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Original Article

Repeated-dose and reproductive/developmental toxicity screening of polyoxymethylene in rats

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ABSTRACT — The Japanese government requires risk assessment of chemicals under the Chemical Substances Control Law (CSCL). Toxicity data for polyoxymethylene (paraformaldehyde; CAS No.: 30525-89-4) for human health are insufficient though the chemical needs a screening assessment under the CSCL. Thus, polyoxymethylene was selected by the Safety Examination of Existing Chemicals and Safety Programmes of the Ministry of Health, Labour and Welfare (MHLW) to assess repeated-dose and reproductive/developmental toxicity. A combined toxicity screening was conducted following the OECD TG422. Male and female rats were administered the test chemical once daily by gavage at doses of 0 (control), 20, 60, or 200 mg/kg bw from 14 days before mating for a total of 28 to 61 days. The 200 mg/kg bw/day dose caused a significant decrease in food consumption. Histopathological examination found ulcers in the forestomach and glandular stomach, and erosion and inflammatory cell infiltration in the submucosa of the glandular stomach at the end of dosing in both sexes. Inflammatory cell infiltration in the submucosa of the glandular stomach was also observed in both sexes after the recovery period. No reproductive and developmental toxicity was observed even at the highest dose. A no-observed-adverse-effect level (NOAEL) for repeated-dose toxicity was 60 mg/kg bw/day, and a NOAEL for reproductive and developmental toxicity was 200 mg/kg bw/day, the highest dose tested.

Key words: Polyoxymethylene, Paraformaldehyde, CAS No. 30525-89-4, Chemical substances control law, Existing chemical substance, Formaldehyde

INTRODUCTION

Polyoxymethylene (paraformaldehyde; CAS No. 30525-89-4), is a polymer of formaldehyde. Formaldehyde is a colorless gas, but it slowly forms polyoxymethylene by condensation as a white precipitate in solutions of formaldehyde. The solid form of polyoxymethylene may have a decree of polymerization range (n) from ~ 8 to 100, or more (Cassidy *et al*., 1983). Polyoxymethylene is degraded to formaldehyde on contact with acids (ICSC, 2021). Thus, formaldehyde is generated from orally-dosed polyoxymethylene by contact with gastric acid. Polyoxymethylene is widely used as a cross-linking agent (Cassidy *et al*., 1983) and in base polymers (plastic and coating) for food utensils, containers, and packaging (MHLW, 2021a). Manufactured and imported polyoxymethylene in Japan was 20,000 - < 30,000 metric tons in 2018 (J-CHECK, 2021). Exposure class for human health

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(total annual amount of environmental release estimated as the sum of emissions during production and usage) was four (10–100 metric tons) in 2019 (J-CHECK, 2021). People can be exposed to polyoxymethylene in the environment and by using products that contain it. The Japanese government performs risk assessments for chemicals released to the environment under the Chemical Substances Control Law (CSCL) (METI, 2015). A screening assessment is required for polyoxymethylene because of its exposure class (4 and higher).

Two endpoints, genotoxicity and general [repeated-dose] toxicity, are mandatory, and two additional endpoints (reproductive/developmental toxicity and carcinogenicity) are indicated. Polyoxymethylene was reported to be mutagenic and clastogenic *in vitro* (MHLW, 2021b), but no data were available to characterize repeated-dose and reproductive/developmental toxicity. Therefore, this chemical was selected by the Safety Examination of Existing Chemicals and Safety Programmes and the MHLW completed the Combined Repeated Dose Toxicity Study with Reproduction/Developmental Toxicity Screening Test (OECD TG 422). The current screening assessment used data obtained to prioritize the chemical for evaluation as a priority assessment chemical substance (PACS). We also discuss the effects of oral formaldehyde, one of the PACSs, on fertility using the results of the present study to fill data gaps and promote human hazard assessment under the CSCL.

MATERIALS AND METHODS

The combined repeated-dose and reproductive/developmental toxicity test was performed at Safety Research Institute for Chemical Compounds Co., Ltd. (Hokkaido, Japan) in compliance with the OECD Guideline 422 Combined Repeated Dose Toxicity Study with Reproduction /Developmental Toxicity Screening Test (OECD, 2016). The study was also compliant with "The Good Laboratory Practice for test facilities conducting tests of New Chemical Substances etc. (March 31, 2011, Yakushokuhatsu 0331 No. 8, Heisei23.03.29 Seikyoku No.6, KampoKihatsu No. 110331010)". Use and care of animals complied with" the Act on Welfare and Management of Animals (Act No. 105, October 1, 1973)", "Standards Relating to the Care and Management of Laboratory Animals and Relief of Pain" (announcement no. 88, Ministry of the Environment, Japan, April 28, 2006; revised August 30, 2013), "Guideline for Animal Experimentation (Japanese Association for Laboratory Animal Science, May 22, 1987)", and "Guideline for Animal Experiment in Facilities under the Jurisdiction of Japanese MHLW" (Notification Kahatsu no. 0601001, June 1, 2006).

Chemicals

Polyoxymethylene (paraformaldehyde; CAS No. 30525-89-4; MITI No. (9)-1941; lot no. SKN5326) 96.2% pure (as formaldehyde) was purchased from FUJIFILM Wako Pure Chemicals Corporation (Osaka, Japan). A general chemical formula of polyoxymethylene is (HCHO)n (n is not specified for this chemical). The chemical was stored at room temperature in a light-shielding airtight container. Its purity and stability were verified by analysis before use. Formulations (4 and 40 mg/mL) were stored for 14 days, and their stability confirmed. Corn oil (lot no. V0P8420; Nacalai Tesque INC, Kyoto, Japan) was used as a vehicle. Free formaldehyde was not detected in this vehicle.

Animals

Sprague–Dawley (Crj: CD [SD]) specific pathogen-free rats were selected for the present study because they were commonly used in earlier toxicity studies, including reproductive and developmental toxicity and historical control data. Male and female rats (9-week-old) were purchased from Charles River Laboratories Japan, Inc., (Kanagawa, Japan). Animals were quarantined for six days and acclimatized to the laboratory; male and female rats in good health were selected for further use. We recorded vaginal smears of each female for 14 days, and only female rats with a normal estrous cycle were selected. Rats were randomly distributed into four groups of 12 male and 12 female rats as mating groups. In addition, five of 12 males that received no chemical and the highest dose were treated as recovery animals. In females, 10 rats that were not mated and received either not chemical or the highest dose were used as non-mating (satellite) groups, and five were included as recovery animals. The test substance was first administered when rats were 11 weeks of age with body weights of 394–494 g (males) and 227–275 g (females). Animals were housed individually in a metal cage except during mating. Rats were fed a basal diet (CRF-1; Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water *ad libitum* and kept in an air-conditioned room at 21°C–23°C, relative humidity of 39%–61%, a 12/12-hr light/dark cycle, and 10–15 air changes per hour.

Dosing

Rats were administered the test chemical once daily by gavage at doses of 0 (control), 20, 60, or 200 mg/kg bw. Doses were determined based on the results of a 14-day dose-finding study performed at 10, 30, 100, and 300 mg/kg bw/day (three rats of each sex per group). Mucous fec-

es in both sexes, and decreased body weight, bodyweight gain, and food consumption in males were observed at 300 mg/kg bw/day. Further hematological examination showed significantly higher reticulocyte and white blood cell counts in both sexes and significantly lower hemoglobin and hematocrit levels in males. Blood chemistry showed high triglyceride levels and low total protein levels in both sexes. Focal dark red or white lesions were found in the glandular stomach at necropsy. Mucous feces were observed in females after administration of 100 mg/kg bw/day. We set the highest dose for the main study at a dose of 200 mg/kg bw/day with a common ratio of three based on these results.

The test chemical was suspended in corn oil and administered to male rats for 28 days beginning 14 days before mating. After 28 days, animals in the recovery group were reared for 14 days without administration of the test chemical. Female rats in the mating group were administered the test chemical for up to 61 days beginning 14 days before mating to day 13 of lactation to cover the time from mating through gestation. Female rats in the satellite group were administered the test chemical for 28 days, and followed by a recovery period of 14 days for the recovery group. The first day of dosing was day 1 of administration, and the day after the final dose was day 1 of recovery. The volume of each dose was adjusted to 5 mL /kg bw based on the latest bodyweight measurement.

