e.g. DG ENTR, DG ENV, and ECVAM (DG JRC), and the Community agencies such as ECHA, EFSA or EMEA through their involvement in guideline generation and acceptance, risk assessment and risk management, and steering of validation of non-animal methods, should play a key supporting role. However, a simple/single solution seems not to be realistic, and multiple approaches will be needed. The pressure to change may have to come from researchers whereby peer pressure and best practices could drive guidance and publications through industrial platforms and scientific societies such as ICH, the International workshop on Genotoxicity testing (IWGT), the Society of Toxicology (SOT) and the European Partnership on Animal Alternatives (EPAA) which then would help push forward changes in law/guidelines (OECD). Taking into account that even in a best-case scenario, changes in guidelines take years, the good will of the parties involved may have to be relied upon or alternative routes will have to be explored in order to move forward. One suggestion made at the workshop was that amendments to the OECD guidelines may be a faster way forward than revising the guidelines.

4. Conclusions

The premise under which the discussions have taken place was that any poorly designed or conducted experiment is a waste of animals and that the modification of methods should not compromise safety standards.

There was agreement amongst the workshop participants that there are many options available to reduce the numbers of animals in *in vivo* genotoxicity studies, and that most of them are in compliance with regulations and scientifically credible, i.e. ready for use. These options include the use of one sex only, one administration and two sampling times *versus* two/three administrations and one sampling time for MN, CA and Comet assays; the omission of a concurrent positive control in routine CA and MN tests; the combination of acute MN and Comet assay studies in case information from more than one endpoint or tissue is needed; and the integration of the MN endpoint into repeat-dose toxicity studies. These options are, to date, not sufficiently utilized and the workshop strongly encouraged the use and promotion of these options. The workshop participants want to encourage the scientific community to present and publish data related to reduction opportunities in order to boost the acceptance level of these approaches. Furthermore, experimental proof is needed and under way to demonstrate the credibility of additional options for reduction, such as the integration of the Comet assay into RDT studies.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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Reproductive and Developmental Toxicity Screening Study of 2,4-Dinitrophenol in Rats

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ABSTRACT: Rats were treated by gavage once daily with 2,4-dinitrophenol (DNP) at 0 (control), 3, 10, or 30 mg/kg bw. Males were dosed for 46 days, beginning 14 days before mating, and females were dosed for 40–47 days, from 14 days before mating to day 3 of lactation. No deaths were observed in males and females of any group. A significant decrease in body weight gain and significant increase in liver weight were found in males and females at 30 mg/kg bw/day. The number of live pups on postnatal days (PNDs) 0 and 4, live birth index, and body weight of live male and female pups on PNDs 0 and 1 were significantly lowered at 30 mg/kg bw/day. External and internal examinations of pups revealed no increased incidence of malformations in DNP-treated groups. On the basis of these findings, we concluded that DNP has general and reproductive/developmental toxicity, but not teratogenicity, under the present conditions. The NOAEL of DNP is considered to be 10 mg/kg bw/day in rats. © 2008 Wiley Periodicals, Inc. Environ Toxicol 24: 74–81, 2009.

Keywords: 2,4-dinitrophenol; reproductive/developmental toxicity; rat

INTRODUCTION

2,4-Dinitrophenol (DNP; CAS No. 51-28-5) is one of the six different isomers of dinitrophenols, and the most commercially important isomer. Commercial dinitrophenol, a mixture of DNP and smaller amounts of 2,3- and 2,6-dinitrophenol, is used in the synthesis of picric acid and picramic acid, and for making dyes, wood preservatives, photographic developers, explosives, and insecticides (ATSDR, 1995). The production volume of DNP exceeded 1 million pounds/year in the U.S. (Scorecard, 2007) and was around 1000 tons in Japan in 2005 (METI, 2006). DNP is used for the same purposes as dinoseb, 2-*sec*-butyl-4,6-dinitrophenol, which was registered as a herbicide and insecticide. DNP was once taken extensively as a weight reduction drug in the 1930s (Simkins, 1937a,b). Thereafter, adverse effects, including cataracts, renal damage, and death due to hyperthermia, were noted in people who took DNP (Beinhauer, 1934; Epstein and Rosenblum, 1935; Goldman and Haber, 1936; Simkins, 1937a,b). DNP was banned for use for this purpose by authorities in the U.S. in 1938 (Parascandola, 1974; Kurt et al., 1986); however, it can be still illicitly purchased in the U.S. as a diet pill via commercial web sites, and incidents, including deaths, have been reported (Miranda et al., 2006). DNP is released into the environment primarily during its manufacture and use, and from waste disposal sites that contain DNP (ATSDR, 1995), and can also form in the atmosphere from the reaction of benzene with NOx in ambient air (Nojima et al., 1983). General population and occupational exposures may occur primarily through the inhalation of ambient air (ATSDR, 1995). According to TRI01 (U.S. EPA, 2001), total on- and off-site release was around 100 000 pounds in

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the U.S. in 2001. IPCS (1996) noted that this substance may be hazardous to the environment and special attention should be given to aquatic organisms.

The toxicity of DNP in mammals is relatively well understood and is summarized by ATSDR (1995). DNP is an uncoupler of oxidative phosphorylation from electron transport in mitochondria, resulting in the release of energy as heat and in increased metabolism of lipids (ATSDR, 1995). Although the areas of reproductive and developmental toxicology are becoming an increasingly important part of the overall toxicology profile of chemicals, only limited reports are available on the reproductive and developmental toxicity of DNP. Only maternal hyperexcitability and hyperthermia were observed at 38.3 mg/kg bw/day in mice given DNP by gavage on gestation days (GDs) 10–12, the susceptible period for dinoseb-induced malformations (Gibson, 1973). In a study to develop a teratogenicity screen (Kavlock et al., 1987), no adverse effects on parturition, survival or growth of offspring were reported even at 125 mg/kg bw/day in mice treated DNP by gavage on GDs 8–12. Decreased viability of pups was found in rats given DNP by gavage twice daily at 20 mg/kg bw beginning 8 days prior to mating and throughout pregnancy and lactation (Wulff et al., 1935). A human clinical study revealed that direct action of DNP was involved because the menstrual changes were striking and occurred soon after DNP treatment before any significant weight loss (Simkins, 1937a,b).

These toxicology reports on DNP were determined to be inadequate to assess the chemical, because they did not follow Good Laboratory Practice (GLP) or did not totally comply with specific testing guidelines (Klimisch et al., 1997; OECD, 2005); therefore, DNP was selected as a target substance for the Safety Examination of Existing Chemicals in Japan (MHLW, 2001) to obtain reliable information on the possible toxic effects in compliance with the OECD Test Guideline and in accordance with the principles of GLP. A reproduction/developmental toxicity screening test of DNP was performed in rats, and the results of this study are reported in this article.

MATERIALS AND METHODS

This study was performed in 2005 at the Safety Research Institute for Chemical Compounds (Sapporo, Japan) in compliance with the OECD guideline 421 Reproduction/Developmental Toxicity Screening Test (OECD, 1995) and in accordance with the principles for GLP (MHLW/METI/MOE, 2004) and "Guidance for Animal Care and Use" of the Safety Research Institute for Chemical Compounds.

Animals

SPF Crl: CD (SD) rats were used in this study. This strain was chosen because it is most commonly used in toxic studies, including reproductive and developmental toxicity studies, and historical control data are available. Males and females at 8 weeks of age were purchased from Atsugi Breeding Center, Charles River Japan (Yokohama, Japan). The rats were acclimated to the laboratory for 14 days prior to the start of the experiment. Male and female rats found to be in good health were selected for use. Vaginal smears of each female were recorded and only females showing a 4- to 6-day estrous cycle were used in the experiment. Male and female rats were distributed on a random basis into four groups of 12 males and 12 females each. Rats were housed individually, except during the acclimation, mating, and nursing periods. From day 17 of pregnancy to the day of sacrifice, individual dams and litters were reared using wood chips as bedding (White Flake; Charles River Japan).

