Although degeneration and hypertrophy of the myocardium or cell infiltration in the heart were observed at 2.5 mg/kg and above in the previous 28-day study (Hirata-Koizumi et al., 2007), such changes were not detected even at the highest dose of 12.5 mg/kg in the present study. Considering that histopathological changes in the heart were also not found in the previous 52-week study of HDBB using young rats (Hirata-Koizumi et al., 2008a) and a 28-day study using young castrated rats (Hirata-Koizumi et al., 2008b), it could not be concluded that preweaning rats were less susceptible to the cardiac effects of HDBB than young rats. In order to investigate the toxicological effects of HDBB on the heart in more detail, the effects on cardiac function (e.g., electrocardiographic parameters, blood pressure, etc.) should be evaluated because they are considered to be more susceptible parameters than histopathology of the heart (Glaister, 1992).

In the present study, some blood biochemical parameters increased in both sexes in the 12.5 mg/kg group. The degree of change was mostly slight, but it was considered to be HDBB related because similar changes were found in previous studies of HDBB (Hirata-Koizumi et al., 2007, 2008a, 2008b). A simultaneous increase in hepatic enzymes (AST, ALP, and LDH) might result from hepatic damage caused by HDBB. Increased BUN suggests renal effects of HDBB, although histopathology of the kidneys was not examined in the present study. As a matter of fact, hypertrophy of the tubular epithelium was noted at 12.5 mg/kg and above in males and at 62.5 mg/kg in females in the previous 28-day study of HDBB using young rats (Hirata-Koizumi et al., 2007).

No effects on the lungs, spleen, and adrenals were found both in previous 28-day and 52-week studies of HDBB using young rats (Hirata-Koizumi et al., 2007, 2008a), whereas decreased weight of these organs was found in preweaning rats given HDBB. In rats, many organs develop rapidly during the early period after birth (Vidair, 2004; Walthall et al., 2005; Zoetis and Hurtt, 2005a). For example, rat lungs have no alveoli at birth, but they develop rapidly, with most lung development complete within the first two weeks after birth (Zoetis and Hurtt, 2005b). It is conceivable that immature and/or rapidly developing organs show different susceptibility from mature organs. Considering these findings together suggests that HDBB might influence these organs, specifically in the preweaning period. Further studies are required to investigate the adverse effects of HDBB on the lungs, spleen, and adrenals during the preweaning period.

Histopathological changes in the liver detected in the current study included nucleolar enlargement, anisokaryosis, increased mitosis, and hypertrophy of hepatocytes. Nucleolar enlargement of hepatocytes indicates the enhancement of protein synthesis and is identified most frequently in hepatocytes that are undergoing rapid cell proliferation (Cattley and Popp, 2002). Anisokaryosis is also considered to correlate at least partly with cell

proliferation. In the present study, nucleolar enlargement, anisokaryosis, and increased mitosis of hepatocytes were observed at 2.5 mg/kg and above in both sexes, whereas hypertrophy of hepatocytes was observed only at the highest dose of 12.5 mg/kg. On the other hand, in the previous 28-day study of HDBB using young rats, hypertrophy of hepatocytes was observed at 0.5 mg/kg and above in males and 12.5 mg/kg and above in females, and increased mitosis of hepatocytes was observed at 62.5 mg/kg and 12.5 mg/kg and above in males and females, respectively, indicating that young rats are more susceptible to the HDBB-induced hypertrophic response of hepatocytes than the mitotic response (Hirata-Koizumi et al., 2007). The higher susceptibility of preweaning rats to such proliferative changes might be associated with dramatic changes of the liver structure during the preweaning period (Alexander et al., 1997).