Observation

All rats were checked daily for clinical signs of toxicity and detailed general condition was recorded weekly. We made functional observations – sensory and motor reactivity to visual, touch, auditory, pain, and proprioceptive stimuli, and air-righting reflex – and tested grip strength of fore and hind limbs in five male and five satellite female rats on day 22 of administration and in five mated females on lactation day 13. We also recorded spontaneous motor activity for 1 hr at 10 min intervals. Body weight of males and satellite females was recorded on days 1, 4, 7, 14, 21, and 28 of administration and in mating females on days 1, 4, 7, and 14 of administration during the premating period; days 0, 7, 14, and 20 of pregnancy; and days 0, 4, 7, 13, and 14 of lactation. Food consumption was recorded on days 4, 7, 14, and 28 for males and satellite females, and on days 4, 7, and 14 of the premating period; on days 7, 14, and 20 of the pregnancy period; on days 4, 7, and 13 of the lactation period in mating females.

Organ weights, gross necropsy, and histopathology

Rats were euthanized by exsanguination from the abdominal aorta under isoflurane anesthesia on the day after final administration in males and satellite females and on day 14 after parturition in females. Animals in the recovery group were euthanized on the day after the end of the recovery period. External and internal macroscopic examination was performed. Blood samples were collected from the abdominal aorta. Brain, spinal cord, pituitary gland, thymus, thyroids, parathyroids, adrenals, spleen, heart, esophagus, stomach, liver, pancreas, submandibular glands, duodenum, jejunum, ileum, cecum, colon, rectum, trachea, lungs and bronchi, kidneys, urinary bladder, testes, epididymides, prostate, seminal vesicles, ovaries, uterus, vagina, eyeballs and Harder glands, mammary gland, femur, mesenteric lymph node, mandibular lymph node, skeletal muscle, and sciatic nerve were removed and fixed in 10% formalin. In addition, the number of implantation sites in the uterus was counted in all mated females. Brain, heart, liver, kidneys, testes, epididymides, seminal vesicles, pituitary gland, thyroids, spleen, thymus, adrenals, prostate, ovaries, and uterus were weighed. For males and females in the main test group and females in the satellite group, specimens of all organs and tissues of the control and high-dose groups fixed and preserved at the end of administration period were prepared by staining with hematoxylin and eosin, and microscopically examined in all animals. Changes were observed in the stomach of males and females in the high-dose group related to test chemical administration. Therefore, the forestomach and glandular stomach of all animals in the low- and middle-dose groups and all animals necropsied at the end of the recovery period were further examined.

Urinalysis, hematology, clinical biochemistry, and hormonal analysis

We sampled fresh urine from five males and five nonmated females using metabolic rat cages (KN-646, B-1; Natsume Seisakusyo Co., Ltd., Tokyo, Japan) for nonfasting and watering conditions (3 hr and 21 hr) on days 26 and 27 of administration and on days 8 and 9 of recovery. The 3 hr urine samples were tested for color, pH, protein, glucose, ketone bodies, bilirubin, occult blood, and urobilinogen by reagent strips, and microscopically for sediments. The 21 hr urine samples were tested for urine volume and specific gravity.

One fraction of blood samples was used for hematology and analyzed with an Automated Hematology Analyzer XT-2000 iV or Automated Blood Coagulation Analyzer CA-620 (Sysmex Corporation, Hyogo, Japan).

Analyses included red blood cell (RBC), platelet, and white blood cell (WBC) counts; hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC); and hemoglobin (HGB), reticulocyte and differential count of WBC, prothrombin time (PT), and activated partial thromboplastin time (APTT). Blood chemistry used serum with an automated system for gel electrophoresis, Epalyzer 2 Junior (Helena Laboratories Japan Co., Ltd., Saitama, Japan) or 7180 automatic analyzers (Hitachi High-Technologies Corporation, Tokyo, Japan). Measurements included aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (γ-GTP), total bile acid (TBA), total protein (TP), albumin (ALB), total bilirubin (T-Bil), urea nitrogen (UN), creatinine (Crea), glucose (GLU), total cholesterol (T-Cho), triglyceride (TG), sodium (Na), potassium (K), chlorine (Cl), calcium (Ca), inorganic phosphate (IP), and protein fraction. Finally, serum isolated from males and pups on postnatal day (PND) 13 was analyzed for thyroxine (T4) (Microplate reader SH-1200, Corona Electric Co., Ltd., Ibaraki, Japan).

Reproductive and developmental toxicity

We evaluated estrous cyclicity using daily vaginal smear samples from each female throughout the premating period. Subsequently, each female was mated with a single male of the same-dose group until copulation or the 2-week mating period elapsed. Successful mating was defined as the presence of sperm in the vaginal smear and/or a vaginal plug. Once insemination was confirmed, we checked for signs of parturition three times a day from day 21 to day 25 of pregnancy. They were allowed to deliver spontaneously and nurse their pups until PND 13. PND 0 was the day that parturition ended by 9:00 a.m. We recorded litter size and the number of live and dead pups. Live pups were sexed and grossly examined. Anogenital distance (AGD) of pups was measured on PND 4, and body weight recorded on PNDs 0, 4, 7, and 13. We counted the number of nipples in male pups on PND 13, then euthanized pups by decapitation or intraperitoneal injection of a 60 mg/mL pentobarbital sodium, and examined pups for external and internal abnormalities.

Statistical analysis

Group means and standard deviations were calculated for grip strength, body weight, body weight gain, food intake, urine volume, hematology, blood chemistry, absolute and relative organ weights, number of offspring, numbers of live and dead offspring, gestation length, body weight, AGD, number of nipples, number of anomalous offspring, and viability index. Bartlett's test was used to analyze the homoscedasticity for males and mating females. One-way analysis of variance was used for equal variance ($p \geq 0.05$), and the Kruskal–Wallis test was used for unequal variance ($p < 0.05$). When one-way analysis of variance showed a significant difference ($p < 0.1$), it was followed with Dunnett's test. When a significant difference was found by Kruskal–Wallis analysis ($p < 0.1$), it was followed using Steel's test method. Quantitative data from satellite females and recovery group males were analyzed using an F test for homogeneity of variance. If variance was homogeneous ($p \geq 0.05$), we analyzed data using Student's t-test. If variance was not homogeneous ($p < 0.05$), we applied Welch's test. Data for detailed clinical observations, reactivity to environmental stimuli, and urinary parameters were analyzed with the Kruskal–Wallis test. When a significant difference was detected ($p < 0.1$) by the Kruskal–Wallis, Steel's test was used to compare control and dose groups. Qualitative data from satellite groups were analyzed with the Wilcoxon rank-sum test. The litter was used as the experimental unit for statistical analysis of the pups. Fisher's exact test was used for incidence of acyclic or irregular estrous cycles, copulation index, fertility index, gestation index, delivery index, sex ratio, the incidence of dams with anomalous offspring, and histopathology. A five percent level was assumed for the threshold of significance.

Hazard assessment of polyoxymethylene for a screening assessment under the CSCL

The screening assessment procedure and hazard classification are reported by Matsumoto *et al*. (2021). A hazard assessment value (*D*-value) is calculated by dividing a no-observed-adverse-effect level (NOAEL) by an uncertainty factor (UF). Hazard classifications of both repeated-dose toxicity and reproductive/developmental toxicity are classified as: class 2 ($D \leq 0.005$ mg/kg/day), class 3 ($0.005 < D \leq 0.05$ mg/kg/day), class 4 ($0.05 < D \leq 0.5$ mg/kg/day), and out of class ($D > 0.5$ mg/kg/day). A UF:600 (species difference (10), individual difference (10), and study duration (6)) as repeated-dose toxicity and the UF:1000 (species difference (10), individual difference (10), database insufficiency (10)) as reproductive/developmental toxicity were used for the OECD TG422 study. Genotoxicity hazard classification of the test chemical was based on Ames test and *in vitro* chromosomal aberration test results reported previously (MHLW, 2021b), based on class 3 (both tests positive), class 4 (one test positive), and out of class (both tests negative).

Table 1. Body weight and bodyweight gain in rats dosed with polyoxymethylene.