Animals were fed on a sterilized basal diet (CRF-1; Oriental Yeast, Tokyo, Japan) and tap water *ad libitum*, and maintained in an air-conditioned room at 22°C ± 3°C, with a relative humidity of 50% ± 20%, a 12-h light/dark cycle and ventilation with 10–15 air changes per hour.

Chemicals and Dosing

DNP is a yellow, odorless solid, very sparingly soluble in cold water and soluble in alcohol, benzene, and aqueous alkaline solution. Its melting point is 112–114°C and molecular weight is 184.1. DNP was obtained from Tokyo Chemical Industry (Tokyo, Japan). The DNP (Lot No. FGH01) used in this study was 84.1% pure (15.9 w/w % moisture content, 99.7% pure after dried) and it was kept in a cool, dark place. The purity converted using the moisture content and stability of the chemical were verified by analysis before the study. DNP was suspended in 1 w/v % methylcellulose solution. The stability of formulations had been confirmed for up to 14 days. During use, the formulations were maintained for less than 14 days, and the concentration was confirmed to be 92.0 to 104.0% of the target. Rats were dosed once daily by gastric intubation with DNP at a dose of 0 (control), 3, 10, or 30 mg/kg bw. Dosage levels were determined based on the results of a 28-day repeat dose oral toxicity test in rats given DNP by gavage at 0, 3, 10, 30, or 80 mg/kg bw/day. Deaths occurred at 80 mg/kg bw/day and decreased locomotor activity and salivation were observed at 30 mg/kg bw/day and more, but no adverse effects were detected at 3 and 10 mg/kg bw/day (Koizumi et al., 2001). Males were dosed for 46 days, beginning 14 days before mating, and females were dosed for 40–47 days, beginning 14 days before mating to day 3 of lactation throughout mating and gestation. The volume of each dose was adjusted to 10 mL/kg bw based on the latest body weight during the administration period in males and during the premating and mating period in females or the body weight on day 0 of pregnancy in females after copulation. Control rats were given 1 w/v % methylcellulose solution only.

Observations

All rats were observed daily for clinical signs of toxicity. The body weight and food consumption were recorded on days 0, 1, 4, 6, 9, and 13 of the premating period and then once a week in males, and on days 0, 1, 4, 6, 9, and 13 of the premating period, on days 0, 1, 3, 5, 7, 10, 14, 17, and 20 of pregnancy, and on days 0, 1, and 4 of lactation in females. The rats were euthanized by exsanguination under anesthesia on the next day of the last administration in males and on day 4 of lactation in females. The external surfaces of the rats were examined. The abdomen and thoracic cavity were opened, and gross internal examination was performed. The brain, heart, liver, kidneys, spleen, adrenal gland, thymus, testes, epididymides, and ovaries were weighed. The numbers of corpora lutea and implantation sites were recorded in females. The testes and epididymides were fixed with Bouin's solution and preserved in 70% ethanol, and other internal organs were stored in 10% neutral-buffered formalin. In control and 30 mg/kg bw/day groups, histopathological evaluations were performed on tissue sections of the testes, epididymides and ovaries, and the stages of spermatogenesis were observed.

Daily vaginal lavage samples of each female were evaluated for estrous cyclicity throughout the premating period. Each female rat was mated overnight with a single male rat of the same dosage group until copulation occurred or the mating period, 2 weeks, had elapsed. During the mating period, daily vaginal smears were examined for the presence of sperm. The presence of sperm in the vaginal smear and/or a vaginal plug was considered evidence of successful mating. Once insemination was confirmed, the females were checked daily for signs of parturition at 9:00, 13:00, and 17:00 from day 21 of pregnancy. Females were allowed to deliver spontaneously and nurse their pups until postnatal day (PND) 4. The day on which parturition was completed by 9:00 was designated as PND 0. Litter size and the numbers of live and dead pups were recorded. Live pups were sexed and grossly examined on PND 0, and individually weighed on PNDs 0, 1, and 4. The pups were euthanized by carbon dioxide inhalation and gross external, including palate, and internal examinations were performed on PND 4.

Data Analysis

Statistical analysis of pups was carried out using the litter as the experimental unit. The body weight, body weight gain, food consumption, absolute and relative organ weights, length of estrous cycle, numbers of corpora lutea, implantation sites, pups delivered and live pups on PNDs 0 and 4, and implantation, delivery, live birth and viability indexes were analyzed with Bartlett's test for homogeneity of variance at the 5% level of significance. If homogeneous, data were analyzed using one-way analysis of variance and Dunnett's multiple comparison test to compare the mean of the control group with that of each dosage group. If not, data were analyzed using the Kruskal–Wallis test and Mann–Whitney *U*-test to compare the mean of the control group with that of each dosage group. The numbers of Sertoli cell, germ cells and germ cells per Sertoli cell in various stages of spermatogenesis were analyzed using the Mann–Whitney *U*-test. Copulation, fertility, gestation and nursing indexes, and sex ratio of pups were analyzed with the Chi-square test and/or Fisher's exact test. The 5% level of probability was used as the criterion of significance.

RESULTS

No deaths were observed in males and females of any group. At 30 mg/kg bw/day, salivation was occasionally observed in three males during the administration period and in one female during pregnancy.

The body weight gains of male and female rats given DNP are shown in Table I. Significant decreases in body weight gain were found on days 0–6, days 13–20, and days 0–45, the whole period of the administration period, in males at 30 mg/kg bw/day. At this dose, a significant decrease in body weight gain was found on days 0–4 during lactation in females. There was no significant difference in food consumption between the control and DNP-treated groups.

Table II shows the organ weight of rats given DNP. The relative weight of the liver in males and absolute and relative weights of the liver in females, the relative weights of the kidneys in males and females, and the relative weight of the heart in females were significantly increased at 30 mg/kg bw/day. The absolute and relative weights of the testes and relative weight of the epididymides were significantly increased at 3 mg/kg bw/day. In females, the weight of ovaries was not affected in DNP-treated groups.

Severe atrophy of seminiferous tubules in the testis, and sperm decrease and luminal cell debris in the epididymis were observed on only the right side of one male at 30 mg/kg bw/day. Slight atrophy of seminiferous tubules in the testes was shown in another male at 30 mg/kg bw/day and in one male of the control group. The number of spermatogonia at 30 mg/kg bw/day was significantly, but slightly, decreased only in stage IX–XI, but not in other stages of spermatogenesis. No changes in the numbers of Sertoli cell and germ cells per Sertoli cell in various stages of spermatogenesis were detected between the control and the DNP-treated group. No histopathological changes in the ovaries were detected at 30 mg/kg bw/day.

Reproductive findings are shown in Table III. There were no significant differences in the length of the estrous cycle, male and female copulation, fertility, gestation and nursing indexes, and gestation length between the control and DNP-treated groups.