In previous studies using young rats (five to six weeks of age), we showed that male rats were much more susceptible to the toxic effects of HDBB than females (Hirata-Koizumi et al., 2007, 2008a). Based on histopathological findings in the liver, which is a major target of HDBB toxicity, differences in susceptibility between sexes was approximately 25 times. Subsequently, we showed that castration markedly reduced the gender-related differences in HDBB hepatotoxicity in rats (Hirata-Koizumi et al., 2008b). Comparing the histopathological findings of the liver observed in the previous 28-day studies using young intact and castrated rats, it became clear that the castration of male rats exerted no effect but that of female rats enhanced the adverse effects of HDBB on the liver, suggesting suppressive effects of estrogen on the hepatotoxicity of HDBB in rats. Despite the marked reduction of gender-related differences in the toxic effects of HDBB by castration, a difference, less than five times, remained in castrated rats. The sexual differences in castrated rats are considered to be due to the exposure to sexual hormones before four weeks of age, when castration was conducted. In the present study, following the administration of HDBB during the preweaning period, similar changes in all examined parameters were observed at the same doses in both sexes. These findings clearly show no gender-related differences in HDBB toxicity in preweaning rats, suggesting that a development at around three to six weeks of age contributes to sexual variations in HDBB toxicity, at least in part.

Gender-related differences in HDBB toxicity were found not only for hepatotoxicity, but also for the reduction of body weight, hematotoxicity, cardiac toxicity, etc., in the previous 28-day and/or 52-week studies using young rats (Hirata-Koizumi et al., 2007, 2008a). Thus, they might be caused by differences in the blood concentration of causative substances (e.g., HDBB or its metabolites) between sexes. A number of reports have been published on the sexual variations in toxicokinetic determinants, such as hepatic metabolism (Gad, 2006) and membrane transporter in various organs, including the kidneys and intestine (Morris et al., 2003). Coleman et al. (1990) reported that

higher sensitivity of male rats to hematotoxicity of dapsone, which is a major component of the multidrug regimen for the treatment of leprosy, was due to the greater capacity for the N-hydroxylation. Another example was an amino acid antitumor agent, acivicin, of which the LD_{50} was much higher in male mice than that in females. McGovren et al. (1981) showed that the plasma half-time was much longer in female mice and speculated that the sexual variation may be related to differences in the renal excretion.

For gender-related differences in toxicokinetic determinants, many mechanistic studies have been reported on the metabolic enzyme cytochrome P450 (CYP) (Waxman and Chang, 2005). In rats, a subset of CYPs is expressed in a sex-dependent fashion. It was reported that ovariectomy reduced the hepatic expression of female-specific/predominant CYPs, but this did not lead to the expression of male-specific CYP enzyme in female rats. If female-specific/predominant metabolic enzymes have an intimate involvement in the detoxication of HDBB, our previous results, showing the higher susceptibility of male young rats to HDBB toxicity than females, and increased susceptibility by castration of females, could be explained. Interestingly, in rat liver, the difference in CYP expression between sexes is not apparent until puberty (Waxman and Chang, 2005). This is consistent with our present results that there was no gender-related difference in HDBB hepatotoxicity in preweaning rats. Mode and Gustafsson (2006) reported that brain centers involved in the hypothalamo-pituitary control of hepatic sex-dependent metabolism in adults are irreversibly programmed by neonatal androgen exposure, which might explain why sexual variation in HDBB toxicity was not completely abolished by castration at four weeks of age.

In order to clarify the cause of gender differences, we are currently performing a toxicokinetic study of HDBB, which includes the identification of metabolites and the related metabolic enzyme as well as measurement of the blood concentration of HDBB both after single and repeated administration of HDBB to young and preweaning rats.

CONCLUSION

The current results showed that oral administration of HDBB to preweaning rats caused hepatotoxicity at 2.5 mg/kg and above in both sexes. The gender-related difference in toxic susceptibility to HDBB, which was observed in young rats, was not detected in preweaning rats.

ACKNOWLEDGMENTS

This study was supported by the Ministry of Health, Labour and Welfare, Tokyo, Japan.

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

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Received 7 December 2007; revised 13 March 2008; accepted 24 March 2008

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 00: 000–000, 2008.

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.