Dose (mg/kg bw/day)		0	20	60	200
MALE					
Number of animals		12	12	12	12
Day	1	441.3 ± 24.8	440.1 ± 24.4	442.1 ± 23.8	442.8 ± 25.6
	4	453.2 ± 27.2	450.3 ± 27.5	453.2 ± 27.7	449.7 ± 25.7
	7	464.9 ± 27.5	460.3 ± 29.7	464.2 ± 31.1	456.9 ± 25.6
	14	493.4 ± 30.7	484.6 ± 36.2	491.2 ± 37.0	478.9 ± 31.1
	21	514.8 ± 31.6	501.8 ± 41.4	512.9 ± 39.1	497.7 ± 33.1
	28	535.8 ± 33.2	525.4 ± 47.8	534.1 ± 43.7	515.8 ± 36.9
Gain		94.5 ± 13.6	85.3 ± 26.9	92.0 ± 23.8	73.1 ± 18.5*
FEMALE (mating group)					
Before mating					
Number of animals		12	12	12	12
Day	1	251.3 ± 12.8	251.3 ± 8.5	253.4 ± 14.5	249.9 ± 12.8
	4	256.8 ± 14.6	255.5 ± 13.0	259.1 ± 14.1	255.5 ± 14.1
	7	259.0 ± 16.3	258.0 ± 12.5	260.3 ± 14.5	257.2 ± 13.0
	14	269.6 ± 20.1	269.3 ± 13.4	270.8 ± 17.6	267.6 ± 15.6
Gain		18.3 ± 11.0	18.0 ± 7.8	17.3 ± 6.0	17.7 ± 7.8
During gestation					
Number of animals		12	10	12	12
Day	0	273.5 ± 19.7	272.8 ± 14.4	280.8 ± 23.8	270.7 ± 13.6
	7	310.6 ± 26.2	307.2 ± 14.7	315.7 ± 22.7	306.6 ± 17.4
	14	349.0 ± 32.3	347.6 ± 18.9	351.8 ± 20.9	347.6 ± 19.0
	20	431.3 ± 38.3	433.9 ± 29.0	435.7 ± 23.0	429.7 ± 25.5
Gain		157.8 ± 19.7	161.1 ± 18.8	154.8 ± 18.1	159.0 ± 19.8
During lactation					
Number of animals		12	10	12	12
Day	0	331.8 ± 31.8	339.4 ± 22.2	338.2 ± 21.2	327.8 ± 24.5
	4	351.0 ± 34.0	350.5 ± 19.3	349.9 ± 23.3	337.6 ± 31.1
	7	355.0 ± 30.3	355.1 ± 19.6	356.0 ± 18.4	345.3 ± 24.5
	13	360.8 ± 27.6	358.2 ± 19.2	362.3 ± 17.5	359.7 ± 17.8
Gain		29.0 ± 11.9	18.8 ± 14.2	24.2 ± 11.0	31.9 ± 16.7

*Significantly different from the control by Dunnett test ($P < 0.05$)
Values represent mean ± SD

RESULTS

No deaths or treatment-related changes were seen in detailed clinical observations, functional observations, grip strength, spontaneous motor activity, or urinalysis in any treated group during administration and recovery. Mucous feces were observed on day 11 of administration in one male rat that received 60 mg/kg bw/day and frequently in almost all animals in both sexes at a dose of 200 mg/kg bw/day during the administration period. After recovery, only one satellite female at 200 mg/kg bw/day showed mucous feces. Body weights were not significantly changed between controls and dose groups (Table 1). Bodyweight gain of male rats significantly decreased after receiving 200 mg/kg bw/day during administration but returned to normal after 14 days of recovery. No other changes were found for bodyweight gain in other treated groups. Food consumption significantly decreased in males on day 4 and in satellite females at a dose of 200 mg/kg bw/day on days 4 and 7 during the administration period, but the decrease was transient (Table 2). No other changes were found for food consumption in other groups.

The results of hematological examination in males and satellite females are shown in Table 3. At 200 mg/kg bw/day, neutrophil count was significantly elevated in males and satellite females, and RBC was significantly decreased in satellite females and tended to decrease in males at the end of treatment. These changes were not present at the end of the recovery period. In addition, WBC, lymphocyte, and monocyte counts tended to increase in males and satellite females at the high dose. Significantly prolonged APTT in males (Table 3) and significantly increased platelet count in mating females at 20 mg/kg bw/day (data not shown) were not dose-dependent and thus not considered toxicological effects.

Table 2. Food consumption in rats dosed with polyoxymethylene.

Dose (mg/kg)		0	20	60	200
MALE					
Number of animals		12	12	12	12
Day	4	24.90 ± 3.29	23.88 ± 2.58	24.13 ± 2.98	21.07 ± 2.17**
	7	24.89 ± 2.56	24.28 ± 2.45	24.93 ± 2.87	22.56 ± 2.19
	14	23.73 ± 2.77	23.08 ± 2.70	24.14 ± 2.30	22.92 ± 2.23
	28	22.68 ± 2.46	22.47 ± 2.45	23.02 ± 1.80	23.86 ± 2.29
FEMALE (mating group)					
Number of animals		12	12	12	12
Before Mating					
Day	4	16.73 ± 2.38	15.92 ± 1.21	16.17 ± 1.39	15.05 ± 1.58
	7	16.66 ± 2.18	16.87 ± 1.60	16.88 ± 1.96	15.93 ± 1.92
	14	16.25 ± 1.75	16.23 ± 1.23	16.44 ± 1.79	15.53 ± 1.96
Number of animals		12	10	12	12
During gestation					
Day	7	20.48 ± 2.44	20.82 ± 1.92	20.69 ± 1.78	20.91 ± 2.36
	14	22.46 ± 2.79	22.12 ± 3.39	21.95 ± 2.10	22.93 ± 2.54
	20	22.46 ± 1.92	22.50 ± 2.37	22.18 ± 1.93	22.31 ± 2.06
Number of animals		12	10	12	12
During lactation					
Day	4	30.58 ± 3.68	28.22 ± 3.62	29.29 ± 3.34	29.16 ± 5.01
	7	42.13 ± 4.19	38.94 ± 5.22	40.48 ± 3.98	42.32 ± 3.26
	13	52.31 ± 3.46	48.10 ± 3.70*	51.58 ± 4.70	54.10 ± 2.94
FEMALE (satellite group)					
Number of animals		10			10
Day	4	17.32 ± 1.34			14.75 ± 0.96##
	7	17.71 ± 1.58			15.60 ± 1.48##
	14	16.32 ± 1.88			15.75 ± 1.70
	21	16.01 ± 1.07			15.76 ± 1.28
	28	15.26 ± 0.83			15.65 ± 1.32

*Significantly different from the control by Dunnett test ($P < 0.05$); **Significantly different from the control by Dunnett test ($P < 0.01$); ##Significantly different from the control by Student t-test ($P < 0.01$)
Values represent mean ± SD

Levels of GLU, TP, and ALB in sera from males that received 200 mg/kg bw/day were significantly diminished at the end of the administration period. Levels of TP were significantly low, while ALB (%) and A/G were significantly elevated in satellite females at the end of treatment, and ALB (%) and A/G were significantly increased at the end of the recovery period (Table 4). T-Cho, ALB, and A/G were significantly increased in satellite females at the high dose at the end of the recovery period. A/G was significantly decreased in mating females at this dose at the end of treatment (Table 5). No significant changes in T4 levels in males between controls and animals receiving the high dose at the end of administration or in pups on PND 13 (data not shown).

Organ weights of rats administered 200 mg/kg bw/day polyoxymethylene showed significantly increased absolute and relative weights of testes in males and adrenals in mating females at the end of treatment. Absolute and relative weights of seminal vesicles tended to increase at the end of treatment and significantly increased at the end of the recovery period. Relative weights of spleen and absolute weight of adrenals were significantly decreased in satellite females at the high dose at the end of recovery, although these changes were not observed at the end of the administration period (data not shown). At a dose of 20 mg/kg bw/day, the relative weights of kidneys were significantly higher in males after treatment, but this effect was not dose-dependent (Table 6).

