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Review

Review of testicular toxicity of dinitrophenolic compounds, 2-*sec*-butyl-4,6-dinitrophenol, 4,6-dinitro-*o*-cresol and 2,4-dinitrophenol

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ABSTRACT

The present review paper summarizes the data available in the literature concerning dinitrophenolic compounds and evaluates male reproductive toxicity in experimental animals. Gavage and feeding doses of 2-*sec*-butyl-4,6-dinitrophenol (dinoseb; CAS No. 88-85-7) manifested testicular toxicity, and 4,6-dinitro-*o*-cresol (DNOC; CAS No. 534-52-1) showed similar but weaker testicular toxicity in laboratory animals. Consecutive doses of dinoseb and DNOC by gavage seemed to induce spermatotoxicity by disturbing spermiogenesis or the maturation process of sperm in the epididymis, and the most probable target cells of spermatotoxicity were thought to be testicular spermatids in rats. Prolonged exposure to dinoseb and DNOC in the diet also induced testicular toxicity in rats. However, the feeding dose of dinoseb irreversibly affected the early stage of spermatogenesis and produced infertility in rats. On the other hand, 2,4-dinitrophenol (DNP; CAS No. 51-28-5) did not show testicular toxicity in laboratory animals according to available literature. Further studies in laboratory animals with nitrophenolic compounds are required for clarification of their testicular toxicity and for risk assessment in humans.

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1. Introduction

Dinitrophenolic compounds have many uses in agriculture as herbicides, insecticides, acaricides and fungicides [1]. Minor differences in chemical structure determine their use, and several compounds have more than one use (Table 1). Although the use of dinitrophenolic compounds as pesticides was banned in many countries due to their serious toxicity [2], they are still trafficked and used in agriculture. In several countries, 2-*sec*-butyl-4,6-dinitrophenol (CAS: 88-85-7; dinoseb) and dinoseb salts are registered for use as herbicides and insecticides [2]. 4,6-Dinitro-*o*-cresol (CAS: 534-52-1; DNOC) and 2,4-dinitrophenol (CAS: 51-28-5; DNP) were once used as weight-reducing agents as well. The main current use of DNOC and DNP is in the plastic industry as an inhibitor of polymerization in styrene and vinyl aromatic compounds [3,4]. It is reported that dinoseb and DNP are high-volume chemicals with production or importation exceeding 1000 tonnes per year in Organisation for Economic Co-operation and Development (OECD) member countries [5]. The annual production of DNOC is ca. 600 tonnes, and 100–200 tonnes are used as an agrochemical. In addition, significant volumes of DNOC are still stocked around the world especially in developing countries [3].

Exposure to dinitrophenolic compounds may occur by direct contact, ingestion and inhalation for users and producers, but potential indirect exposure via the environment for consumers is

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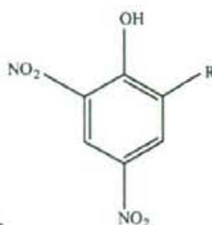
Table 1



Scheme of dinitrophenolic compounds

Chemical name	CAS number	R	Agricultural use
2,4-Dinitrophenol (DNP)	51-28-5	H	I
4,6-Dinitro-o-cresol (DNOC)	534-52-1	CH_3	A, F, H, I
2-sec-Butyl-4,6-dinitrophenol (Dinoseb)	88-85-7	$CH(CH_3)CH_2CH_3$	H, I

A: acaricide; F: fungicide; H: herbicide; I: insecticide.

also anticipated. Dinoseb, DNOC and DNP have been detected in groundwater [3,4,6], and DNOC has been identified in extracts of rain and snow [3]. The use of DNP and DNOC as diet pills was discontinued in the US by the end of 1938 [4]; however, DNP was introduced in a bodybuilding magazine in the late 1990s [7], and it has managed to steadily gain popularity in some bodybuilders and athletes to rapidly lose body fat. The average daily dose of DNP or DNOC for man was about 3 mg/kg bw/day in 1930s [3,8], and the current average intake of DNP seems to be 200–400 mg/day according to a commercial web site [9].