TABLE I. Body weight gains of male and female rats given DNP

Dose (mg/kg bw/day)	0 (Control)	3	10	30
No. of males	12	12	12	12
Initial body weight (g)[a]	373.3 ± 19.9	373.9 ± 16.9	375.2 ± 20.2	375.2 ± 18.1
Body weight gain during dosing (g)[a]				
Days 0–6	25.9 ± 4.9	21.8 ± 9.4	24.2 ± 6.3	17.4 ± 9.2*
Days 6–13	28.1 ± 8.5	21.5 ± 6.0	27.0 ± 6.3	22.8 ± 7.6
Days 13–20	26.4 ± 6.6	23.1 ± 4.9	21.6 ± 8.1	18.6 ± 8.9*
Days 20–27	23.2 ± 5.4	27.6 ± 7.7	27.8 ± 4.9	21.6 ± 6.2
Days 27–34	25.5 ± 4.5	23.3 ± 7.3	26.8 ± 6.6	19.3 ± 6.4
Days 34–41	19.0 ± 5.6	17.8 ± 4.3	21.5 ± 7.1	18.3 ± 8.8
Days 41–45	11.1 ± 5.0	13.0 ± 5.7	11.3 ± 5.2	8.4 ± 7.7
Days 0–45	159.2 ± 26.0	148.2 ± 21.6	160.0 ± 33.6	126.5 ± 34.7*
No. of females	12	12	12	12
Initial body weight (g)[a]	229.8 ± 9.9	229.4 ± 11.9	228.3 ± 8.0	228.9 ± 13.8
Body weight gain during pre-mating (g)[a]				
Days 0–6	14.8 ± 7.2	16.9 ± 9.0	14.9 ± 7.1	12.6 ± 6.3
Days 6–13	11.3 ± 7.5	13.8 ± 7.9	13.0 ± 7.7	7.6 ± 6.2
Days 0–13	26.1 ± 11.5	30.7 ± 5.8	27.9 ± 10.7	20.2 ± 9.6
Body weight gain during pregnancy (g)[a]				
Days 0–7	41.4 ± 8.0	42.8 ± 8.4	40.5 ± 8.5	47.8 ± 7.5
Days 7–14	38.3 ± 7.6	45.2 ± 10.6	40.7 ± 7.9	40.5 ± 5.8
Days 14–20	77.8 ± 10.3	83.4 ± 10.0	76.6 ± 14.4	74.8 ± 6.6
Days 0–20	157.6 ± 17.6	171.4 ± 16.0	157.9 ± 23.6	163.1 ± 10.0
Body weight gain during lactation (g)[a]				
Days 0–4	32.4 ± 16.3	27.3 ± 7.0	23.8 ± 10.1	15.5 ± 12.0**

During pregnancy and lactation, data from females treated with 3, 10 or 30 mg/kg bw/day were obtained from only 11 females because one female in each group did not become pregnant.

* Significantly different from the control group, $p < 0.05$.

** Significantly different from the control group, $p < 0.01$.

[a] Values are the mean ± SD.

The developmental findings in rats given DNP are presented in Table IV. There were no significant differences in the implantation, delivery and viability indexes, numbers of corpora lutea and pups delivered, and sex ratio and body weight on PND 4 of live pups between the control and DNP-treated groups. At 30 mg/kg bw/day, significant decreases were noted in the number of live pups on PNDs 0 and 4, live birth index, and body weight of live male and female pups on PNDs 0 and 1. The number of implantation sites was significantly high at 3 mg/kg bw/day. External and internal examinations of pups revealed dilatation of the cerebral ventricle of one pup in the control group.

DISCUSSION

In the present study in rats, DNP was given to males during the premating and mating periods and to females during the premating, mating, pregnancy, and early lactation periods.

As stated above, DNP was used as a weight-reduction agent in the 1930s (Simkins, 1937a,b). Weight loss was achieved because energy was released as heat by uncoupling of electron transport from ATP synthesis (ATSDR, 1995). The decreased body weight gain unaccompanied with decreased food consumption observed at 30 mg/kg bw/day seems to be consistent with the action of DNP as a metabolic activator. Higher relative weight, but not absolute weight, of the heart in females at 30 mg/kg bw/day is considered to be secondarily due to the lowered body weight on the day of scheduled sacrifice, not to the direct effects of DNP. In the present study, the increased relative kidney weights were observed in both sexes at 30 mg/kg bw/day. In our previous 28-day repeat dose toxicity study of DNP, renal mineralization at the corticomedullary junction was found in rats of both sexes given at 80 mg/kg bw/day (Koizumi et al., 2001). The renal damages were reported in humans took DNP (Beinhauer, 1934; Goldman and Haber, 1936; Simkins, 1937a,b). We concluded that the kidney is one of the target organs for DNP toxicity, and increased kidney weight might be due to the test substance treatment. Liver weights at 30 mg/kg bw/day increased regardless of the absolute and relative weights and sex in the present study. These data indicate that the NOAEL for the general toxicity of DNP is 10 mg/kg bw/day.

In the present study, atrophy of seminiferous tubules in the testis and slight change in the number of spermatogonia

TABLE II. Absolute and relative organ weights of male and female rats given DNP

		Dose (mg/kg bw/day)			
		0 (Control)	3	10	30
No. of males		12	12	12	12
Body weight	(g)	537.7 ± 39.1	526.2 ± 34.6	537.3 ± 50.9	502.9 ± 50.7
Liver	(g)	19.01 ± 2.06	18.62 ± 213	18.85 ± 2.57	19.96 ± 2.92
	(%)	3.53 ± 0.24	3.54 ± 0.25	3.50 ± 0.19	3.95 ± 0.23**
Kidneys	(g)	3.57 ± 0.53	3.74 ± 0.30	3.73 ± 0.43	3.78 ± 0.52
	(%)	0.66 ± 0.08	0.71 ± 0.05	0.70 ± 0.04	0.75 ± 0.05**
Heart	(g)	1.50 ± 0.15	1.44 ± 0.10	1.51 ± 0.17	1.44 ± 0.16
	(%)	0.28 ± 0.02	0.27 ± 0.02	0.28 ± 0.02	0.29 ± 0.01
Testes	(g)	3.34 ± 0.27	3.58 ± 0.28*	3.46 ± 0.14	3.29 ± 0.49
	(%)	0.62 ± 0.05	0.68 ± 0.05*	0.65 ± 0.06	0.66 ± 0.10
Epididymides	(g)	1.34 ± 0.13	1.43 ± 0.12	1.42 ± 0.07	1.27 ± 0.18
	(%)	0.25 ± 0.02	0.28 ± 0.02*	0.27 ± 0.03	0.25 ± 0.04
No. of females		12	11[a]	11[a]	11[a]
Body weight	(g)	351.3 ± 21.3	348.7 ± 15.3	345.8 ± 17.2	338.2 ± 19.0
Liver	(g)	14.84 ± 1.69	14.82 ± 1.10	14.54 ± 1.35	16.30 ± 1.21*
	(%)	4.22 ± 0.33	4.25 ± 0.24	4.21 ± 0.38	4.83 ± 0.30*
Kidneys	(g)	2.24 ± 0.20	2.25 ± 0.20	2.28 ± 0.24	2.39 ± 0.14
	(%)	0.64 ± 0.03	0.65 ± 0.05	0.66 ± 0.06	0.71 ± 0.05*
Heart	(g)	1.05 ± 0.09	1.07 ± 0.07	1.06 ± 0.08	1.09 ± 0.11
	(%)	0.30 ± 0.02	0.31 ± 0.02	0.31 ± 0.02	0.32 ± 0.03*
Ovaries	(mg)	116.5 ± 18.7	109.7 ± 13.3	110.7 ± 18.3	110.8 ± 12.5
	(10^{-3} %)	33.05 ± 4.02	31.58 ± 4.61	32.11 ± 5.70	32.77 ± 3.18

Weight values are the mean ± S.D.
* Significantly different from the control group, $p < 0.05$.
** Significantly different from the control group, $p < 0.01$.
[a] One female in each of the 3, 10, and 30 mg/kg bw/day groups did not become pregnant.

only in the limited stage were observed at 30 mg/kg bw/day. These changes are likely to be spontaneous, because the incidence of atrophy was very low, the atrophy was also observed in the control group, and no changes were detected in the numbers of Sertoli cells and germ cells per Sertoli cell. We previously noted that dinoseb, a dinitrophenol herbicide, caused a decrease in sperm motility, and an increase in the rates of sperm with abnormal tail and head following administration by gavage for 42 days at 7.0 mg/kg bw/day in rats (Matsumoto et al., 2007). Takahashi et al.