Histopathological findings of stomachs for males and females dosed with 200 mg/kg bw/day showed ulcers in the forestomach and glandular stomach, and erosion, and inflammatory cell infiltration in the submucosa of the glandular stomach after treatment. Inflammatory cell infiltration was seen in 3/5 males and 4/5 females with or without a decrease in severity after the recovery period (Table 7).

Reproductive and developmental findings showed no significant change in incidence of abnormal estrus cycles,

Table 3. Hematological findings in male and satellite female rats dosed with polyoxymethylene at the end of the treatment period.

Dose (mg/kg bw/day)	0	20	60	200
MALE				
Number of animals	5	5	5	5
RBC (10^4/μL)	883.2 ± 23.5	887.6 ± 29.0	872.4 ± 49.7	848.0 ± 14.8
HGB (g/dL)	15.60 ± 0.56	15.54 ± 0.55	15.58 ± 0.59	15.32 ± 0.50
HCT (%)	43.68 ± 1.72	43.48 ± 1.84	43.12 ± 1.76	43.12 ± 1.54
MCV (fL)	49.42 ± 0.67	49.00 ± 2.21	49.48 ± 1.36	50.84 ± 1.43
MCH (pg)	17.64 ± 0.21	17.54 ± 0.74	17.88 ± 0.53	18.08 ± 0.36
MCHC (g/dL)	35.72 ± 0.29	35.76 ± 0.29	36.14 ± 0.18	35.56 ± 0.56
Reticulocytes (%)	3.462 ± 0.382	3.404 ± 0.087	3.300 ± 0.401	4.192 ± 0.555
Platelets (10^4/μL)	105.50 ± 12.93	96.12 ± 12.22	102.14 ± 11.02	121.94 ± 10.94
WBC (10^2/μL)	87.42 ± 18.52	73.50 ± 17.67	90.24 ± 10.36	110.86 ± 28.53
Neutrophils (10^2/μL)	17.88 ± 5.39	13.38 ± 5.20	21.10 ± 2.39	30.88 ± 6.76**
Lymphocytes (10^2/μL)	64.24 ± 15.05	55.34 ± 16.67	63.80 ± 12.32	73.40 ± 23.93
Monocytes (10^2/μL)	4.00 ± 2.11	3.68 ± 0.74	4.12 ± 1.29	5.30 ± 1.84
Eosinophils (10^2/μL)	1.26 ± 0.36	1.08 ± 0.25	1.22 ± 0.39	1.22 ± 0.48
Basophils (10^2/μL)	0.04 ± 0.05	0.02 ± 0.04	0.00 ± 0.00	0.06 ± 0.05
PT (sec)	17.78 ± 0.76	20.50 ± 2.11	18.56 ± 4.59	19.10 ± 1.92
APTT (sec)	18.70 ± 1.04	20.92 ± 0.18$	18.96 ± 2.14	18.14 ± 2.31
FEMALE (satellite group)				
Number of animals	5			5
RBC (10^4/μL)	856.0 ± 45.6			787.4 ± 45.8#
HGB (g/dL)	15.50 ± 0.72			14.68 ± 0.58
HCT (%)	43.14 ± 1.84			41.10 ± 1.63
MCV (fL)	50.44 ± 1.25			52.26 ± 1.42
MCH (pg)	18.10 ± 0.28			18.64 ± 0.50
MCHC (g/dL)	35.92 ± 0.35			35.72 ± 0.11
Reticulocytes (%)	2.564 ± 0.563			3.326 ± 0.538
Platelets (10^4/μL)	105.86 ± 8.31			106.20 ± 9.99
WBC (10^2/μL)	54.36 ± 20.06			70.72 ± 26.91
Neutrophils l (10^2/μL)	9.62 ± 4.02			18.32 ± 4.86#
Lymphocytes (10^2/μL)	42.44 ± 16.62			49.52 ± 21.47
Monocytes (10^2/μL)	1.42 ± 0.38			1.94 ± 0.77
Eosinophils (10^2/μL)	0.88 ± 0.26			0.92 ± 0.43
Basophils l (10^2/μL)	0.00 ± 0.00			0.02 ± 0.04
PT (sec)	15.42 ± 0.52			15.40 ± 0.54
APTT (sec)	14.60 ± 1.76			13.24 ± 0.58

#Significantly different from the control by Student t-test ($P < 0.05$); **Significantly different from the control by Dunnett test ($P < 0.01$); $Significantly different from the control by Steel test ($P < 0.05$); Values represent mean ± SD

length of estrus cycles, number of estruses, copulation index, fertility index, number of days required for copulation, number of implantations, delivery index, gestation length, gestation index, numbers of offspring delivered, live newborns, or dead newborns of parental animals. For offspring, we found no significant changes in sex ratio, viability index on PND 0, 4, or 13, general conditions, incidence of external anomalies, male and female bodyweight on PND 0, 4, 7, or 13, and necropsy findings in treated animals (Table 8). A thread-like tail was observed in one pup that received 60 mg/kg bw/day, but this abnormality was considered spontaneous. No significant changes in AGD on PND 4 in either sex or in the number of nipples on PND 13 in male pups were observed.

DISCUSSION

The main toxicological findings were significantly decreased food consumption in males and satellite females, decrease body weight gain in males, and mucosal damage accompanied with inflammatory cell infiltration in the stomach at the highest dose of 200 mg/kg/day. Inflammatory cell infiltration in the submucosa of the glandular stomach was consistently detected at

Table 4. Biochemical findings in male and satellite female rats dosed with polyoxymethylene.