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Contract grant sponsor: Ministry of Health, Labor and Welfare, Japan

Published online in Wiley InterScience (www.interscience.wiley.com).
DOI 10.1002/tox.20398

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

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 高生産量化学物質点検プログラム：第 26 回初期評価会議概要

OECD High Production Volume Chemicals Programme: Summary of 26th SIDS Initial Assessment Meeting

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要旨：第 26 回の OECD 高生産量化学物質初期評価会議が、2008 年 4 月 16-18 日にフランスのパリで開催された。この会議では計 24 物質の初期評価文書について審議され、12 物質の初期リスク評価結果および評価結果に基づく措置に関する勧告が合意された。日本は、政府が原案を作成した Benzoic acid, 4-methyl-（CAS:99-94-5）および国際化学工業協会協議会（ICCA）が原案作成した Sodium sulfite（CAS:7757-83-7）の初期評価文書を提出し合意が得られた。本稿では、第 26 回初期評価会議の討議内容の概要を報告する。

キーワード：経済協力開発機構、高生産量化学物質、SIDS 初期評価会議、リスク評価

Abstract: The 26th SIDS (Screening Information Data Set) Initial Assessment Meeting was held in Paris, France on 16th-18th April 2008. The initial assessment documents of 24 substances were discussed, and the results of initial assessment and the recommendation for 12 substances were approved at the meeting. The Japanese Government submitted the initial assessment documents for two substances, benzoic acid, 4-methyl-（CAS: 99-94-5）prepared by the Japanese Government and sodium sulfite（CAS: 7757-83-7） prepared by International Council of Chemical Association (ICCA), and both documents were approved at the meeting. This paper reports the summary of the 26th SIDS Initial Assessment Meeting.

Keywords: OECD, HPV, SIDS Initial Assessment Meeting, Risk Assessment

化学生物総合管理　第 4 巻第 2 号 (2008.12)　237-245 頁
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受付日：2008 年 6 月 16 日　　受理日：2008 年 8 月 21 日

はじめに

経済協力開発機構（OECD: Organisation for Economic Co-operation and Development）では、高生産量化学物質「（少なくとも加盟国の 1 ヶ国において年間 1,000 トンを超えて生産または輸入されている化学物質(HPV: High Production Volume Chemical）」に対し加盟各国の分担により、初期リスク情報を収集・評価する HPV 点検プログラムを行っている。加盟各国は企業と協力しつつ、それぞれ担当する化学物質のリスクの初期評価に必要なスクリーニング情報データセット（SIDS: Screening Information Data Set）の項目の情報収集や試験を行い、初期評価文書として、初期評価プロファイル（SIAP: SIDS Initial Assessment Profile）、初期評価レポート（SIAR: SIDS Initial Assessment Report）および網羅的資料集（Dossier: SIDS Dossier）の 3 文書を作成し、初期評価会議（SIAM: SIDS Initial Assessment Meeting）に提出して審議を受けている。このプログラムは、1990 年の理事会決定に基づき、化学物質による有害な作用からヒトおよび環境を保護するとともに、各国の化学物質規制の体制整備・国際協調の場を提供する環境保健安全プログラムの一環として行なわれている。OECD の化学物質対策における HPV 点検プログラムの位置づけ、今までの成果および初期評価文書作成方法などの詳細は江馬（2006）が報告している。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初期評価文書については、高橋他（2006a, b, c; 2007a, b, c）が報告している。また、第 1 から第 18 回までの SIAM の概要については松本他（2006）を参照されたい。

1993 年の第 1 回 SIAM から 2000 年 3 月の第 10 回 SIAM までは、加盟国政府が提案国となり審議を行ってきたが、1998 年秋に国際化学工業協会協議会（ICCA: International Council of Chemical Association）が HPV 点検プログラムへの参加を表明し、第 11 回 SIAM (2001 年)から産業界が ICCA イニシアティブとして初期評価文書の作成に協力している。これらの ICCA イニシアティブの初期評価文書は、担当国政府を通じて提出されている。しかし、2005 年 12 月に行われた第 14 回既存化学物質タスクフォース（既存化学物質政策についての方針決定機関）は、スポンサー国（初期評価書文書原案作成を担当する単独または複数の国）が決まらない物質について、産業界が直接初期評価文書を提出することに合意した。