TABLE III. Reproductive findings in rats given DNP

	Dose (mg/kg bw/day)			
	0 (control)	3	10	30
No. of rats (male/female)	12/12	12/12	12/12	12/12
Length of estrous cycle (days)[a]	3.9 ± 0.3	4.0 ± 0.1	4.1 ± 0.3	4.0 ± 0.0
Copulation index (%)[b] male, female	100, 100	100, 100	100, 100	100, 100
Fertility index (%)[c]	100	92	92	92
Gestation index (%)[d]	100	100	100	100
Gestation length (days)[a]	22.7 ± 0.5	22.7 ± 0.5	22.7 ± 0.5	22.7 ± 0.5
Nursing index (%)[e]	100	100	100	100

[a] Values are the mean ± SD.
[b] Number of animals with successful copulation/number of animals mated × 100.
[c] Number of pregnant females/number of females with successful copulation × 100.
[d] Number of females with live pups/number of pregnant females × 100.
[e] Number of females with live pups on lactation day 4/number of females with live pups delivery × 100.

TABLE IV. Developmental findings in rats given DNP

	Dose (mg/kg bw/day)			
	0 (control)	3	10	30
No. of litters	12	11	11	11
No. of corpora lutea[a]	15.5 ± 1.7	16.8 ± 1.2	15.5 ± 2.8	16.3 ± 1.6
No. of implantation sites[a]	14.8 ± 1.5	16.6 ± 1.1*	14.7 ± 1.8	15.4 ± 1.3
Implantation index (%)[b]	95.9	99	95.6	94.8
Delivery index (%)[c]	95.8	92.9	94	91.1
No. of pups delivered[a]	14.3 ± 2.0	15.5 ± 1.6	13.9 ± 2.3	14.0 ± 1.3
PND 0				
No. of live pups[a]	14.3 ± 2.0	15.3 ± 1.8	13.6 ± 2.4	11.1 ± 3.2**
Sex ratio of live pups (male/female)	83/88	80/88	87/63	61/61
Live birth index (%)[d]	100	98.8	97.8	79.7**
PND 4				
No. of live pups[a]	14.1 ± 2.0	15.2 ± 1.7	13.5 ± 2.3	10.9 ± 3.2**
Viability index (%)[e]	98.8	99.5	98.7	98.4
Body weight of male pups (g)[a]				
PND 0	6.89 ± .067	6.91 ± 0.72	6.57 ± 0.62	6.09 ± 0.69*
PND 1	7.54 ± 0.78	7.63 ± 0.88	7.25 ± 0.79	6.61 ± 0.92*
PND 4	11.18 ± 1.21	10.86 ± 1.39	10.74 ± 1.23	9.87 ± 1.53
Body weight of female pups (g)[a]				
PND 0	6.49 ± 0.72	6.51 ± 0.66	6.23 ± 0.57	5.76 ± 0.73*
PND 1	7.09 ± 0.86	7.20 ± 0.83	6.94 ± 0.68	6.21 ± 0.99*
PND 4	10.54 ± 1.37	10.29 ± 1.38	10.18 ± 1.12	9.16 ± 1.64
Morphological examinations of pups on PND 4				
No. of pups (litters) examined	169 (12)	167 (11)	148 (11)	120 (11)
Dilatation of cerebral ventricle	1 (1)	0 (0)	0 (0)	0 (0)

PND, postnatal day.
* Significantly different from the control group, $p < 0.05$.
** Significantly different from the control group, $p < 0.01$.
[a] Values are the mean ± SD.
[b] Number of implantation sites/number of corpora lutea × 100.
[c] Number of live pups born/number of implantation sites × 100.
[d] Number of live pups on lactation day 0/number of pups born × 100.
[e] Number of live pups on lactation day 4/number of live pups on lactation day 0 × 100.

(2003, 2004) compared the testicular toxicity of dinitrophenolic compounds, dinoseb, 4,6-dinitro-*o*-cresol (DNOC) and DNP. In the *in vitro* rat Sertoli-germ cell coculture system, DNP decreased germ cell viability only at the highest concentration of 10^{-4} M (Takahashi et al., 2003). In rats given DNP by gavage at 30 mg/kg bw/day for 5 days, DNP caused a slight increase in the incidence of tailless sperm (Takahashi et al., 2004). The authors noted that the spermatotoxicity of DNP was very weak compared with that of dinoseb and DNOC; however, the mode of action of DNP toxicity closely resembled that of dinoseb and DNOC (Takahashi et al., 2004). It is suggested that the induction of sperm toxicity by dinitrophenolic compounds is involved in the uncoupling effect (Linder et al., 1982; Takahashi et al., 2004). The uncoupling action of DNP is weaker than that of dinoseb and DNOC in liver mitochondria *in vitro* and their toxicities tend to increase with increasing uncoupling potency (Ilivicky and Casida, 1969); therefore, it appears that a lack of sperm toxicity of DNP is due to the weak uncoupling potency of this compound.

With regard to reproductive parameters, no effects of DNP on estrous cyclicity, length of gestation, copulation, fertility and nursing indexes, and reproductive organ weights were observed. As for developmental parameters, decreases in the live birth index, and the numbers of live pups on PNDs 0 and 4, and body weights of live pups on PNDs 0 and 1 were detected at 30 mg/kg bw/day; however, there was no increased incidence of pups with malformations in DNP-treated groups. These findings indicate that DNP is toxic to the survival and growth of offspring during the pre- and postnatal periods, and has developmental toxicity, but not teratogenicity, at 30 mg/kg bw/day. In the present study, maternal adverse effects were observed during early lactation, as evidenced by decreased body weight gain at 30 mg/kg bw/day, and these phenomena might affect the survival and growth of offspring. Koizumi et al. (2001) noted that DNP directly gavaged to pups on PNDs 4-21 caused decreased body weight gain and death at 20 and 30 mg/kg bw/day, respectively, although the exposure levels of DNP to pups after direct administration is thought

to be much higher than to offspring after maternal administration. Consideration of these findings suggests that adverse effects on the survival and growth of offspring are due to a combination of direct effects of DNP and/or its metabolites and altered maternal physiology.

DNP produced dose-related hyperthermia resulted from the uncoupling of oxidative phosphorylation action (Tainter and Cutting, 1933; Pugh and Stone, 1968; ATSDR, 1995). Hyperthermia is known to be teratogenic and embryolethal in rats (Cockroft and New, 1978; Germain et al., 1985), and rectal temperature at 41.0°C, an elevation of 2.5°C, for 1 h was the threshold combination for teratogenic potential (Germain et al., 1985). In the present study, intrauterine death of offspring, as evidenced by a lowered live birth index, increased at 30 mg/kg bw/day, but no pups with malformations were found in DNP-treated groups. The possibility that elevation of body temperature participates in the developmental toxicity of DNP persists. Further studies are needed to clarify the relationship between increased body temperature and developmental toxicity of DNP.

In conclusion, DNP shows general and reproductive/developmental toxicity, but not teratogenicity, under the present study conditions. The NOAEL of DNP for general and reproductive/developmental toxicity was 10 mg/kg bw/day in rats.