	Administration period				Recovery period	
Dose (mg/kg bw/day)	0	20	60	200	0	200
MALE						
Number of animals	5	5	5	5	5	5
AST (IU/L)	64.6 ± 4.8	63.4 ± 4.3	84.2 ± 25.8	73.6 ± 16.5	68.8 ± 9.9	67.6 ± 8.2
ALT (IU/L)	35.2 ± 14.6	26.4 ± 3.3	38.6 ± 13.3	36.2 ± 11.9	29.2 ± 7.4	26.8 ± 1.8
ALP (IU/L)	413.2 ± 66.6	452.0 ± 148.1	365.8 ± 40.2	389.0 ± 103.8	335.8 ± 70.9	357.8 ± 55.3
γ-GTP (IU/L)	0.44 ± 0.11	0.46 ± 0.11	0.36 ± 0.13	0.40 ± 0.12	0.38 ± 0.08	0.34 ± 0.15
T-Bil (mg/dL)	0.068 ± 0.013	0.064 ± 0.005	0.076 ± 0.022	0.056 ± 0.005	0.056 ± 0.005	0.054 ± 0.011
TBA (μmol/L)	18.90 ± 8.52	13.94 ± 6.78	23.18 ± 14.90	22.38 ± 15.49	8.46 ± 3.59	14.48 ± 10.63
GLU (mg/dL)	164.6 ± 18.8	162.6 ± 39.6	152.4 ± 14.2	134.8 ± 5.3$^{\$}$	156.0 ± 16.4	145.4 ± 20.1
T-Cho (mg/dL)	52.4 ± 6.0	47.8 ± 10.6	57.2 ± 13.7	55.6 ± 5.8	52.2 ± 18.2	53.4 ± 7.7
TG (mg/dL)	28.0 ± 12.9	29.2 ± 25.4	28.4 ± 10.9	40.0 ± 19.7	39.0 ± 17.8	32.6 ± 18.1
TP (g/dL)	5.46 ± 0.23	5.34 ± 0.09	5.40 ± 0.10	5.04 ± 0.19**	5.36 ± 0.30	5.42 ± 0.22
UN (mg/dL)	11.14 ± 1.69	10.76 ± 1.25	11.42 ± 0.80	12.94 ± 0.73	15.26 ± 1.22	14.26 ± 2.03
Crea (mg/dL)	0.306 ± 0.036	0.286 ± 0.023	0.320 ± 0.037	0.320 ± 0.032	0.338 ± 0.011	0.318 ± 0.050
Na (mEq/L)	144.2 ± 0.4	144.6 ± 1.1	144.4 ± 0.5	144.4 ± 0.5	143.8 ± 1.3	144.6 ± 0.9
K (mEq/L)	4.600 ± 0.149	4.506 ± 0.088	4.488 ± 0.239	4.668 ± 0.136	4.714 ± 0.293	4.586 ± 0.163
Cl (mEq/L)	103.8 ± 0.4	104.4 ± 1.3	104.2 ± 1.3	103.6 ± 1.5	103.6 ± 0.5	104.0 ± 1.2
Ca (mg/dL)	9.38 ± 0.16	9.16 ± 0.23	9.24 ± 0.38	9.10 ± 0.21	9.22 ± 0.19	9.24 ± 0.15
IP (mg/dL)	6.32 ± 0.66	6.34 ± 0.44	6.32 ± 0.41	6.18 ± 0.24	6.24 ± 0.36	6.40 ± 0.31
A/G	0.884 ± 0.096	0.904 ± 0.057	0.876 ± 0.091	0.874 ± 0.081	0.884 ± 0.055	0.910 ± 0.036
ALB (g/dL)	2.552 ± 0.129	2.534 ± 0.105	2.514 ± 0.113	2.344 ± 0.148*	2.510 ± 0.097	2.582 ± 0.112
ALB (%)	46.76 ± 2.59	47.40 ± 1.59	46.56 ± 2.60	46.56 ± 2.35	46.88 ± 1.50	47.66 ± 1.00
α1-G (%)	23.10 ± 2.86	21.22 ± 3.16	23.26 ± 2.30	22.42 ± 1.26	23.86 ± 1.07	23.82 ± 2.11
α2-G (%)	8.72 ± 0.68	9.60 ± 0.90	9.24 ± 1.12	9.78 ± 1.22	8.28 ± 0.47	8.06 ± 1.33
β-G (%)	16.50 ± 0.86	16.24 ± 0.61	16.08 ± 1.60	17.34 ± 1.86	16.64 ± 0.30	16.12 ± 1.06
γ-G (%)	4.92 ± 1.43	5.54 ± 1.29	4.86 ± 1.42	3.90 ± 1.13	4.34 ± 1.37	4.34 ± 0.89
FEMALE (satellite group)						
Number of animals	5			5	5	5
AST (IU/L)	70.2 ± 14.8			55.8 ± 6.6	59.3 ± 6.7	60.2 ± 4.9
ALT (IU/L)	36.2 ± 16.5			23.4 ± 3.6	27.8 ± 7.0	25.0 ± 5.0
ALP (IU/L)	183.2 ± 36.0			163.6 ± 52.2	200.4 ± 75.2	178.6 ± 66.9
γ-GTP (IU/L)	1.00 ± 0.33			0.84 ± 0.36	0.46 ± 0.09	0.46 ± 0.17
T-Bil (mg/dL)	0.060 ± 0.019			0.070 ± 0.010	0.086 ± 0.024	0.094 ± 0.024
TBA (μmol/L)	12.48 ± 1.83			17.26 ± 7.31	13.70 ± 4.03	16.70 ± 6.60
GLU (mg/dL)	148.4 ± 21.2			124.2 ± 14.8	152.8 ± 10.8	147.6 ± 13.1
T-Cho (mg/dL)	63.2 ± 8.2			66.2 ± 11.7	68.0 ± 8.4	81.6 ± 9.5$^{\#}$
TG (mg/dL)	10.6 ± 5.5			20.2 ± 9.7	18.6 ± 5.8	19.2 ± 5.3
TP (g/dL)	6.04 ± 0.31			5.48 ± 0.44$^{\#}$	6.00 ± 0.25	6.14 ± 0.28
UN (mg/dL)	14.06 ± 2.04			13.84 ± 2.35	16.42 ± 2.12	15.86 ± 0.78
Crea (mg/dL)	0.318 ± 0.033			0.358 ± 0.044	0.362 ± 0.045	0.362 ± 0.024
Na (mEq/L)	142.2 ± 0.8			142.2 ± 0.4	142.2 ± 0.8	143.2 ± 0.8
K (mEq/L)	4.140 ± 0.279			4.140 ± 0.113	4.784 ± 0.894	4.302 ± 0.244
Cl (mEq/L)	105.6 ± 1.5			104.4 ± 0.9	106.0 ± 1.2	104.4 ± 1.1
Ca (mg/dL)	9.56 ± 0.34			9.20 ± 0.23	9.46 ± 0.27	9.44 ± 0.11
IP (mg/dL)	5.56 ± 0.43			5.72 ± 0.47	5.38 ± 0.63	5.26 ± 0.36
A/G	1.126 ± 0.063			1.238 ± 0.051$^{\#}$	1.072 ± 0.094	1.242 ± 0.115$^{\#}$
ALB (g/dL)	3.198 ± 0.184			3.032 ± 0.282	3.102 ± 0.122	3.394 ± 0.157$^{\#}$
ALB (%)	52.98 ± 1.43			55.32 ± 1.03$^{\#}$	51.70 ± 2.22	55.32 ± 2.28$^{\#}$
α1-G (%)	18.58 ± 1.08			17.06 ± 1.86	17.90 ± 3.24	16.82 ± 1.58
α2-G (%)	7.60 ± 0.45			8.58 ± 1.00	8.08 ± 1.65	7.42 ± 0.26
β-G (%)	14.42 ± 0.53			15.46 ± 1.17	15.38 ± 1.04	14.60 ± 0.74
γ-G (%)	6.42 ± 1.40			3.58 ± 0.69$^{\#\#}$	6.94 ± 1.26	5.84 ± 0.86

*Significantly different from the control by Dunnett test ($P < 0.05$); **Significantly different from the control by Dunnett Test ($P < 0.01$); $^{\#}$Significantly different from the control by Student t-test ($P < 0.05$); $^{\#\#}$Significantly different from the control by Student t-test ($P < 0.01$); $^{\$}$Significantly different from the control by Steel test ($P < 0.05$)

Values represent mean ± SD

Table 5. Biochemical findings at the end of treatment in mating female rats dosed with polyoxymethylene.

Dose (mg/kg bw/day)	0	20	60	200
FEMALE (mating group)				
Number of animals	5	5	5	5
AST (IU/L)	135.4 ± 58.5	155.8 ± 59.5	131.0 ± 72.2	133.2 ± 39.6
ALT (IU/L)	78.2 ± 23.6	82.4 ± 28.2	66.2 ± 16.2	72.8 ± 16.6
ALP (IU/L)	416.2 ± 193.2	500.0 ± 115.0	439.8 ± 117.7	418.6 ± 177.9
γ-GTP (IU/L)	0.84 ± 0.27	1.60 ± 1.74	0.68 ± 0.28	0.68 ± 0.41
T-Bil (mg/dL)	0.056 ± 0.015	0.056 ± 0.011	0.052 ± 0.013	0.046 ± 0.013
TBA (μmol/L)	32.78 ± 16.29	34.44 ± 23.61	22.64 ± 10.49	23.30 ± 11.22
GLU (mg/dL)	152.2 ± 17.1	152.4 ± 18.4	152.2 ± 16.7	131.2 ± 13.0
T-Cho (mg/dL)	102.4 ± 14.7	102.4 ± 13.0	99.8 ± 9.0	100.0 ± 8.4
TG (mg/dL)	82.4 ± 31.2	110.2 ± 43.6	85.8 ± 20.8	65.8 ± 22.6
TP (g/dL)	5.66 ± 0.29	6.04 ± 0.30	5.66 ± 0.19	5.84 ± 0.30
UN (mg/dL)	24.40 ± 8.11	29.92 ± 9.37	22.58 ± 3.88	25.64 ± 8.13
Crea (mg/dL)	0.390 ± 0.092	0.444 ± 0.090	0.398 ± 0.037	0.400 ± 0.076
Na (mEq/L)	139.4 ± 3.2	138.8 ± 1.6	140.6 ± 1.3	140.2 ± 1.9
K (mEq/L)	4.420 ± 0.973	4.412 ± 0.588	3.902 ± 0.344	4.322 ± 0.157
Cl (mEq/L)	99.2 ± 3.1	96.4 ± 1.8	98.8 ± 1.5	98.8 ± 2.7
Ca (mg/dL)	9.74 ± 0.82	10.10 ± 0.38	9.48 ± 0.36	9.84 ± 0.38
IP (mg/dL)	8.72 ± 3.14	9.34 ± 1.17	7.60 ± 0.86	8.28 ± 1.57
A/G	0.972 ± 0.111	0.984 ± 0.043	0.972 ± 0.028	0.844 ± 0.071*
ALB (g/dL)	2.776 ± 0.097	2.994 ± 0.190	2.792 ± 0.099	2.666 ± 0.143
ALB (%)	49.16 ± 3.01	49.54 ± 10.3	49.30 ± 0.71	45.72 ± 2.21
α1-G (%)	19.72 ± 3.58	20.12 ± 1.36	19.70 ± 3.08	22.02 ± 2.89
α2-G (%)	11.12 ± 0.44	10.98 ± 1.18	11.00 ± 1.75	10.88 ± 0.43
β-G (%)	16.70 ± 0.97	15.86 ± 0.41	16.10 ± 1.04	17.48 ± 1.24
γ-G (%)	3.30 ± 0.72	3.50 ± 0.63	3.90 ± 0.68	3.90 ± 1.17