第 26 回 SIAM は 2008 年 4 月 15 日-18 日にフランスのパリで開催され、加盟国から 35 名、産業界から 28 名の約 60 名が参加し、24 物質の初期評価文書についての審議が行われた。日本からは、政府専門家(3 名)、オブザーバー（1 名）および産業界(2 名)が出席した。本稿では第 26 回 SIAM での討議内容として、第 25 回 SIAM 以降の HPV 点検プログラムの進捗状況、初期評価文書の審議結果および本プログラムの全般的な懸案事項に関する討議内容について報告する。なお、本稿は第 26 回 SIAM の会議報告書（OECD 2008a）を参照して作成した。

1．第 25 回 SIAM 以降の HPV 点検プログラム進捗状況

(1) 初期評価文書の公開状況

SIAM で合意された初期評価文書は、既存化学物質タスクフォースおよび化学物質の安全管理の全般的な方針を決定する「OECD 化学品委員会および化学品・農薬・バイオテクノロジー作業部会合同会合（Joint Meeting）」に提出して承認を得る。承認が得られた SIAP については、OECD が HPV データベース(OECD 2008b)を通じて公開している。Dossier は IUCLID（International Uniform Chemical Information Database）というデータベースを用いて作成されているが、出力方法をエクスポートファイルにすることによって、生データのやり取りが可能となる。SIAR および Dossier については国連環境計画(UNEP: United Nations Environment Programme)が、エクスポートファイルについては、OECD がそれぞれウェブサイトで公開している（UNEP 2008;OECD 2008c）。第 25 回 SIAM 以降、UNEP からの公式発表は滞っており、UNEP からの公式発表総数は第 24 回 SIAM 開催時と同様 398 物質であった。

SIAM における環境影響とヒト健康影響についての勧告は、FW(The substance is a candidate

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for further work）または LP（The substance 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。FW となる理由には追加試験が必要とされる場合の他、曝露情報の調査、詳細なリスク評価、リスク管理などが必要と判断される場合がある。しかし、これらの具体的な対応は各国に任されており、日本では評価結果を参考に必要があれば化学物質審査規制法（化審法）や化学物質把握管理促進法（PRTR 法）などの各法や各省の取り組みのなかに取り込むことになっている。SIAM で合意された勧告についてはその根拠と共に解釈することが望まれており、評価内容と合わせて参照する必要がある。

(2) 最終版の初期評価文書提出状況について

SIAM が終了した後、スポンサー国または産業界は SIAM での審議をもとに最終版の初期評価文書（SIAR、Dossier およびエクスポートファイル）を作成し、SIAM 後 3 ヶ月を目途に OECD 事務局に提出することになっている。最終版の初期評価文書の提出が 6 ヶ月以上滞っている場合、スポンサー国または産業界は状況説明と提出予定期日を示す必要がある。今回の SIAM に先立って日本および米国が提出予定日を報告した。また、ドイツは会議の場で進捗状況を報告し、英国は提出予定日の記載されたリストを OECD 事務局に提出した。

既存化学物質タスクフォースは、最終文書の提出が滞っている物質について、早急に出版を済ませるよう SIAM に勧告しているが、現在未処理の文書は 200 物質を超えている。OECD 事務局は、スポンサー国が ICCA イニシアティブの修正版文書を確認する作業を手伝う人員（2 名）を 2008 年 7 および 8 月に用意していることを報告した。OECD 事務局は、スポンサー国がこの機会を有効に活用するよう奨励した。

(3) 既存化学物質タスクフォースおよび Joint Meeting の報告

第 16 回既存化学物質タスクフォース（2007 年 11 月）は、OECD HPV 点検プログラムの今後の展開について討議し、（定量的）構造活性相関「(Q)SAR : (Quantitative) Structure-Activity Relationships」の使用や、特定のエンドポイントのみを評価する手法（選択的評価 : Targeted assessment）や評価すべき物質の優先順位をつけるためのツール（優先順位設定ツール : Priority setting tools）を用いた物質選定などの利用を検討した。また、初期評価文書から勧告の記述を削除することについても検討された。2008 年 2 月に行われた Joint Meeting は、HPV 点検プログラムの中長期的な展望について次のように結論した。

