the harvest time. In addition, on the gaschromatograms of these samples, the peak intensity of pyrethrins I and II was much lower comparing with those of standard extract. It was also found that samples which showed higher percentages of pyrethrins I and II than those of the WSPE also gave higher content of total pyrethrins than those by the acidmetries.

5) The assay values were corrected by multiplying these values by the ratios (the percentage of prethrins I and II in WSPE/those of each samples) which were obtained gaschromatographically. By such a correction, fairly good agreement of the assay values between the phosphoric acid method and acidmetries was obtained.

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Teratologic Evaluation of Tsumacide (*m*-Tolyl-*N*-Methylcarbamate) in the Rat. Mineo Yasuda (Department of Anatomy, Kyoto Prefectural University of Medicine, Kyoto) Received September 21, 1972. *Botyu-Kagaku*, 37, 161, 1972.

24. ラットにおけるツマサイドの催奇形性の評価

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ツマサイド（*m*-tolyl-*N*-methylcarbamate）の催奇形作用をラットを用いて検索した．MTMCを80および4000ppmの割合で飼料にまぜ，妊娠8日より15日までSD-JCLラットに連続経口投与した．妊娠末期胎仔および生後6週の育成仔を観察したところ，ツマサイドの催奇形作用は検出されなかった．

Tsumacide® (*m*-tolyl-*N*-methylcarbamate) is an insecticide for control of planthoppers and leafhoppers in paddy rice fields, and has been widely used in Japan. Reported here are effects of this product having been given to pregnant rats upon the fetal and postnatal development of the offspring.

Materials and Methods

SD-JCL rats, obtained from Japan CLEA Co., Ltd., were used. They were housed in wire-bottom cages. The diet, ground CE-2 laboratory chow made by Japan CLEA Co., Ltd., was supplied in unrestricted amounts, and bottled water for drinking was freely available. The room temperature was maintained at 22±1°C and relative humidity at 55±5%. The room was kept dark from 10 AM to 9 PM.

Virgin females of 12 to 16 weeks of age, approaching estrus, were caged with males from 9 AM to 3 PM. Pregnancy was considered to have started (day 0) at 12 AM when sperm were found in the vaginal smears at 3 PM. On day 8, pregnant rats were randomly allotted to test groups.

Tsumacide, assay 97.6%, was supplied by Mitsubishi Chemical Industries Ltd. From day 8 to day 15 of pregnancy, Tsumacide was mixed with the diet at levels of 80 and 4000 ppm; the high level of 4000 ppm was estimated to be near the maximum nontoxic dose for adult female SD rats after a preliminary subchronic toxicity study of 2 weeks. In this preliminary study, body weight, general behavior and gross autopsy findings were used as indices of toxicity.

Rats were weighed at the time of sperm detection, and everyday from day 8 to day 21 of gestation. Daily consumption of food and water was also measured from day 8 to day 21.

About three-quarters of the dams in each group were killed on day 21 of gestation by cervical dislocation. Immediately after death, the uterine contents were examined, and the number and position of viable fetuses, dead fetuses and resorption sites were recorded. Viable fetuses were removed from the uterus, sexed, examined for externally visible malformations and cleft palate, and weighed. Then, about half of the fetuses in each litter were fixed in Bouin's fluid and the remaining half were placed in 95%

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ethanol. The Bouin-fixed specimens were hand-sectioned with a razor blade and examined for internal anomalies[1]; ventricular septal defects were carefully sought for with the technique described by Inomata and Yasuda[2]. The skeleton of the ethanol-fixed fetuses was examined after staining by the Alizarin red technique[1]. Skeletal deformities which were invariably involved in certain external malformations were not recorded as skeletal malformations. Some morphological and numerical deviations such as cervical rib and lumbar rib, which have not rarely been observed in the SD-JCL rat, were regarded as normal variations.

The remaining quarter of the dams were allowed to litter naturally, and the postnatal development of the young was observed. Shortly after parturition, the number of live and dead offspring, and the presence of gross abnormalities were recorded. The live young were branded for identification, sexed, weighed and allowed to suckle the mother. Further record was made of the number, weight and condition of all offspring, weekly, up to 6 weeks post partum. When the young were weaned at 4 weeks of age, the dam was killed, and the number of implantation sites in the uterus was compared with the number of newborns counted at the first examination. The weanlings were raised up to 6 weeks of age. Then, they were killed by chloroform overdosage and examined for external and internal anomalies.