*Significantly different from the control by Dunnett test ($P < 0.05$)
Values represent mean ± SD

the end of the recovery period, but incidence and degree were reduced and healing was observed. Further, changes observed in hematology, such as significantly increased neutrophil count in males and satellite females, significantly decreased RBC in satellite females, and decreased RBC in males may be related to mucosal damage with inflammation and bleeding in the stomach. These toxicological findings are similar to toxicities exhibited by formaldehyde. Known toxicological effects of formaldehyde by oral exposure included: histopathological effects (hyperkeratosis and slight focal atrophic gastritis) in the forestomach of rats receiving 125 mg/kg bw/day in drinking-water (generated from 95% paraformaldehyde) over a period of 4 weeks (Til *et al*., 1988); reduction in weight gain in rats administered 150 mg/kg bw and dogs administered 100 mg/kg bw of formaldehyde (generated from 95% paraformaldehyde) for 90 daily doses (Johannsen *et al*., 1986); histopathological effects (papillary epithelial hyperplasia, hyperkeratosis, and/or focal ulceration in the forestomach and focal chronic atrophic gastritis, ulceration and/or glandular hyperplasia in the glandular stomach) in rats exposed to 82–109 mg/kg bw/day formaldehyde (generated from 95% paraformaldehyde) in drinking water for up to 2 years (Til *et al*., 1989); histopathological changes (squamous cell hyperplasia with or without hyperkeratosis in the forestomach and hyperplasia in the glandular stomach) in rats provided 50 mg/kg bw/day formaldehyde (generated from 80% paraformaldehyde) in a 2-year drinking-water study (Tobe *et al*., 1989). Polyoxymethylene is decomposed to formaldehyde by gastric acid, and toxicological responses to polyoxymethylene are similar to formaldehyde, namely irritant effects in the stomach.

Decreased serum levels of TP, ALB, and A/G in males and females were seen at the end of administration and recovery periods. Decreased TP and ALB were consistently observed in previous studies conducted with oral exposure of formaldehyde in rats (Til *et al*., 1988, 1989; Tobe *et al*., 1989). However, changes observed in this study were not considered to be toxicologically important because no related findings were seen in the liver. Reduced protein and GLU levels may be related to decreased food consumption after oral administration of polyoxymethylene.

Table 6. Organ weights of rats dosed with polyoxymethylene.

		Administration period				Recovery period	
Dose (mg/kg bw/day)		0	20	60	200	0	200
MALE							
Number of animals		7	12	12	7	5	5
Body Weight	(g)	512.0 ± 38.7	501.2 ± 46.5	509.9 ± 41.9	486.6 ± 44.2	537.2 ± 14.8	524.6 ± 18.8
Liver	AB (g)	13.383 ± 1.619	13.281 ± 1.881	13.493 ± 2.556	12.031 ± 1.586	12.712 ± 0.402	12.618 ± 1.407
	RE (g/100g)	2.607 ± 0.166	2.642 ± 0.189	2.629 ± 0.306	2.467 ± 0.198	2.366 ± 0.058	2.404 ± 0.235
Kidney	AB (g)	3.047 ± 0.331	3.315 ± 0.303	3.271 ± 0.293	3.103 ± 0.255	3.182 ± 0.305	3.276 ± 0.291
	RE (g/100g)	0.596 ± 0.061	0.663 ± 0.051*	0.643 ± 0.040	0.640 ± 0.051	0.594 ± 0.058	0.622 ± 0.051
Heart	AB (g)	1.513 ± 0.065	1.513 ± 0.114	1.446 ± 0.071	1.446 ± 0.138	1.494 ± 0.048	1.512 ± 0.096
	RE (g/100g)	0.296 ± 0.020	0.304 ± 0.024	0.284 ± 0.014	0.297 ± 0.015	0.280 ± 0.007	0.286 ± 0.011
Spleen	AB (mg)	754.4 ± 87.7	710.3 ± 90.1	752.7 ± 117.9	666.7 ± 142.7	694.4 ± 103.2	7678 ± 59.2
	RE (mg/100g)	148.126 ± 20.255	142.199 ± 15.518	147.183 ± 14.539	136.231 ± 22.579	129.506 ± 20.751	146.594 ± 13.754
Thymus	AB (mg)	286.6 ± 72.6	315.5 ± 87.4	336.5 ± 106.2	270.1 ± 70.1	310.0 ± 77.2	278.8 ± 52.0
	RE (mg/100g)	55.666 ± 11.994	62.826 ± 17.158	66.060 ± 20.447	55.631 ± 13.788	57.604 ± 13.860	53.206 ± 9.854
Adrenal	AB (mg)	73.3 ± 11.1	67.5 ± 11.1	73.3 ± 11.4	77.1 ± 14.8	61.4 ± 15.6	68.0 ± 11.7
	RE (mg/100g)	14.294 ± 1.700	13.547 ± 2.285	14.386 ± 1.894	15.891 ± 2.677	11.400 ± 2.763	12.938 ± 2.020
Pituitary gland	AB (mg)	13.76 ± 2.68	14.07 ± 1.96	12.81 ± 1.19	13.24 ± 2.98	13.94 ± 3.01	12.74 ± 2.48
	RE (mg/100g)	2.687 ± 0.507	2.808 ± 0.315	2.520 ± 0.235	2.709 ± 0.476	2.588 ± 0.514	2.420 ± 0.419
Thyroid	AB (mg)	24.83 ± 3.83	24.69 ± 5.16	23.25 ± 3.71	25.01 ± 4.56	21.52 ± 5.34	20.72 ± 4.71
	RE (mg/100g)	4.884 ± 0.917	4.920 ± 0.928	4.580 ± 0.778	5.161 ± 1.012	4.012 ± 1.030	3.954 ± 0.925
Testis	AB (g)	3.257 ± 0.138	3.488 ± 0.274	3.358 ± 0.204	3.610 ± 0.203*	3.266 ± 0.373	3.354 ± 0.229
	RE (g/100g)	0.640 ± 0.068	0.703 ± 0.081	0.661 ± 0.071	0.744 ± 0.060*	0.606 ± 0.059	0.642 ± 0.049
Epididymis	AB (g)	1.403 ± 0.092	1.455 ± 0.146	1.341 ± 0.126	1.434 ± 0.165	1.404 ± 0.114	1.474 ± 0.113
	RE (g/100g)	0.276 ± 0.023	0.293 ± 0.024	0.264 ± 0.028	0.296 ± 0.036	0.262 ± 0.023	0.282 ± 0.016
Prostate	AB (mg)	796.6 ± 97.5	685.7 ± 157.3	680.5 ± 148.6	711.4 ± 159.7	808.4 ± 103.3	770.4 ± 177.9
	RE (mg/100g)	155.463 ± 13.556	138.434 ± 36.414	135.163 ± 35.263	145.214 ± 25.543	150.618 ± 20.436	147.028 ± 33.821
Seminal vesicle	AB (g)	2.407 ± 0.253	2.353 ± 0.261	2.366 ± 0.237	2.520 ± 0.481	2.418 ± 0.151	2.720 ± 0.120##
	RE (g/100g)	0.470 ± 0.048	0.471 ± 0.056	0.468 ± 0.063	0.519 ± 0.088	0.450 ± 0.033	0.520 ± 0.031##
Brain	AB (g)	2.223 ± 0.127	2.209 ± 0.099	2.204 ± 0.085	2.221 ± 0.123	2.196 ± 0.040	2.166 ± 0.079
	RE (g/100g)	0.437 ± 0.035	0.443 ± 0.047	0.433 ± 0.037	0.459 ± 0.029	0.412 ± 0.008	0.414 ± 0.017
FEMALE (mating group)							
Number of animals		12	10	12	12		
Body Weight	(g)	330.2 ± 24.7	329.2 ± 13.8	335.1 ± 19.8	324.0 ± 17.0		
Liver	AB (g)	11.363 ± 0.883	11.486 ± 0.995	11.612 ± 0.588	11.078 ± 0.571		
	RE (g/100g)	3.448 ± 0.205	3.490 ± 0.262	3.474 ± 0.223	3.424 ± 0.201		
Kidney	AB (g)	2.200 ± 0.245	2.060 ± 0.088	3.265 ± 3.817	2.163 ± 0.137		
	RE (g/100g)	0.666 ± 0.047	0.625 ± 0.024	0.955 ± 1.054	0.670 ± 0.051		
Heart	AB (g)	1.103 ± 0.034	1.087 ± 0.060	1.090 ± 0.061	1.077 ± 0.091		
	RE (g/100g)	0.335 ± 0.022	0.331 ± 0.020	0.328 ± 0.019	0.333 ± 0.019		
Spleen	AB (mg)	570.6 ± 59.0	553.8 ± 70.5	609.5 ± 69.2	563.5 ± 59.3		
	RE (mg/100g)	173.306 ± 18.365	169.024 ± 26.988	182.853 ± 25.445	174.471 ± 22.040		
Thymus	AB (mg)	237.9 ± 85.5	218.6 ± 85.4	251.0 ± 68.1	205.7 ± 43.1		
	RE (mg/100g)	72.123 ± 25.940	66.226 ± 24.575	74.986 ± 19.674	63.726 ± 14.113		
Adrenal	AB (mg)	75.0 ± 10.4	72.8 ± 10.4	82.5 ± 11.6	86.0 ± 8.3*		
	RE (mg/100g)	22.738 ± 2.613	22.201 ± 3.668	24.672 ± 3.562	26.631 ± 3.120*		
Pituitary gland	AB (mg)	15.53 ± 3.29	14.65 ± 2.75	15.79 ± 2.03	15.38 ± 2.04		
	RE (mg/100g)	4.716 ± 0.987	4.465 ± 0.905	4.712 ± 0.538	4.747 ± 0.591		
Thyroid	AB (mg)	17.09 ± 2.78	18.12 ± 3.82	15.71 ± 2.51	14.98 ± 2.43		
	RE (mg/100g)	5.173 ± 0.738	5.500 ± 1.142	4.706 ± 0.803	4.641 ± 0.827		
Ovary	AB (mg)	104.8 ± 19.4	105.5 ± 14.0	104.4 ± 18.5	103.1 ± 11.1		
	RE (mg/100g)	31.799 ± 5.581	32.140 ± 4.722	31.232 ± 5.745	31.819 ± 3.080		
Uterus	AB (mg)	557.8 ± 105.0	604.3 ± 76.0	573.8 ± 92.5	537.1 ± 45.0		
	RE (mg/100g)	169.566 ± 34.310	184.133 ± 26.840	172.153 ± 32.029	165.838 ± 11.711		
Brain	AB (g)	2.032 ± 0.060	1.995 ± 0.076	2.032 ± 0.089	2.033 ± 0.087		
	RE (g/100g)	0.618 ± 0.047	0.608 ± 0.038	0.609 ± 0.037	0.629 ± 0.038		