Most dinitrophenolic compounds are absorbed well by the skin, gastrointestinal tract or lung. Dinitrophenolic compounds shows moderate to strong acute oral toxicity with LD_{50} values in the range of 25–46 mg/kg bw (dinoseb), 26–34 mg/kg bw (DNOC) and 50–71 mg/kg bw (DNP) in rats [1,10]. The major systems prone to toxicity are the hepatic, renal and nervous systems [11]. The basic mechanism of toxicity of nitrophenolic compounds is thought to be stimulation of oxidative metabolism in cell mitochondria by the uncoupling of oxidative phosphorylation [12]. Early symptoms include hyperthermia, sweating, headache and confusion. Severe exposure may result in restlessness, seizures, coma and death [11–13].

In the previous review, we showed that the administration of dinoseb to maternal animals produced developmental toxicity including teratogenicity [14]. We also reported that rats dosed with dinoseb by gavage for a total of 42 days decreased sperm motility and increased the rates of abnormal sperm, tails and heads at 7.0 mg/kg bw/day [15]. Sperm parameters are considered to be sensitive indicators of fertility [16]. In fact, the feeding dose of dinoseb to male rats for 11 weeks induced low fertility at 225 ppm (15.6 mg/kg bw/day) and infertility at 300 ppm (22.2 mg/kg bw/day) due to low or no spermatogenesis [17].

In Sertoli-germ cell co-cultures, dinoseb caused degenerative alterations in pre-pachytene and pachytene spermatocytes and Sertoli cells, while DNOC and DNP affected pre-pachytene and pachytene spermatocytes [18]. These findings suggested the possibility that dinitrophenolic compounds can produce testicular toxicity with a similar mode of action in laboratory animals and humans. However, a recent study by Takahashi et al. [19] reported no testicular toxicity in Crl:CD(SD) rats treated with DNP by gavage up to 30 mg/kg bw/day for 46 days. There were differences in testicular toxicity between dinoseb and DNP although they showed similar toxicity in Sertoli-germ cell co-cultures. In this review, we attempt to clarify the testicular toxicity of dinitrophenolic compounds in laboratory animals, and possible mechanisms involved in the testicular toxicity are discussed.

2. Testicular toxicity of dinitrophenolic compounds

The available literature for dinitrophenolic compounds shown in Table 1 was searched and reviewed for male reproductive toxicity. Only statistically significant effects are summarized below unless noted otherwise.

2.1. Testicular toxicity of dinoseb

Table 2 shows a summary of the testicular toxicity of dinoseb. When male Crj:CD(SD)IGS rats were administered dinoseb (0, 0.78, 2.33, or 7.0 mg/kg bw/day) by gavage for 42 days, motile sperm rate, progressive sperm rate, straight line velocity and viability rate were lower than the controls, and the amplitude of lateral head displacement, abnormal sperm rate and abnormal tail rate were higher than the controls at both the end of the administration and 14-day recovery periods at 7.0 mg/kg bw/day [17]. However, there were no dose related effects on spermatogenesis at the stages of spermatogonia and spermatocytes in this study.

Sato et al. [20] showed that 3-consecutive-day administration of dinoseb by gavage at 7.5 mg/kg bw/day reduced number of sperm, sperm motility, path velocity, curvilinear velocity, and amplitude of lateral head displacement in Crj:CD(SD)IGS rats. These effects were observed 19 days after the last administration, but not 12 days after the last administration. Similarly, another spermatotoxicity study, in which male Jcl:SD rats were treated with dinoseb for 5 days at 7.5 mg/kg bw/day, showed no effects on sperm parameters in the cauda epididymis 3 days after the final dosing, but reduced sperm motility and increased incidence of tailless sperm were noted 14 days after the final dosing [21]. No testicular histopathological alteration was observed in this study. These findings suggest that consecutive dose of dinoseb by gavage affects sperm by disturbing spermiogenesis or maturation processes of sperm in the epididymis in rats.

Sato et al. [20] also demonstrated that gavage dose of dinoseb at 7.5 mg/kg bw/day three times per week (Monday/Wednesday/Friday) for 3 weeks did not affect any sperm parameters in Crj:CD(SD)IGS rats. In another study, a single oral dose of dinoseb did not cause any alteration of sperm parameters 2 and 14 days after the administration at 17.5 mg/kg bw, but five daily doses of dinoseb at 7.5 mg/kg bw/day altered sperm morphology both 3 and 13 days after the last administration and caused a decreased number of sperm and the percentage motile sperm at 13 days after the last administration in SD rats [22].