Among the obtained experimental data, those which were considered empirically to be samples from the normal distribution, for example, maternal weight and food and water comsumption, were analyzed using t tests for comparisons between means after calculations of the mean and the standard error of each group for each day. The data relating to fetuses or postnatal young were analyzed in the following way in order that biases resulting from the litter effect[3] might be minimized. First, the percentage or mean for each litter was calculated. Then, the average of these percentages or means in each group was computed. The distribution of a percentage or a mean for litters treated with Tsumacide was compared with the appropriate control distribution by use of the Wilcoxon rank sum test[4].

Results

No marked changes in behavior or appearance were observed in the mothers given Tsumacide, whereas body weight gain in the dams fed on the diet containing 4000 ppm of Tsumacide was slightly less than in the controls during the period of Tsumacide administration (Fig. 1).

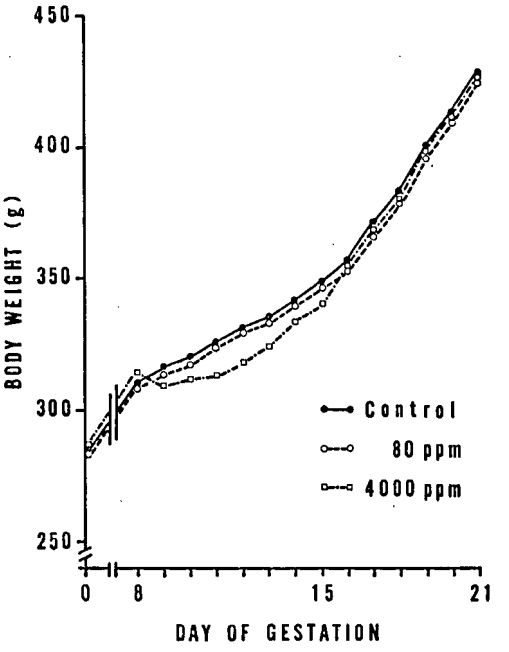

Fig. 1. Body weight of pregnant rats given Tsumacide from day 8 to day 15 of gestation.

Food intake of these dams also decreased at that time (Fig. 2). After the period of Tsumacide administration, however, the food and water consumptions of the dams in the high dose group exceeded those of the control dams (Figs. 2 and 3).

The effects of Tsumacide upon rat fetuses observed at term are summarized in Tables 1 and 2. No significant differences were observed between the treated groups and the control group with regard to the litter size, fetal mortality and fetal weight. External examination of the fetuses revealed one case each of myeloschisis, tail defect and umbilical hernia in the control, 80 ppm and

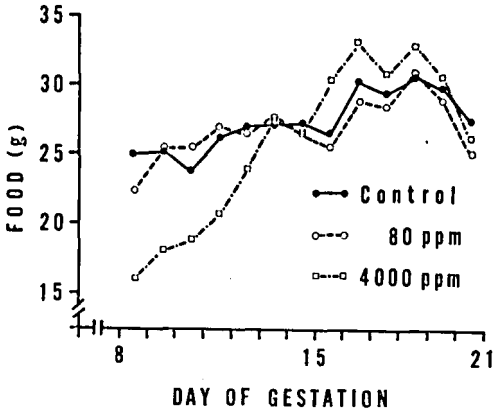

Fig. 2. Food consumption of pregnant rats given Tsumacide from day 8 to day 15 of gestation.

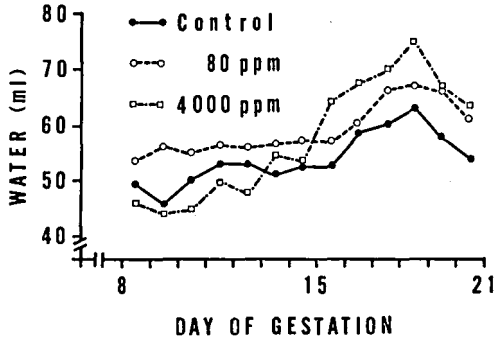

Fig 3. Water consumption of pregnant rats given Tsumacide from day 8 to day 15 of gestation.

4000 ppm groups, respectively. Cases with split vertebral body, the only abnormal skeletal finding, were observed in the control and 80 ppm groups in low incidence. Visceral examination by Wilson's method revealed sporadic occurrences of ventricular septal defect and hydronephrosis in the treated groups as well as the control group. These external, skeletal and visceral malformations were not considered to have correlation with the Tsumacide feeding, because these malformations have been known to occur spontaneously in the SD-JCL rat, and because no dose-response relationship in the incidence of these malformations was found in the present experiment.

A summary of the postnatal development of the young from dams treated with Tsumacide is presented in Table 3. No effect of Tsumacide was evident on each parameter given in Table 3. At weaning all young showed no abnormalities in the external differentiation, auditory function and behavior. Autopsy of the young at 6 weeks of age revealed two cases of hydronephrosis in the control group and one case of hydronephrosis in the group with 4000 ppm.

