.

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