In a feeding study of dinoseb at 75, 150, 225 or 300 ppm (0, 3.8, 9.1, 15.6 or 22.2 mg/kg bw/day), sperm counts and morphologically normal sperm were decreased at 150–300 ppm after 11 weeks

Table 2
Testicular toxicities in animals given dinoseb.

Species	Dose	Route	Exposure time	Effect observed	Reference
Crj:CD(SD)IGS rat	7.0 mg/kg	Gavage	42 days	↑The amplitude of lateral head displacement, ↑abnormal sperm rate and ↑abnormal tail, ↓sperm motility, ↓progressive sperm rate, ↓straight line velocity and ↓viability	[15]
Crj:CD(SD)IGS rat	7.5 mg/kg	Gavage	3 days	↓No. of sperm, ↓motile sperm, ↓path velocity, ↓curvilinear velocity and ↓amplitude of lateral head displacement	[20]
	7.5 mg/kg	Gavage	9 doses/3 weeks	No effects on sperm	
Jcl:SD rat	7.5 mg/kg	Gavage	5 days	↓Weights of seminal vesicle, prostate and epididymis, ↓sperm motility and ↑tailless sperm	[21]
SD rat	7.5 mg/kg	Gavage	5 days	↓Sperm motility, ↓no. of sperm and ↑abnormal sperm	[22]
	17.5 mg/kg	Gavage	1 day	No effects on sperm	
Sharman rat	150 ppm (9.1 mg/kg)	Diet	71–77 days	Sperm counts, ↓morphologically normal sperm, ↑adjusted weight of seminal vesicle and ↓sperm content of caudae and vasa deferentia	[17]
	225 ppm (15.6 mg/kg)	Diet	71–77 days	Infertility, ↓adjusted weights of testis and epididymides, ↑adjusted weight of seminal vesicle, ↓sperm content of caudae and vasa deferentia, sperm counts and↓ morphologically normal sperm	
	300 ppm (22.2 mg/kg)	Diet	71–77 days	↓Adjusted weight of seminal vesicle and above observed effects (225 ppm) except ↑adjusted weight of seminal vesicle	
	300 ppm (22.2 mg/kg)	Diet	10 days	No effects	
			20 days	↓Morphologically normal sperm, ↓adjusted weights of seminal vesicles and prostate	
			30 days	↓Morphologically normal sperm	
			50 days	↓Morphologically normal sperm, ↓adjusted weights of seminal vesicles	
SD rat	125–175 ppm	Diet	25 days	↓Sperm motility	[23]
Sharman rat[a]	200 ppm	Diet	153 days	Diffuse tubular atrophy of the testes	[24]
Rat	0.05% (13.5 mg/kg)	Diet	21 days	No histopathological changes in the testis	[26]
	0.02% (5.4 mg/kg)	Diet	6 months	No histopathological changes in the testis	
CD (SD) rat[a]	1–10 mg/kg	Diet	3-generation	No effects on reproduction	[6,25]
CD-1 mouse[a]	1–10 mg/kg	Diet	2 years	Testicular atrophy/degeneration with hypospermatogenesis	[6,25]

[a] Only abstract or secondary literature is available.

administration in Sherman rats [17]. In addition, spermatozoa were not found in sections of the epididymides at 300 ppm. None of 5 males fed 300 ppm and only 1 of 10 males fed 225 ppm produced litters after mating with non-treated females. There was little or no remission of these effects after a 16-week recovery period. Histopathological changes to spermatogonia, spermatocytes, spermatids and sperm in the testes were observed after 20 or 30 days administration at 300 ppm, and severe damage to the spermatogonia was observed after 50 days treatment at 300 ppm.