- 今後のプログラムの発展が、過去に国や地域と OECD HPV 点検プログラムの間でとられた調和を乱してはならない。
- （定量的）構造活性相関は全ての加盟各国が使用を認めた場合にのみ、試験結果の代用として使用されるべきである。
- 選択的評価（部分的評価）はスクリーニングとしての性格をもっていることを常に銘記すべきである。
- 優先順位設定ツールによって除外され、有害性が低いと考えられた化学物質であっても、全てのエンドポイントについて評価する候補物質となり得る余地を残す必要がある。
- 選択的評価によって低有害性と推定され、物質選定から除外することは、SIAM で十分な経験を集積し、より決定的な手法がまとまるまでは、non-HPV に限定して適用すべきである。
- 初期評価文書の勧告については、将来、削除される場合もあり得る。

(4) CDG 上での審議状況

OECD HPV 点検プログラムでは、SIAM での対面討議の他、オンライン会議用掲示板（CDG : Committee Discussion Group）を用いて審議をすることが可能である。第 22 回 SIAM で審議

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された物質カテゴリー：PFOA（CAS: 335-67-1、3825-26-1）は、米国/ICCA（ヒト健康影響）とドイツ/ICCA（環境影響）が初期評価文書を提出したが、HPV ではないため本プログラムの通常の評価物質として扱われず勧告も定められなかった。しかし、SIAM 後に CDG 上で審議した結果、ヒト健康影響・環境影響共に FW という結論で合意が得られた。

2．第 26 回 SIAM での審議状況

(1) 初期評価文書の審議結果

初期評価文書は加盟各国が初期評価文書の原案をCDGに掲載し、CDG上での事前討議（コメントの提出、コメントへの返答、コメントに応じたSIAPの修正）およびSIAMでの対面討議で審議される。第26回SIAMでの初期評価文書の審議は、CDGでの事前討議を基に修正したSIAPを用いて行われた。日本は日本政府が原案を作成したBenzoic acid, 4-methyl-（CAS: 99-94-5）および国際化学工業協会協議会（ICCA）が原案作成したSodium sulfite（CAS: 7757-83-7）の初期評価文書を提出した。今回の会議では、12物質の初期リスク評価結果および評価結果に基づく措置に関する勧告が合意された（表1）。中でも、次の物質については、通常の審議と異なる点があったため特筆する。

1）C5 Aliphatics（CAS: 78-78-4、109-66-0、287-92-3）

米国／ICCAが担当した物質カテゴリー（C5 Aliphatics；CAS: 78-78-4、109-66-0、287-92-3）については、全身毒性に対する直鎖構造のC５とCyclopentane（CAS: 287-92-3）の毒性をRead-acrossを用いて推定することの正当性をさらに明確に示すよう求められた。修正したSIAPが、SIAM後にCDGで審議され合意された。ヒト健康影響については、有害性はあるものの高曝露でのみ認められる一過性の毒性であるためLPと結論された。環境影響については、n-Pentan(CAS: 109-66-0)および2-Methylbutane(CAS: 78-78-4)は有害性が認められるものの良分解性・低蓄積性のためLPとされ、Cyclopentaneについては、難分解性のためFWと結論された。なお、n-Pentanは第13回SIAM（2001年11月）でノルウェー：eu（欧州連合でのリスク評価文書を基にしたことを意味する）のスポンサーのもと審議され、LPという結論で合意されていた。合意された初期評価文書も既に公開されているが、今回は物質カテゴリーを構成する物質として再審議された。

2) Formates（CAS: 64-18-6、107-31-3、141-53-7、540-69-2、544-17-2、590-29-4、20642-05-1）