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【特集】

OECD 化学物質対策の動向（第 15 報）

―第 25 回、第 26 回 OECD 高生産量化学物質初期評価会議

（2007 年ヘルシンキ、2008 年パリ）

Progress on OECD Chemicals Programme (15) - SIAM 25 in Helsinki, 2007 and SIAM 26 in Paris, 2008

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要旨：第 25 回 OECD 高生産量化学物質初期評価会議（SIAM 25）が 2007 年 10 月にフィンランド・ヘルシンキで開催され、日本が提出した 1 物質（1,3-ジ-o-トリルグアニジン：CAS 番号 97-39-2）の初期評価プロファイル（SIAP）について合意が得られた。また、SIAM 26 が 2008 年 4 月にフランス・パリで開催され、日本が提出した 2 物質（p-トルイル酸：CAS 番号 99-94-5、亜硫酸ナトリウム：CAS 番号 7757-83-7）の SIAP について合意が得られた。本稿では本会議で合意の得られたこれらの物質の初期評価文書について紹介する。

キーワード：OECD、HPV プログラム、SIDS 初期評価会議

Abstract: The 25th Screening Information Data Set (SIDS) Initial Assessment Meeting (SIAM 25) was held in Helsinki, hosted by Finland. The initial assessment documents of 1,3-di-*o*-tolylguanidine (CAS number: 97-39-2) were submitted by the Japanese Government without the collaboration with International Council of Chemical Associations (ICCA). SIAM 26 was held at the Organisation for Economic Co-operation and Development (OECD) headquarters in Paris, France. The initial assessment documents of two substances, *p*-toluic acid (CAS number: 99-94-5) and sodium sulfite (CAS number: 7757-83-7) were submitted by the Japanese Government with or without the collaboration with ICCA. All SIDS Initial Assessment Profiles (SIAPs) of the substances were agreed at the meetings. In this report, the documents of these substances are introduced.

Keywords: OECD, HPV programme, SIDS Initial Assessment Meeting

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1　はじめに

経済協力開発機構（Organisation for Economic Co-operation and Development : OECD）では、1992 年に始まった高生産量化学物質点検プログラム（High Production Volume Chemical (HPV) Programme）により、加盟各国における高生産量化学物質の安全性の評価を行っている（長谷川ら 1999a、江馬 2006）。日本政府は初回より評価文書を提出しており、2001 年からは国際化学工業協会協議会（International Council of Chemical Associations : ICCA）による評価文書の原案作成に伴い日本化学工業協会加盟企業も評価文書の原案作成に参加している。第 24 回までの初期評価会議（Screening Information Data Set (SIDS) Initial Assessment Meeting : SIAM）において日本政府が担当し結論および勧告が合意された化学物質の評価文書のヒトの健康影響または環境影響・曝露情報部分については既に紹介してきた（長谷川ら 1999b、2000、2001 ; 高橋ら 2004、2005a、2005b、2006a、2006b、2006c、2007a、2007b、2007c、2008）。また、第 19 回 SIAM（SIAM 19）から SIAM 27 の各会議内容、SIAM 1 から SIAM 18 までの会議の結果の概要についても紹介してきた（松本ら 2005a、2005b、2006a、2006b、2007a、2007b、2007c、2008a、2008b、2009）。

本稿では SIAM 25、SIAM 26 で合意に至った日本担当物質の評価文書の概要を紹介する。なお、OECD ガイドラインに則した毒性試験についてはガイドライン番号を示したが、遺伝毒性に関しては 1 物質に対して多種の試験が行われることもあり、結果のみ簡潔に示すこととした。

2　SIAM 25 および SIAM 26 で合意された日本担当物質の初期評価内容

我が国は、2007 年 10 月にヘルシンキ（フィンランド）で開催された SIAM 25 では 1 物質、2008 年 4 月にパリ（フランス）で開催された SIAM 26 では 2 物質の初期評価文書を提出し、それら全ての初期評価結果および勧告が合意された。

SIAM における合意は FW（The chemical is a candidate for further work.）または LP（The chemical is currently of low priority for further work.）として示されている。FW は「今後も追加の調査研究作業が必要である」、LP は「現状の使用状況においては追加作業の必要はない」ことを示す。

2 - 1　SIAM 25 について

（1）1,3-ジ-o-トリルグアニジン

英名 1,3-Di-*o*-tolylguanidine (97-39-2)（日本政府）

1）曝露状況

本物質は主にタイヤに用いる加硫促進剤として使用される。本物質は閉鎖系で製造・加工されるため、職業曝露の可能性は低い。本物質は加硫プロセスにおいて分解されるため、最終ゴム製品には含まれず、消費者曝露の可能性も低い。また、本物質に関して現在利用可能な測定データはないが、製造・加工過程における排水から環境中への排出量は、排水処理が行われているために少ないと考えられる。

2）環境影響

媒体別分配割合の予測（Mackay-Type Level III Fugacity Model による）の結果、本物質が大気に放出された場合は主に土壌（95.8%）と水圏（4.2%）に分布し、水圏に放出された場合は主に水圏（98.2%）に残留し、土壌に放出された場合は主に土壌（98.3%）に残留し、大気・土壌・水圏に放出された場合は主に土壌（86.5%）と水圏（13.3%）に分布する。本物質は容易に生分解しないが、水生生物における生物濃縮性は低い（BCF : 34 [計算値]）。

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水生生物に対する急性毒性について、魚類の半数致死濃度（LC_{50}）は 19 mg/L（96 時間、OECD TG 203）、ミジンコの LC_{50} は 7.2 mg/L（48 時間、OECD TG 202）、藻類の半数影響濃度（EC_{50}）は 8.9 mg/L（72 時間、生長速度法：OECD TG 201）であった。慢性毒性については、ミジンコの最大無影響濃度（NOEC）は 2.8 mg/L（21 日間、繁殖阻害：OECD TG 211）、藻類の NOEC は 2.3 mg/L（72 時間、生長速度法：OECD TG 201）であった。

3）健康影響

本物質は、ほ乳類中枢神経系における選択的シグマ受容体リガンドとして知られ、腹腔内、皮下、静脈内、または、中脳黒質内への注入により、低体温、疼痛行動の減少、回転性行動異常、自発運動の低下などの行動変化がマウスやラットで認められている。

ラットの単回経口投与毒性試験（OECD TG 401）での LD_{50} は雄で 85.3 mg/kg bw、雌では 56.0 mg/kg bw であった。

また、二次資料であり信頼性は評価できないが、本物質には皮膚に対する刺激性は無いが、眼に対する刺激性が認められたことが報告されている。

ラットに 0、7.5、15、30 または 60 mg/kg bw/day を強制経口投与した 28 日間反復経口投与毒性試験（OECD TG 407）において、投与期間中に 60 mg/kg bw/day の雄 1/12 例および雌 7/12 例の死亡が認められた。30 mg/kg bw/day 以上の雌雄に散瞳および流涎がみられ、60 mg/kg bw/day の雌雄に振戦、自発運動の低下、緩徐呼吸、体温低下および下腹部汚染が認められた。死亡例では腹臥位、側臥位およびあえぎ呼吸も認められた。60 mg/kg bw/day の雄で投与 8 日より 28 日まで、雌では投与 8 日と 15 日に体重の低値がみられた。60 mg/kg bw/day の雌雄で投与 2、8、15 および 28 日に摂餌量の低値がみられた。30 mg/kg bw/day 以上の雄に尿量の増加傾向、15 mg/kg bw/day 以上の雌に尿量の増加がみられ、この変化に伴って浸透圧および比重の低値が認められた。30 mg/kg bw/day 以上の雄に APTT の短縮が認められた。60 mg/kg bw/day の雌雄に血中総蛋白質の低値、GPT 活性およびカリウムの高値が認められ、さらに、雄ではアルブミンの低値、アルカリフォスファターゼおよび尿素窒素の高値が、雌では GOT 活性およびナトリウムの低値、トリグリセライドの高値が認められた。また、30 mg/kg bw/day 以上の雌に総コレステロールおよびリン脂質の高値が認められた。30 mg/kg bw/day 以上で雌に肝臓の相対重量の高値が認められた。死亡した 60 mg/kg bw/day の雌 1 例に肉眼的に腺胃粘膜の単発性の淡赤色点、組織学的に腺胃に軽度のびらんが認められた。生存例では、60 mg/kg bw/day で雌 1 例に肝細胞肥大が認められたが、同群で投与期間中に死亡が発生したことにより回復群が設けられなかったことから、回復性は確認できなかった。その他の変化はいずれも 14 日間の休薬により回復しており、回復性は良好であった。30 mg/kg bw/day でみられた散瞳、流涎、総コレステロールおよびリン脂質の高値から、**反復投与毒性の NOAEL** は雌雄ともに **15 mg/kg bw/day** とされた。