Similarly, a feeding dose of dinoseb (0, 125, 150 or 175 ppm) to male SD rats for 25 days showed reduced sperm motility in all treatment groups [23]. Hall et al. [24] provide a brief summary of a subchronic feeding study of dinoseb, in which Sherman rats were fed a diet with 0, 50, 100, 150, 200, 300, 400 or 500 ppm of dinoseb for 153 days. Mortality was observed at 300, 400 and 500 ppm and only animals treated with dinoseb up to 200 ppm were evaluated. Diffuse tubular atrophy of the testes was observed particularly at 200 ppm. No further details were available for this study.

The findings in the feeding dose studies suggest that prolonged exposure to dinoseb in feed affects the early stage of spermatogenesis in rats. Consistency was confirmed in a 2-year feeding study in CD-1 mice. Testicular atrophy or degeneration with hypospermatogenesis was observed at 1, 3, or 10 mg/kg bw/day [6,25]. However, a three-generation feeding study showed no effects on male reproductive toxicity in SD(CD) rats given dinoseb at 1, 3, and 10 mg/kg bw/day for 29 weeks [6,25]. An old study showed no histopathological changes in the testes of rats fed dinoseb in the diet for 21 days at 0.05% (13.5 mg/kg bw/day) or for 6 months at 0.02% (5.4 mg/kg bw/day) [26]. No further information was available.

2.2. Testicular toxicity of DNOC

A summary of testicular toxicity of DNOC is shown in Table 3. DNOC was administrated to Jcl:SD rats by gavage at 0, 4, 7.5 or 15 mg/kg bw/day for 5 consecutive days [21]. Examination at 3 days after the last dosing revealed no treatment-related alterations in histopathology of the testis and in sperm parameters. DNOC administration resulted in reduced sperm motility and increased the incidence of tailless sperm in the cauda epididymis, but there were no testicular histopathological alterations at 14 days after the last

Table 3
Testicular toxicities in animals given DNOC.

Species	Dose	Route	Exposure time	Effect observed	Reference
Jcl:SD rat	15 mg/kg	Gavage	5 days	↓Motile sperm and ↓morphologically normal sperm	[21]
Jcl:SD rat	10 mg/kg	Gavage	5 days	↑Peeled sperm	[27]
	15 mg/kg			↑Tailless sperm	
Riv:Tox(M)Wistar rat	20 mg/kg	Diet	90 days	↓Spermatogenesis and ↓relative weights of testes and prostate	[29]
Rat	0.10% (27 mg/kg)	Diet	6 months	No histopathological changes in the testis	[26]
SD(CD) rat[a]	7.20 –10.1 mg/kg	Diet	2 generations	No effects on reproduction	[3]
(C3H × C57BL/6) F1 mouse	3–12 mg/kg	Gavage	5 days	No effects on testes weight, sperm count or sperm abnormalities	[28]
(C3H × C57BL/6) F1 mouse	3–12 mg/kg	i.p.	5 days	No effects on testes weight, sperm count or sperm abnormalities	
CFLP mouse	10 mg/kg	i.p.	Single dose	↑Dominant lethal value and damage in the germ cells	[30]

[a] Only abstract or secondary literature is available.

dosing. These results indicate that consecutive gavage dose of DNOC can also affect sperm by disturbing spermiogenesis or maturation process of sperm in the epididymis in rats.

Subsequently, Takahashi et al. [27] showed that the target cells of DNOC spermatotoxicity are likely to be testicular spermatids. DNOC was administered to Jcl:SD rats by gavage at 0, 10 or 15 mg/kg bw/day for 5 days. One day after the last dosing, there were elongated spermatids that looked normal but lacked the mitochondrial sheath at the proximal end of the middle piece. Fourteen days after the last dosing, there were increases in the number of peeled sperm at 10–15 mg/kg bw/day and the number of tailless sperm at 15 mg/kg bw/day. The authors suggest that the elongated spermatids may develop into tailless sperm when they reach the cauda epididymis. In the preceding study [15], dinoseb did not affect spermatogonia and spermatocytes, but the number of abnormal sperm was increased. Therefore, spermatids may also be the target cells of dinoseb spermatotoxicity.