Discussion

Because of increasing warnings against environmental pollution with pesticides and herbicides, it has been required to test these kinds of chemicals for reproductive and teratogenic effects. Among carbamate insecticides, 1-naphthyl meth-

Table 1. Effects upon rat fetuses of Tsumacide administered orally from day 8 to day 15 of gestation.

Statistic	Dose (ppm)		
	0	80	4000
No. litters	17	19	19
No. implants	278	286	290
Litter size, mean ± SE	16.35 ±0.54	15.05 ±0.85	15.26 ±0.60
No. resorptions	21	20	15
Percent resorptions	7.6	9.5	5.4
No. live fetuses	257	266	275
Wt. male fetuses, mean±SE(g)	4.565 ±0.074	4.683 ±0.060	4.703 ±0.061
Wt. female fetuses, mean±SE(g)	4.328 ±0.066	4.507 ±0.081	4.446 ±0.050
No. malformed fetuses	4	10	2

Table 2. Number and types of malformations observed among rat fetuses after oral administration of Tsumacide from day 8 to day 15 of gestation.

Statistic	Dose (ppm)		
	0	80	4000
No. fetuses examined for external malformations	257	266	275
Myeloschisis	1		
Tail defect		1	
Umbilical hernia			1
Percent fetuses with external malformations	0.5	0.5	0.4
No. fetuses examined for skeletal malformations	133	138	141
Split vertebral body	1	2	
Percent fetuses with skeletal malformations	0.7	2.0	0
No. fetuses examined for visceral malformations	124	128	134
Ventricular septal defect	1	2	
Hydronephrosis	1	5	1
Percent fetuses with visceral malformations	1.7	5.5	0.9

Table 3. Effects upon postnatal development of rat offspring of Tsumacide administered orally from day 8 to day 15 of gestation.

Statistic	Dose (ppm)		
	0	80	4000
No. litters	5	7	6
No. implants	74	113	101
No. born	73	107	86
Percent born*	98.8	94.6	85.0
Percent perinatal death**	8.8	0.8	0
Percent weaned***	96.6	79.0	98.8
Mean wt. ±SE (g)			
At birth: Male	6.14 ±0.21	6.10 ±0.13	6.10 ±0.23
Female	5.85 ±0.19	5.82 ±0.14	5.71 ±0.17
At weaning: Male	81.4 ±4.1	84.2 ±3.3	86.0 ±5.0
Female	75.6 ±3.6	78.3 ±2.4	78.5 ±4.5
At 6 weeks: Male	195.6 ±7.4	181.9 ±6.0	193.9 ±12.1
Female	158.7 ±2.9	149.4 ±3.0	149.7 ±7.0

*: Percent of No. implants **: Percent of No. born ***: Percent of No. liveborn

ylcarbamate, known also as Sevin and carbaryl, has been intensively investigated for its possible adverse effects on reproduction and embryogenesis, with somewhat inconsistent results[5]. Tsumacide has not previously been evaluated for its teratogenicity.

The present experiment was intended to predict the degree of safety of Tsumacide residue in the diet for man. Thus, to test animals, the substance was given by dietary feeding. The intake of Tsumacide calculated from food consumption and body weight was about 6 mg/kg/day for the

dose level of 80 ppm and 280 mg/kg/day for 4000 ppm. Because of the slight decrease in the maternal body weight gain, the dose level of 4000 ppm was interpreted to be slightly toxic to the dams.

With regards to the residue of Tsumacide in the food rice, the following data were available[6]: Unpolished rice contained Tsumacide at a concentration of 0.02 ppm when harvested 15 days after 6 applications of Tsumacide microgranules (2%, 4 kg/10 a), or 0.067 ppm 19 days after 6 applications of Tsumacide emulsifiable concentrate (30%, ×500, 150 *l*/10 a). If 500 g of rice containing 0.1 ppm of Tsumacide are taken daily, the amount ingested would be 50 μg/man/day, or about 1 μg/kg/day.

The high dose level employed in the present experiment is about 300,000 times the amount ingested by man. Even at this high dose level no embryotoxic effects were detected in the rat. The results indicate that the residue of Tsumacide in the diet has little possibility of affecting human embryonic development.

Summary

Tsumacide (*m*-tolyl-*N*-methylcarbamate) was studied in a teratology experiment in rats. Tsumacide was mixed with food and given to pregnant SD-JCL rats from day 8 to 15 of gestation at levels of 80 and 4000 ppm (about 6 and 280 mg/kg/day, respectively). No signs of drug induced teratogenicity were detected in fetuses examined at term and in young autopsied at 6 weeks of age.

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