*Significantly different from the control by Dunnett test ($P < 0.05$); ##Significantly different from the control by Student t-test ($P < 0.01$) AB: Absolute weight; RE: Relative weight

Values represent mean ± SD

Table 7. Histopathological findings in stomachs of rats dosed with polyoxymethylene.

	Administration period				Recovery period	
Dose (mg/kg bw/day)	0	20	60	200	0	200
MALE						
Number of animals	7	12	12	7	5	5
Ulcer, forestomach						
Not remarkable	7	12	12	6		
Severe	0	0	0	1		
Ulcer, glandular stomach						
Not remarkable	7	12	12	5		
Moderate	0	0	0	2		
Erosion, glandular stomach						
Not remarkable	7	12	12	3		
Slight	0	0	0	1		
Mild	0	0	0	3		
Infiltration, inflammatory cells, submucosa, glandular stomach						
Not remarkable	7	12	12	4	5	2
Slight	0	0	0	0	0	3
Mild	0	0	0	3	0	0
FEMALE (mating group)						
Number of animals	12	10	12	12	-	-
Ulcer, forestomach						
Not remarkable	12	10	12	8		
Severe	0	0	0	4		
Ulcer, glandular stomach						
Not remarkable	12	10	12	11		
Moderate	0	0	0	1		
Erosion, glandular stomach						
Not remarkable	11	10	12	11		
Slight	1	0	0	1		
Infiltration, inflammatory cells, submucosa, glandular stomach						
Not remarkable	12	10	12	4		
Slight	0	0	0	7 **		
Mild	0	0	0	1 **		
FEMALE (satellite group)						
Number of animals	5			5	5	5
Ulcer, glandular stomach						
Not remarkable	5			4		
Moderate	0			1		
Erosion, glandular stomach						
Not remarkable	5			1		
Slight	0			4*		
Infiltration, inflammatory cells, submucosa, glandular stomach						
Not remarkable	5			2	5	1
Slight	0			3	0	4*

*Significantly different from the control by Fisher's exact test ($P < 0.05$); ** Significantly different from the control by Fisher's exact test ($P < 0.01$)

Significantly increased absolute and relative weights of adrenals without histopathological changes were observed, with dose-dependency at the end of dosing in mating females at the high dose. Individual values of adrenal weight were within or close to baseline laboratory norms: absolute weight, 76.1 ± 9.5 mg (mean ± SD); relative weight: 22.752 ± 2.927 mg/100 g bw (mean ± SD); n = 54, 2017–2021). These effects were not observed in males or satellite females. Therefore, changes in adrenal weight were considered toxicologically insignificant. Absolute and relative weights of testes were significantly increased at the end of treatment at the high dose. Also, absolute and relative weights of seminal vesicles tended to increase at the end of administration and increases

Table 8. Reproductive and developmental findings for rats dosed with polyoxymethylene.

Dose (mg/kg bw/day)		0	20	60	200
Number of animals		12	10	12	12
Gestation length	(day)	22.17 ± 0.39	22.10 ± 0.32	22.17 ± 0.39	22.42 ± 0.51
Gestation index	(%)	100	100	100	100
Number of implantations	(%)	14.9 ± 1.2	14.9 ± 2.5	15.3 ± 1.4	15.2 ± 1.6
Delivery Index	(%)	94.51 ± 5.44	88.95 ± 10.41	93.92 ± 6.03	92.88 ± 10.39
Number of offspring		14.1 ± 1.2	13.3 ± 2.9	14.3 ± 1.7	14.1 ± 2.1
Number of live newborns	M	6.3 ± 2.1	7.0 ± 2.2	7.1 ± 1.6	6.0 ± 1.9
	F	7.7 ± 2.5	6.0 ± 2.4	7.2 ± 2.2	7.8 ± 1.9
	Total	14.0 ± 1.1	13.0 ± 2.7	14.3 ± 1.6	13.8 ± 2.6
Sex ratio	live-born	45.2	53.8	49.7	43.6
	delivered pups	45.0	54.1	49.4	43.8
Number of dead newborns		0.1 ± 0.3	0.3 ± 0.5	0.1 ± 0.3	0.3 ± 1.2
Viability index (PND0) (%)		99.48 ± 1.79	98.02 ± 3.26	99.48 ± 1.79	97.43 ± 8.89
Viability index (PND4) (%)		98.33 ± 4.14	98.41 ± 3.49	98.13 ± 4.45	100.00 ± 0.00
Viability index (PND13) (%)		100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
Body Weight (male pups)					
PND0		6.611 ± 0.812	6.827 ± 0.624	6.619 ± 0.357	7.046 ± 0.694
PND4		10.678 ± 1.639	11.173 ± 1.117	10.674 ± 1.085	10.926 ± 1.834
PND7		18.242 ± 2.867	18.756 ± 1.449	18.527 ± 1.746	18.422 ± 2.243
PND13		34.573 ± 4.378	34.673 ± 2.372	34.973 ± 3.263	34.266 ± 3.208
Body Weight (female pups)					
PND0		6.301 ± 0.711	6.539 ± 0.655	6.203 ± 0.342	6.640 ± 0.680
PND4		10.121 ± 1.310	10.513 ± 1.154	9.898 ± 1.011	10.460 ± 1.809
PND7		17.958 ± 1.970	17.541 ± 1.633	17.303 ± 1.766	17.452 ± 2.157
PND13		34.443 ± 2.560	32.877 ± 2.338	33.321 ± 3.013	32.839 ± 3.035