雌雄ラットに交配前 2 週間から交配期間を含め、雄では 49 日間、雌では分娩後哺育 4 日まで（40〜49 日間）、0、8、20 または 50 mg/kg bw/day を強制経口投与した経口投与簡易生殖毒性試験（OECD TG 421）では、投与期間中に 50 mg/kg bw/day の雄 2/12 例および雌 3/12 例の死亡が認められた。生存例では、20 mg/kg bw/day 以上の雌雄で流涎、20 mg/kg bw/day 以上の雌および 50 mg/kg bw/day の雄で散瞳、自発運動の低下、緩徐呼吸、腹臥位および振戦が認められた。さらに、50 mg/kg bw/day の雌雄では体重増加量の減少と摂餌量の低値がみられ、20 mg/kg bw/day の雌においても摂餌量の低値が認められた。剖検、器官重量および病理組織学検査では本物質投与の影響は認められなかった。親動物の生殖機能に関しては、性周期、黄体数、着床痕数、着床率、交尾率、受胎率および交尾所要日数に本物質投与の影響は認められなかった。また、50 mg/kg bw/day で産児数、出産生児数、出生率および雌雄生児の生後 0 日の体重の低値、外表異常出現率の高値、新生児の生後 4 日の生存率の低値が認められた。妊娠期間、死産率、出産率、生児の性比および型別外表異常出現率については本物質投与の影響は

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認められなかった。これらのことから、反復投与毒性の NOAEL は雌雄ともに 8 mg/kg bw/day、生殖発生毒性の NOAEL は親動物の生殖能力に関しては 50 mg/kg bw/day、児の発生・発育に関しては 20 mg/kg bw/day とされた。

妊娠 6-19 日の妊娠ラットに 0、10、20 または 40 mg/kg bw/day を強制経口投与した出生前発生毒性試験（OECD TG 414）では、投与期間中に 40 mg/kg bw/day の雌 4/24 例の死亡が認められた。20 mg/kg bw/day 以上で散瞳、40 mg/kg bw/day では自発運動の低下、脱毛、緩徐呼吸、腹臥位および振戦も認められた。20 mg/kg bw/day 以上で母動物の体重増加量の減少、40 mg/kg bw/day では摂餌量の低値が認められた。母体毒性の NOAEL は 10 mg/kg bw/day とされた。また、40 mg/kg bw/day で妊娠子宮重量の低値、着床後胚死亡の増加、生存胎児数の減少、胎児体重および胎盤重量の低値が認められた。生存胎児の検査では、40 mg/kg bw/day で外表異常の出現率増加、20 mg/kg bw/day 以上で骨格異常の出現率増加が認められた。特に 40 mg/kg bw/day では、短指、短尾、尾椎・指節骨・中手骨の異常の出現率増加が認められ、骨化遅延もみられた。これらのことから、本物質は母体に毒性影響を及ぼす用量において発生毒性を示すことが明らかとなった。発生毒性の NOAEL は 10 mg/kg bw/day とされた。

細菌を用いる復帰突然変異試験は陰性であり、チャイニーズ・ハムスター培養細胞を用いる染色体異常試験において S9mix 非存在下では陰性であったが、S9mix 存在下では陽性であった。また、*in vivo* 小核試験は陰性であった。これらのことから、本物質は *in vivo* において遺伝毒性を示さないとされた。

4）結論と勧告

本物質は健康に対して有害性（急性経口毒性、反復投与毒性、発生毒性）を示すが、現況においては人体への曝露量が少ないので、健康影響について LP と勧告された。また、環境に対しても有害性（魚類・ミジンコ・藻類への急性毒性：1～100 mg/L）を示すが、現況においては環境への曝露量が少ないので、環境影響について LP と勧告された。

2 - 2　SIAM 26 について

（1）p-トルイル酸

英名 *p*-Toluic acid (99-94-5)（日本政府）

1）曝露状況

本物質は感光色素、蛍光染料、染料の中間体として使用されている。本物質は閉鎖系で製造・加工されるため、職業曝露の可能性は低い。また、本物質は中間体であるので、消費者曝露の可能性も低い。

2）環境影響

媒体別分配割合の予測（Mackay-Type Level III Fugacity Model による）の結果、本物質が大気・土壌・水圏に放出された場合は主に土壌（69.8%）と水圏（28.5%）に分布し、残りは大気（1.6%）に分布する。本物質は容易に生分解し、水生生物における生物濃縮性も低い（BCF：3.16 [計算値]）。

水生生物に対する急性毒性について、魚類の LC_{50} は 64 mg/L（96 時間、OECD TG 203）、ミジンコの EC_{50} は 42 mg/L（48 時間遊泳阻害、OECD TG 202）、藻類の EC_{50} は 74 mg/L（72 時間、生長速度法：OECD TG 201）であった。慢性毒性については、ミジンコの NOEC は 3.2 mg/L（21 日間、繁殖阻害：OECD TG 211）、藻類の NOEC は 46 mg/L（72 時間、生長速度法：OECD TG 201）であった。

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3）健康影響

単回経口投与での LD_{50} はマウスの雄で 2,340 mg/kg bw、雌では 2,484 mg/kg bw、また、ラットの雄で 3,113 mg/kg bw、雌では 2,115 mg/kg bw であった。

刺激性に関する利用可能なデータはないが、本物質は酸性であることから皮膚や眼に対して刺激性を持つことが考えられる。本物質はヒトに皮膚感作性を示した。また、異性体（パラ、メタ、オルト）間で交差感作性が認められた。

ラットに 0、100、300 または 1,000 mg/kg bw/day を強制経口投与した 28 日間反復経口投与毒性試験（OECD TG 407）において、死亡例はみられなかった。1,000 mg/kg bw/day において、雌雄で一過性の流涎、尿検査値の変化が認められ、雌では摂餌量の高値が認められたが、これらの変化は本物質の局所刺激性により引き起こされた変化であり、本物質の全身毒性による影響ではないと考えられた。1,000 mg/kg bw/day で雌に血小板数の減少傾向、血中総蛋白質の低値、GOT 活性の高値が認められた。これらのことから、反復投与毒性の NOAEL は雄で 1,000 mg/kg bw/day、雌で 300 mg/kg bw/day とされた。