On the other hand, no effects were found on sperm morphology, sperm counts or testicular weights at 30 days after the last administration in (C3H × C57BL/6) F1 mice given DNOC by gavage or by intraperitoneal injection at 3–12 mg/kg bw/day for 5 days [28]. These inconsistent results may be due to the length of the post-administration period. There is a possibility that dose-related effects on spermatids, the most probable target cells, cannot be detected at 30 days after the last dosing of DNOC. Another possibility is a difference in excretion, which means that DNOC is excreted at a slower rate in rats than in mice [3].

Spermatogenesis and relative organ weights of the testes and prostate were decreased in a 90-day feeding study, in which Riv:Tox(M)Wistar rats were exposed to 20 mg/kg bw/day DNOC in the diet [29]. These findings suggest that prolonged exposure to DNOC in the diet can also induce testicular toxicity. However, an old study showed no histopathological changes in the testes in rats fed DNOC in the diet at 0.10% (27 mg/kg bw/day) for 6 months [26]. No further detailed information was available.

Intraperitoneal injection of 10 mg/kg bw DNOC into male CFLP mice increased the frequency of chromosomal aberrations in male germinal cells 20 days after administration [30]. DNOC-treated males were mated with untreated nulliparous females for 8 weeks after the treatment. New females were caged with the males every week. The number of living embryos was decreased with a dominant lethal value of 16% at the sixth week, indicating DNOC possibly affect the survival of offspring in mice. However, a two-generation feeding study revealed that there were no effects on reproduction in SD(CD) rats given DNOC at 7.20–10.1 mg/kg bw/day [3].

2.3. Testicular toxicity of DNP

Table 4 shows a summary of testicular toxicity of DNP. Unlike dinoseb and DNOC, DNP dose studies did not show testicular toxicity in rats. Crl:CD(SD)IGS rats treated with DNP by gavage at 80 mg/kg bw/day for 28 days showed increased relative organ weight of the testes, but histopathological changes in the testes, epididymis and prostate were not observed. The number of sperm in the epididymis was not affected [31,32]. Therefore, the increased relative organ weight of the testes was considered to be due to reduced body weight on the day of scheduled killing.

DNP was administrated to Jcl:SD rats by gavage at 0, 7.5, 15 or 30 mg/kg bw/day for 5 consecutive days [21]. Organ weights of the testes, epididymis, seminal vesicles and prostate were not affected at 3 days after the last dosing. Tailless sperm in the cauda epididymis was only increased slightly at 14 days after the last dosing, but there were no statistically significant effects. Similarly, organ weights of the testes and epididymis and numbers of Sertoli cells and of germ cells per Sertoli cell were not affected in Crl:CD(SD) rats treated by gavage with DNP at a dose of 0, 3, 10 or 30 mg/kg bw/day for a total of 46 days [19]. These findings indicate that a gavage dose of DNP was unlikely to possess testicular toxicity in rats under these test conditions.

Negative results were also observed in capsule and feeding dose studies. No gross or histological evidence of treatment-related testicular damage was reported following DNP treatment of dogs exposed to 5 or 10 mg/kg bw/day by capsules 6 days a week for 6 months [8] or rats exposed to 0.10% (27 mg/kg bw/day) in the diet for 6 months [26]. Small testes and testicular atrophy were observed in rats given 0.20% DNP (54 mg/kg bw/day) in the diet for 24 days. However, the authors stated that it was difficult to distinguish between direct toxic effects and secondary effects due to reduced body weight gain.

3. Discussion and conclusion

Consecutive gavage doses of dinoseb manifested testicular toxicity in rats at 7.0–7.5 mg/kg bw/day, and DNOC showed similar but weaker testicular toxicity in rats at 10–15 mg/kg bw/day. Consecutive gavage dose of DNP showed no testicular toxicity up to 80 mg/kg bw/day in rats. Consecutive doses of dinoseb and DNOC by gavage seemed to affect sperm by disturbing the spermiogenesis or maturation processes of sperm in the epididymis, and the most probable target cells of toxicity were thought to be testicular spermatids in rats.