米国／ICCAが担当した物質カテゴリー(Formates; CAS: 64-18-6、107-31-3、141-53-7、540-69-2、544-17-2、590-29-4、20642-05-1）は、ギ酸、ギ酸塩およびギ酸メチルで構成されるが、ギ酸メチルがエステルとメタノールに代謝・分解されることから、カテゴリーを構成する物質として正当であるか否かが議論された。スポンサーは初期評価文書とは別にカテゴリーとしての正当性を示す文書を提示し、ギ酸メチルは体内では酵素によってギ酸に加水分解されることから、ヒト健康影響の観点からカテゴリーに入れるべきであるとした。SIAMはギ酸メチルをカテゴリーに入れることに合意し、SIAM後にCDGを通じて初期評価文書の最終精査を行い合意が得られた。ヒト健康影響については、有害性が認められるものの職業曝露がコントロールされていることからLPと結論された。ただし、ギ酸メチルのみはメタノール（代謝物）の有害性が懸念されFWと結論された。環境影響については、有害性が認められるものの良分解性・低蓄積性のためLPとされた。

3) Hexafluorosilicilic acid（CAS: 16961-83-4）・Ammonium hydrogen fluoride（CAS: 1341-49-7）

NL/ICCAが担当したHexafluorosilicilic acid（CAS: 16961-83-4）およびAmmonium hydrogen fluoride（CAS: 1341-49-7）については、Sodium fluoride（CAS: 7681-49-4）の生殖発生毒

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性の試験結果をサポートデータして利用するに当たって、より詳細な情報が必要であると勧告された。スポンサーはSodium fluorideの生殖発生毒性の主要試験情報（RSS: Robust Study Summary）を提出することになった。修正した初期評価文書については、SIAM後にCDGで審議されることになった。

（2）HPV 点検プログラムにおける全般的な議題

1）SIAP のテンプレートについて

Joint Meeting が SIAP のテンプレート作成を勧告したことを受け、前 SIAM において OECD 事務局および SIAM 議長がフランス、スイス、英国および米国の有志者と共にテンプレート作成を行うことに合意した。SIAP のテンプレートを作成する目的は、基本となる文章の構造や表現をエンドポイントごとに用意することによって、より明瞭な SIAP が作成できるようになることである。また、テンプレートの利用は文書作成にかける時間を節約できることになる。今回の SIAM では、SIAP テンプレートおよびテンプレート導入に伴う HPV 点検プログラムのマニュアルのガイダンス修正案について討議された。SIAM は、環境影響に関する記述として扱われていた物性情報を、個別のパラグラフにして SIAP の最初の部分に移動することに合意した。その他、数国からのコメントがあったが、OECD 事務局は会議後にもコメントを提出するよう加盟各国に勧告した。修正した SIAP テンプレートおよび HPV 点検プログラムのマニュアルは、承認を得るために既存化学物質タスクフォースに提出される。

2)OECD HPV点検プログラムの発展について

HPV 点検プログラムでは、EU のリスク評価文書や IPCS の国際簡潔化学物質評価文書（CICAD : Concise International Chemical Assessment Document）などを SIAR の代わりに提出することが許可されている。2008 年 2 月に行われた Joint Meeting は、米国の新しい評価文書形式(Hazard Characterizations)を SIAR の代わりとして提出することを承認した。Hazard Characterizations は、US チャレンジプログラムで情報収集された化学物質の有害性を示す文書であり、2007 年の夏から公開が始まり現在では約 200 物質についての文書が公開されている（EPA 2008）。また、2007 年 10 月に行われた既存化学物質タスクフォースにおいて、米国は Hazard Characterizations を HPV 点検プログラムに提出するまでのフレームワークを提示し、経済産業諮問委員会（BIAC : Business and Industry Advisory Committee）などがその提出に貢献できることを示唆した。