雌雄ラットに交配前 2 週間から交配期間を含め、雄では 42 日間、雌では分娩後 4 日まで、0、100、300 または 1,000 mg/kg bw/day を強制経口投与した経口投与簡易生殖毒性試験（OECD TG 421）では、300 mg/kg bw/day 以上で雌に体重増加量の減少が認められた。1,000 mg/kg bw/day で精巣上体尾部に精子の少ない管腔の増加が認められ、管腔内の細胞残屑がわずかに増加した例もみられた。また、生殖発生毒性については、1,000 mg/kg bw/day で受胎率が低下し、300 mg/kg bw/day 以上で着床率の低下、産児数の減少が認められた。さらに、1,000 mg/kg bw/day では産児数の減少に伴う出産生児数および哺育 4 日における生児数の減少が認められた。これらのことから、反復投与毒性の NOAEL は雄で 300 mg/kg bw/day、雌で 100 mg/kg bw/day、生殖毒性の NOAEL は 100 mg/kg bw/day とされた。

細菌を用いる復帰突然変異試験では陰性、チャイニーズ・ハムスター培養細胞を用いる染色体異常試験では陽性であった。また、*in vivo* 小核試験は陰性であった。これらのことから、本物質は *in vivo* において遺伝毒性を示さないとされた。

4）結論と勧告

本物質は健康に対して有害性（感作性、反復投与毒性、生殖毒性）を示すが、現況においては人体への曝露量が少ないので、健康影響について LP と勧告された。また、環境に対しても有害性（魚類・ミジンコ・藻類への急性毒性：1～100 mg/L）を示すが、本物質は容易に生分解し、生物濃縮性も低いので、環境影響について LP と勧告された。

（2）亜硫酸ナトリウム

英名 Sodium sulfite (7757-83-7)（原案作成：ICCA 日本企業）

必要に応じて、亜硫酸ナトリウム（Na_2SO_3）以外の硫黄(IV)（以下、S(IV)とする）化合物（亜硫酸水素塩、亜硫酸塩、二亜硫酸塩、二酸化硫黄）のデータが利用された。また、二亜硫酸ナトリウム(7681-57-4)についてはすでに HPV プログラムで評価されている。

1）曝露状況

本物質は、化学工業、皮革加工工業、写真工業、高分子工業、紙パルプ工業において使用され、毛髪染料や食品添加物としても使用されている。本物質を含む粉塵による職業曝露が考えられ、作業者は保護具着用が推奨される。亜硫酸塩は、含硫アミノ酸の代謝物や中間体として、汚染大気中の SO_2 を吸入した際の代謝物として、そして、食品添加物である亜硫酸塩剤の摂取から、体内に存在している。WHO（世界保健機関）による亜硫酸塩全体としての一日摂取許容量は 0.7 mg/kg bw（SO_2 換算）である。

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2）環境影響

本物質は、水溶液中で完全にイオンに解離するため、水圏からの揮発や土壌/沈殿物への吸着を生じにくい。亜硫酸塩は速やかに酸化して硫酸塩となり、その際に水中の酸素を消費するため、水生生物への影響評価においては酸素欠乏の影響も検討する必要がある。

水生生物に対する急性毒性について、被験物質に亜硫酸カリウム（K_2SO_3）を用いた魚類の LC_{50} は亜硫酸ナトリウム換算で 170～370 mg/L（96 時間、通気あり）、二亜硫酸ナトリウム（$Na_2S_2O_5$）を用いたミジンコの EC_{50} は亜硫酸ナトリウム換算で 118 mg/L（48 時間）、亜硫酸ナトリウムを用いた 3 種の藻類の EC_{50} は 63～126 mg/L（96 時間）であった。慢性毒性については、二亜硫酸ナトリウム（$Na_2S_2O_5$）を用いたミジンコの NOEC は亜硫酸ナトリウム換算で 13 mg/L（21 日間、繁殖阻害）であった。また、被験物質として亜硫酸水素ナトリウム（$NaHSO_3$）を 18 種の藻類に一定濃度 12.6 mg/L（亜硫酸ナトリウム換算）で 24 時間曝露すると光合成を 0～33%抑制した。

3）健康影響

本物質は胃腸管から速やかに吸収される。本物質の主な代謝物は硫酸塩であり、多くの組織で亜硫酸酸化酵素により形成される。亜硫酸塩に由来する硫黄の組織への蓄積は、胃、皮膚、毛髪、腸、腎臓で高い。本物質は代謝後、主に尿中に速やかに排泄される。

単回経口投与の LD_{50} はラットで 3,560 mg/kg bw 以上、マウスで 820～920 mg/kg bw であった。エアロゾルでの急性吸入投与では、モルモットに気管支収縮を引き起こし、最小毒性濃度（LOAEC）は 0.204 mg/m^3 であった。

本物質には眼や皮膚に対する刺激性は認められなかった（OECD TG 404 および 405）。

ヒトにおいて、クリーム剤を局所的に塗布した際に酸化防止剤として含まれた本物質により皮膚炎を示したケースがある。また、アトピー性皮膚炎患者 1,762 人のうち 1.4%が本物質のパッチテストで陽性を示した。また、S(IV)化合物の吸入または経口摂取により過敏症を示したケースがあった。本物質は食品添加物として幅広く使われていることから、これらの反応は感受性の高い個体に限定的にあらわれたと考えられる。

290 日間、イヌに S(IV)のエアロゾル 0.3 mg/m^3 を全身曝露した試験では、肺機能にはわずかな変化（肺コンプライアンスや拡散能のわずかな減少）しか認められなかったが、呼吸気道で細菌の防御機能障害、鼻腔における増殖性/炎症性変化、喉頭/気管/肺胞における繊毛細胞の成長阻害が認められたことから、エアロゾルでの反復吸入投与毒性の LOAEC は 0.3 mg S(IV)/m^3（亜硫酸ナトリウム 1.2 mg/m^3 相当）とされた。

90 日間、ラットに 0、1、2 または 4%の亜硫酸ナトリウム（雄で 0、620、1,670 または 3,230 mg/kg bw/day、雌で 0、650、1,190 または 3,070 mg/kg bw/day）を混餌投与した試験において、4%で雄に体重増加量の減少、精巣と脳の相対重量の増加が認められ、反復経口投与毒性の NOAEL は雄で 2%（1,670 mg/kg bw/day 相当）、雌で 4%（3,070 mg/kg bw/day 相当）とされた。また、精巣については絶対重量と組織に影響はみられず、雄の生殖能力への影響は認められなかった。

2 年間、3 世代の雌雄ラットに 0、0.125、0.25、0.5、1.0 または 2.0%の二亜硫酸ナトリウム（$Na_2S_2O_5$）を混餌投与した試験において、1.0%（亜硫酸ナトリウム 300mg/kg bw/day 相当）以上で前胃と腺胃の過形成/炎症が認められ、慢性毒性の NOAEL は 0.5%（亜硫酸ナトリウム 144 mg/kg bw/day 相当）とされた。また、最高用量までどの世代にも生殖能力の低下や生殖器官の組織学的変化は認められず、生殖毒性の NOAEL は 2.0%（亜硫酸ナトリウム 625 mg/kg bw/day 相当）とされた。

妊娠 8-20 日の間、0、0.32、0.63、1.25、2.5 または 5%の亜硫酸ナトリウム七水和物（$Na_2SO_3 \cdot 7H_2O$）を妊娠ラットに混餌投与した試験において、5%で母体に体重増加量の減少が認められ、

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母体毒性の NOAEL は 2.5%（亜硫酸ナトリウム 1,050 mg/kg bw/day 相当）とされた。また、最高用量の 5%でも催奇形性は認められず、催奇形性の NOAEL は 5%（亜硫酸ナトリウム 1,650 mg/kg bw/day 相当）とされた。

亜硫酸ナトリウムは、細菌を用いる復帰突然変異試験、S9mix 非存在下で行われたチャイニーズ・ハムスター培養細胞を用いる染色体異常試験、その他の *in vitro* 試験において陰性であった。また、本物質の *in vivo* 試験は行われていないが、本物質以外の亜硫酸塩での *in vivo* 試験の結果を考慮して、*in vivo* において本物質の遺伝毒性はないとされた。