Prolonged exposure to dinoseb and DNOC in the diet also induced testicular toxicity in rats at 9.1–22.2 and 20 mg/kg bw/day, respectively. However, a feeding dose of dinoseb irreversibly affected the early stage of spermatogenesis and produced infertility in rats at 22.2 mg/kg bw/day. In a 2-year feeding study in mice, testicular atrophy or degeneration with hypospermatogenesis was observed at 1–10 mg dinoseb/(kg bw day). However, male reproductive toxicity was not affected in rats up to 10 mg/kg bw/day for 29 weeks (three generations). No treatment-related testicular damage was reported following DNP treatment at 27 mg/kg bw/day in the diet for 6 months in rats.

In Sertoli-germ cell co-cultures, dinoseb caused degenerative alternations in pre-pachytene and pachytene spermatocytes and Sertoli cells, while DNOC and DNP affected pre-pachytene and pachytene spermatocytes [18]. Testicular effects observed in laboratory animals are thought to be caused by the uncoupling effects rather than due to a body weight loss or body temperature increase [17]. The uncoupling effects of dinoseb are stronger than that of DNP and DNOC in mouse liver and brain cells [33]; this may explain why

Table 4
Testicular toxicities in animals given DNP.

Species	Dose	Route	Exposure time	Effect observed	Reference
Crl:CD(SD)IGS rat	80 mg/kg	Gavage	28 days	No effects on sperm or sex organs	[31,32]
SPF Crl:CD(SD) rat	30 mg/kg	Gavage	46 days	No effects on sperm or sex organs	[19]
Jcl:SD rat	30 mg/kg	Gavage	5 days	No effects on sperm or sex organs	[21]
Rat	0.10% (27 mg/kg)	Diet	6 months	No histopathological changes in the testis	[26]
	0.20% (54 mg/kg)	Diet	24 days	Small testes and testicular atrophy	
Dog	5–10 mg/kg	Capsules	6 days/week for 6 months	No histopathological changes in the testis or epididymis	[8]

strong testicular toxicity was observed in dinoseb treated animals. However, the uncoupling effects of DNP were stronger than DNOC in mouse brain cells [33]. Acute toxicities in animals tend to increase with increasing uncoupling potency; however, a good correlation between *in vivo* and *in vitro* studies was not established, indicating that other factors such as absorption, distribution, metabolism and excretion may also play a role in the toxicity.

Generally, phenols tend to be absorbed rapidly and distributed throughout the body, and excretion occurs over a period of weeks [34]. DNP is metabolized rapidly to less toxic metabolites by reduction of nitro groups to amine groups [4]. Reduction of the nitro groups is also main detoxification pathway in DNOC, but oxidation of the alkyl substituent was also noted in rats and rabbits [35]. Dinoseb is metabolized by side-chain oxidation in mice, rats and dogs, followed by nitro-reduction in rats, but reduction of the nitro group was not observed in dogs and mice [34,36,37]. There were several unknown metabolites and conjugates after the administration of dinoseb and DNOC [35,36]. Some of metabolites of dinoseb and DNOC may also play a role for the testicular toxicity of these compounds. In fact, some metabolites of dinoseb (not identified) caused toxicity in the liver, kidney, spleen and blood of rats [34].

Sperm motility was reduced by gavage dosing of dinoseb and DNOC [16,20–22], and a suggestive reduction in sperm motility was also observed after DNP administration in rats [21]. Uncoupling agents such as DNP, pentachlorophenol (PCP) and carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) reduced mitochondrial membrane potential by attacking the proton gradient across the inner mitochondrial membrane [38,39]. Reduced sperm motility was correlated with reduced mitochondrial membrane potential caused by uncoupling agents when glucose was not present [38,40].

Of interest, *m*-dinitrobenzene (DNB), a structurally similar compound to DNP but not an uncoupling agent, causes Sertoli cell vacuolization and germ cell apoptosis in rats [41,42] but not in hamsters [43]. An intermediate metabolite, *m*-nitrosonitrobenzene, produced toxicity in rat Sertoli-germ cell co-cultures while metabolites of DNB, *m*-nitroaniline (NA) and *m*-nitroacetanilide (NAA), do not provoke testicular toxicity [44]. Seminiferous tubules isolated from hamsters were more capable of reductively metabolizing DNB to NA and NAA than that from rats, and DNB induced ATP depletion in rat seminiferous tubules, but not in hamster tubules [45].