第 26 回 SIAM では、米国が 2 文書についての事例報告を行った。SIAM は Hazard Characterizations の文書を OECD の HPV 点検プログラムに提出することを承認した。OECD 事務局は、手続き方法等は確立されていないものの、理論上 Hazard Characterizations の文書はどの国からでも OECD の HPV 点検プログラムに提出し得ると述べた。Hazard Characterizations の文書には生産量、用途および曝露についての情報は含まれていないが、これらの情報は EPA の Web から入手が可能であるかもしれないと米国は述べた。また、BIAC が情報を収集し、Hazard Characterizations に記述を追加する可能性もあるとした。オーストラリアは、Hazard Characterizations に記述を追加することに特別問題はない旨コメントした。英国は、現在の文書形式で RSS が満たされていると述べ、また化学物質の分類に関する項目は OECD の HPV 点検プログラムに提出される際に容易に削除できる旨コメントした。

3) 試験及び評価に関する統合的アプローチのワークショップについて

2007 年 12 月にワシントン DC において、試験及び評価に関する統合的アプローチ（IATA : Integrated Approaches to Testing and Assessment）についてのワークショップが開催された。このワークショップの目的は、様々な法規制や評価プログラムの条件を満たす総合的な評価アプローチを新たに模索することであった。異なる 3 つのグループの化学物質（Triadimefon「抗真

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菌剤」；Sulfosuccinates「食品における界面活性剤」；Ethylene glycols「HPV のカテゴリー物質」）について、①急性水生毒性、②慢性水生毒性、③皮膚刺激性、④皮膚感作性、⑤がん原性、⑥生殖発生毒性について評価し、事例研究を行った。ワークショップでは、現在使用されている評価方法（*in vivo* および *in vitro* による試験、（定量的）構造活性相関、Read across および カテゴリー評価）の有用性を様々な角度から検討した。ワークショップの報告は、2008 年 5 月に OECD Series on Testing and assessment No.88（OECD 2008d）として公開されている。ワークショップでは以下の事柄に関する勧告がまとめられた。

- （定量的）構造活性相関を規制的政策に関する意思決定や評価に利用するために、様々なエンドポイントについて定量的な予測法を更に開発していくこと。
- OECD のマニュアルの化学物質のグループ化に関するガイダンス（カテゴリーアプローチ）を、農薬・殺生物剤、芳香剤、香料剤などの評価の経験を含めるように拡大すること。
- Read-across の頑強性を向上させるために、体内動態（ADME：absorption, distribution, metabolism and excretion）や環境中での変化についての情報を使用するためのガイダンスを作成すること。
- 量的なエンドポイントについては Read-across を用いて数値を算出し、または不確実性を確定するなどガイドダンス文書を向上させること。

Joint Meeting はワークショップの結論と勧告を承認し、勧告を遂行するための準備を進めることについても承認した。

おわりに

OECD の HPV 点検プログラムにおける評価手法は、"Learning by Doing" の考え方に基づいて常に変革してきたが、今回の SIAM では物質カテゴリーの構成物質選定や、サポートデータの利用など、新しい評価手法の利用方法について議論された。また、米国の Hazard Characterizations の導入や Targeted assessment や Priority setting tools などの新たな評価手法も紹介され、本プログラムの効率化・加速化への貢献が期待された。

勧告の判定については前回の会議に引き続き、環境影響またはヒト健康影響に対する有害性が認められ、かつ曝露情報が不足している、または高曝露が予測される物質については FW と結論される傾向にあった。本会議に日本が提出した Sodium sulfite（CAS: 7757-83-7）は、動物を用いた試験での有害性は低かったものの、感作性および呼吸器系への影響が化学物質に対する感受性の高い人の一部に認められたことから、ヒト健康影響については FW と結論された。このことから、ヒトに対する報告に重きが置かれていることが伺えた。一方、環境影響またはヒト健康影響に対する有害性の低いもの、或いは有害性は認められるが低曝露が予測される物質（ヒト健康影響）および速やかに生分解される物質（環境影響）などは、LP と結論される傾向にあった。

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化学生物総合管理　第 4 巻第 2 号 (2008.12)　237-245 頁
連絡先：〒158-8501 世田谷区上用賀 1-18-1　E-mail: hirose@nihs.go.jp
受付日：2008 年 6 月 16 日　　受理日：2008 年 8 月 21 日