PND, postnatal day; Values represent mean ± SD

reached significant levels at the end of recovery. Testicular toxicity of formaldehyde was previously reported after oral administration (Cassidy *et al*., 1983; Til *et al*., 1989) and inhalation (Zhou *et al*., 2011; Ozen *et al*., 2005; Golalipour *et al*., 2007). A single oral dose of formaldehyde, 200 mg/kg bw, increased the incidence of abnormal sperm in rats (Cassidy *et al*., 1983). An increased relative weight of testes and an instance of Leydig-cell tumor were observed in a rat in a two-year drinking-water study. However, the author did not consider these changes to be formaldehyde-related (Til *et al*., 1989). Thus, changes in the male reproductive organs were considered responses to oral exposure of polyoxymethylene. However, no histopathological changes were seen in the present study in these organs, and no effects on fertility were found. Individual values for organ weights were within or close to baseline values – absolute weight of testes: 3.406 ± 0.303 g (mean ± SD); relative weight of tested: 0.620 ± 0.074 g/100 g bw; n = 165, 2017–2021, and absolute weight of seminal vesicle: 2.307 ± 0.311 g; relative weight of seminal vesicle: 0.426 ± 0.077 g/100 g bw; n = 100, 2017–2021]. Therefore, we consider effects on male reproductive organs dose-related but not adverse. No effects on reproductive and developmental processes were found in this study. A two-generation rat study of inhaled formaldehyde showed teratogenicity [Chinese paper with English summary] (Tang *et al*., 2006) and a study in rabbits showed meromelia, encephalocele, oligodactyly, umbilical hernia, and short tail in newborns (Saraj and A. Al, 2009). Conversely, no teratogenicity was reported by oral formaldehyde administration in two previous prenatal developmental toxicity studies in mice (Marks *et al*., 1980; Seidenberg *et al*., 1986). Our findings are consistent with these results. No gross internal and external anomalies were found in pups at a dose level where maternal toxicity was observed. Prolonged pregnancy was observed previously in females exposed to formaldehyde for 10 to 15 days before mating, then mated with non-exposed males by inhalation [Russian paper with English summary] (Gofmekler and Bonashevskaia, 1969). This effect was not observed in the present study. Adverse responses to formaldehyde differ by route of exposure for reproductive/developmental toxicity, but also other endpoints, such as carcinogenicity. These differences may be related to the first contact place, or absorption, distribution, metabolism, and elimination (ADME), which depends on the route of exposure. Details of such differences are not currently available. Still, the current study

Table 9. Summary results of hazard assessment for polyoxymethylene.

Hazard	Repeated-dose toxicity (general toxicity)	Reproductive/developmental toxicity	Genotoxicity (MHLW, 2021b)	
Toxicity test	Combined repeated-dose and reproductive/developmental toxicity screening test in rats (0, 20, 60, and 200 mg/kg bw/day)		Ames test in *Salmonella typhimurium* (TA100, TA1535, TA98, TA1537) & Escherichia coli (WP2 uvrA)	*In vitro* mammalian chromosome aberration test with Chinese hamster lung cell line (CHL/IU)
Endpoint	Decreased food consumption, histopathological changes in the stomach	No changes up to 200 mg/kg/day	Reverse mutation	Chromosomal aberration
POD	NOAEL 60 mg/kg bw/day	NOAEL 200 mg/kg bw/day	ND	ND
UF	600 [species difference (10) × individual difference (10) × study duration (6)]	1000 [species difference (10) × individual difference (10) × database insufficiency (10)]	ND	ND
D-value/genotoxic judgment	0.1 mg/kg/day	0.2 mg/kg/day	Positive	Positive
Hazard class	Class 4 ($0.05 < D \leq 0.5$)	Class 4 ($0.05 < D \leq 0.5$)	Class 3	

POD, point of departure; UF, uncertainty factor; ND, not determined; NOAEL, no-observed-adverse-effect level

confirms that oral administration of polyoxymethylene does not affect reproduction and development under study conditions and may suggest that oral administration of formaldehyde is unlikely to affect fertility in rats at a dose that induces general toxicity.

A NOAEL for repeated exposure to polyoxymethylene is 60 mg/kg bw/day based on decreased body weight gain and food consumption and histopathological changes in the stomach., A NOAEL for reproductive/developmental toxicity is 200 mg/kg bw/day (the highest dose tested) for both sexes. Consequently, *D*-values were calculated as 0.1 mg/kg bw/day (60 mg/kg bw/day/UF:600) for repeated-dose toxicity and 0.2 mg/kg bw/day (200 mg/kg bw/day/UF:1000) for reproductive and developmental toxicity. These values indicate that the chemical is class 4 for repeated-dose toxicity, class 4 for reproductive and developmental toxicity, and class 3 for genotoxicity (Table 9). A prioritization matrix was developed for the screening assessment under the CSCL (Fig. 1). The lowest class of 3 for polyoxymethylene indicates medium priority as a human health hazard.

We report the results of an OECD TG 422 study and hazard assessment of polyoxymethylene. Our earlier work on hazard assessment of other substances is also available in scientific journals (Kawashima *et al*., 2020; Matsumoto *et al*., 2021; Igarashi *et al*., 2020, 2018a, 2018b). Study reports are accessible via the Japan Existing Chemical Database (JECDB, 2021). This paper provides data necessary for screening assessment of polyoxymethylene and summarizes helpful data for filling the

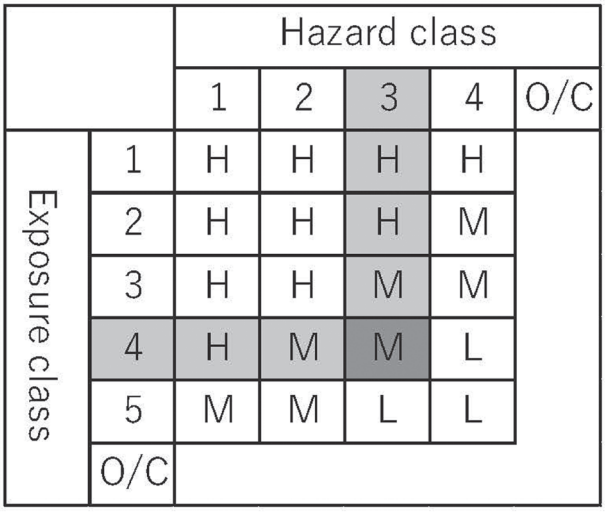

Fig. 1. Prioritization matrix for screening assessments of possible human health effects of existing chemicals under the Japanese Chemical Substances Control Law (CSCL). Priority is divided into four classes: high (H), medium (M), low (L), and out-of-class (O/C) based on both hazard and exposure. Priority "H" automatically requires a more detailed risk assessment, while priority "M" requires expert judgment to assess whether a more detailed risk assessment is required. Priority of human health of polyoxymethylene was designated as medium based on exposure class 4 and hazard class 3.

data gap for effects on fertility of formaldehyde by oral exposure. Formaldehyde is classified as a PACS to recognize its carcinogenic potential by inhalation [IARC: carcinogenic to humans, Group 1 (IARC, 2012)] under the CSCL. Thus, we recommend that official experts review all available toxicity data on both polyoxymethylene and formaldehyde and determine if polyoxymethylene should be classified as a PACS.

Notice: Conclusions expressed in this paper are the authors' own and should not be considered as Japanese government conclusions under the CSCL.

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Conflict of interest---- The authors declare that there is no conflict of interest.

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