長期間、ラットやマウスに二亜硫酸ナトリウム（$Na_2S_2O_5$）を混餌/飲水投与した試験において発ガン作用は認められなかったので、亜硫酸ナトリウムが発ガン性を示す可能性も低いとされた。

4）結論と勧告

本物質は健康に対して有害性（気道反応、皮膚感作性）を示し、また、曝露の可能性を否定できないので、健康影響については FW と勧告され、職業曝露量及び消費者曝露量に関する調査が推奨された。また、環境に対しては有害性（藻類への急性毒性：1～100 mg/L）を示すが、容易に生分解し、魚類における濃縮性も低いので、環境影響については LP と勧告された。

3　おわりに

本稿では、SIAM 25 及び SIAM 26 で合意された日本担当物質の初期評価文書について紹介した。SIAM で SIAP を合意された物質の初期評価文書はインターネットの OECD web サイト（http://cs3-hq.oecd.org/scripts/hpv/）で入手が可能である。電子出版までの手順については、江馬（2006）に記載されている。

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Terminology of developmental abnormalities in common laboratory mammals (version 2)☆,☆☆

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ABSTRACT

This update (version 2) of the *Terminology of developmental abnormalities in common laboratory mammals (version 1)* by Wise et al. [Wise LD, Beck SL, Beltrame D, Beyer BK, Chahoud I, Clark RL, Clark R, Druga AM, Fueston MH, Guittin P, Henwood SM, Kimmel CA, Lindstrom P, Palmer AK, Petrere JA, Solomon HM, Yasuda M, York RG. Terminology of developmental abnormalities in common laboratory mammals (version 1). Teratology 1997;55:249–92] incorporates improvements and enhancements to both content and organization of the terminology, to enable greater flexibility in its application, while maintaining a consistent approach to the description of findings. The revisions are the result of an international collaboration among interested organizations, advised by individual experts and the outcomes of several workshops. The terminology remains organized into tables under the broad categories of external, visceral, and skeletal observations, following the manner in which data are typically collected and recorded in developmental toxicity studies. This arrangement of the tables, as well as other information provided in appendices, is intended to facilitate the process of specimen evaluation at the laboratory bench level. Only the commonly used laboratory mammals (i.e., rats, mice, rabbits) are addressed in the current terminology tables. The inclusion of other species that are used in developmental toxicity testing, such as primates, is considered outside the scope of the present update. Similarly, categorization of findings as,

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for example, "malformation" or "variation" remains unaddressed, in accordance with the overall principle that the focus of this document is descriptive terminology and not diagnosis/interpretation. The skeletal terms have been augmented to accommodate cartilage findings.

1. Introduction

This publication is the first update (i.e., "Version 2") to the *Terminology of developmental abnormalities in common laboratory mammals (Version 1)* by Wise et al. [1]. It builds upon past efforts to assemble an internationally harmonized source of common nomenclature for use in describing observations of fetal and neonatal morphology. Improvements and enhancements to the content and organization of the Version 1 terminology are provided, to enable a greater degree of flexibility in its application, while maintaining a consistent approach to the description of findings. The terminology should be of particular usefulness for submissions of developmental toxicity data to regulatory agencies, while also having broader applicability in research.

Historically, Version 1 was compiled under the auspices of the International Federation of Teratology Societies (IFTS), which included member groups from North America, Europe, and Asia. It was based upon a glossary previously published by the Middle Atlantic Reproduction and Teratology Association (MARTA) [2]. Additional input was provided by the Midwest Teratology Association (MTA) and the IFTS International Committee on Nomenclature (which included the Italian Nomenclature Working Group, the U.K. Foetal Pathology Terminology Group, and the French Teratology Association Nomenclature Working Group). Following the publication of Wise et al. [1], a Japanese translation of the terminology paper was also published [3].

Over time, the laboratories and regulatory agencies have gained practical experience in the application and interpretation of the internationally harmonized terminology presented in Version 1. Additionally, several international terminology workshops were held in Berlin from 1998 to 2007, some of which have been summarized in the published literature [4–6]. An image-based atlas that can serve as an illustrative resource for the harmonized terminology has also been compiled [e.g., 7].

The present Version 2 document represents a broad international collaboration among experts in the field of fetal and neonatal developmental toxicology.

2. Organization of Version 2

Most of the principles outlined in the Version 1 glossary [1] were adhered to in Version 2. These include the following:

- Terms describe morphological changes observed grossly or with the aid of a dissecting microscope.
- Only the terminology for common laboratory mammals (i.e., rats, mice, rabbits) is included. While it is recognized that other species (e.g., primates, guinea pigs) may be used in developmental assessments, their inclusion was considered outside the scope of the current update.
- The term "abnormalities" is used to denote changes in the specimen under examination relative to the perceived "norm" of control specimens for a particular species and developmental stage. It is recognized that this norm could change over time, and that, therefore, careful on-going monitoring of historical control data within each laboratory will be necessary to define the current standards.
- The glossary includes terms that describe observations in both fetal and neonatal animals.

Purely descriptive terms are preferentially used; Version 1 specified that diagnostic terms or terms that may imply a mechanism are generally not preferred unless the mechanism of the abnormality is known. This concept has been more rigorously applied in Version 2 than in Version 1, resulting in the relegation of what is often thought of as commonly used medical terminology to a "synonym or *related term*" status. As an illustration of this concept, the term "small" would be preferred to "hypoplasia" (the latter being defined as incomplete or underdevelopment of an organ or tissue).

Also in general accordance with the principle of using descriptive rather than diagnostic terms, findings are not categorized as, for example, "malformations" or "variations". The definition and use of such categorizations are left to the discretion of the individual laboratories.

3. External, visceral, skeletal, and maternal–fetal tables

The glossary remains organized into broad categories of external, visceral, and skeletal observations, following the manner in which fetal data are typically collected and recorded in developmental toxicity studies. In addition, a table of maternal–fetal observations has been included. It is recognized that some observations could justifiably be placed in more than one of these categories. To obviate unnecessary duplication, each observation appears in only one category in the current presentation. Within each table, the findings are organized into regions, structures, or organs in a generally cranial to caudal sequence. Observations within each section are then listed alphabetically; for the skeletal observations, they are also listed alphabetically within subsections of Structural (S) or Ossification (O) findings. Overall, the organization of the tables, as well as other information provided as appendices are intended to facilitate the process of specimen evaluation at the laboratory bench level.

Each table is divided into columns designating the general "Region/Organ/Structure", then into "Observations," "Synonyms or *Related Terms*" (where related terms are identified by the use of italicized text), "Non-preferred Terms," "Definitions," and "*Notes*" (where notes are presented in italicized text). In the Version 2 tables, the Observations are expanded into two columns. This allows organization of the terminology for a specific Region/Organ/Structure into (1) specific substructures or locations and (2) associated findings or anomalies, thereby allowing for more precision in characterization.

Although Version 1 provided a numerical code number for each observation, with the intention that these codes would provide a means of tracking future revisions to specific terms, this concept has not been pursued in Version 2. This decision was made because it became apparent that (1) the numerical code system would be complicated to maintain, owing to the large number of new observation terms added to the glossary, and (2) it did not appear to provide substantive overall value for most laboratory users. The code numbers have therefore been relegated to a less significant position in the tables. They have not been completely abandoned, to allow for the possibility that some laboratories have already organized their observation recording systems according to the Version 1 Code Numbers. However, the reorganization and synthesis of terminology that has occurred in Version 2 has inevitably resulted in the attrition of some Version 1 code numbers. For example, the Version 1 skeletal term "Skull, General, Extra ossification site" (code