There have been no mechanistic studies on the testicular toxicity of dinoseb and DNOC. However, like DNB, there is a possibility that metabolic activation via reductive metabolism of a nitrogroup may be responsible for the testicular toxicity of dinoseb and DNOC in laboratory animals, although dinitrophenolic compounds and DNB showed different cytotoxicity in rat Sertoli-germ cell cocultures [18]. Understanding the basic mechanisms involved in male germ cell toxicity is necessary step to prevent reproductive failure. Further mechanistic studies on nitrophenolic compounds and compounds with similar structures are required to clarify the testicular toxicity of these compounds. No information on male reproductive toxicity was obtained for other dinitrophenolic compounds with an alkyl substituent, but similar toxicity to dinoseb and DNOC can be expected from their structures.

DNP did not show testicular toxicity in laboratory animals according to the available literature, and possibly related mechanisms were discussed. However, the histopathology of the testes could be inadequate in these DNP studies due to shorter length of exposure. The long-duration studies with DNP were conducted on dogs in 1934 and on rats in 1948 when the standard of testicular pathology is likely to be below current standard. Therefore, further studies which meet the current standard of testicular pathology [46] must be required to clarify the testicular effects of DNP.

It is noted that the efficiency of sperm production and the epididymal spermatozoal reserves of humans are considerably lower than those of conventional animal models [47]. Working [48] also described that the human male is of relatively low fertility and thus may be at greater risk from reproductive toxicants than males of the common laboratory animal model species. Furthermore, Parker [49] commented that rodent males produce sperm in numbers that greatly exceed the minimum requirement for fertility while sperm production in human males appears to be much closer to the infertility threshold; therefore, less severe reductions in sperm counts may affect human fertility significantly. These considerations indicate that definitive animal studies of chemical compounds that are suspected to have testicular toxicity are needed to assess the risk to reproduction in humans. Further histopathological studies of the testes in laboratory animals given nitrophenolic compounds and compounds with similar structures could help us to understand the testicular toxicity of these compounds, because histopathology is acknowledged as the most sensitive endpoint for detecting testicular toxicity [46].

Conflict of interest statement

There is no conflict 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 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. 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 14 報）

－第 23 回、第 24 回 OECD 高生産量化学物質初期評価会議（2006 年済州、2007 年パリ）

Progress on OECD Chemicals Programme (14) – SIAM 23 in Jeju, 2006 and SIAM 24 in Paris, 2007

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要旨：第 23 回 OECD 高生産量化学物質初期評価会議（SIAM 23）が 2006 年 10 月に韓国・済州で開催され、日本が提出した 2 物質の初期評価文書について合意が得られた。また、SIAM 24 が 2007 年 4 月にフランス・パリで開催され、日本が提出した 2 物質および物質カテゴリーを構成する 1 物質の初期評価文書については全ての評価結果の合意が得られた。本稿では本会議で合意の得られたこれらの物質および物質カテゴリー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 23rd Screening Information Data Set (SIDS) Initial Assessment Meeting (SIAM 23) was held in Jeju, hosted by Korea. The initial assessment documents of two substances (CAS numbers: 88-09-5, 111-41-1) at SIAM 23 were submitted by the Japanese Government with or without the International Council of Chemical Associations (ICCA) and all of them were agreed at the meeting. SIAM 24 was held at the Organisation for Economic Co-operation and Development (OECD) headquarters in Paris, France. The initial assessment documents of two substances (CAS numbers: 88-85-7, Mixture of 110-30-5, 5136-44-7, 5518-18-3) and one substance (CAS: 7782-63-0) as a member of a chemical category (iron salts and their hydrates) at SIAM 24 were submitted by the Japanese Government with or without ICCA and all of them were agreed at the meeting. 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）加盟各国における高生産量化学物質（High Production Volume Chemical : HPV）について、1992年に始まったOECD高生産量化学物質点検プログラム（HPV Programme）により安全性の評価が行われている（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、第22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）。また、第19回SIAM（SIAM 19）からSIAM 24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）。

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においても2001年から、日本政府に加え日本化学工業協会加盟企業も評価文書の原案を作成している。

評価文書は、物性、曝露情報、健康影響および環境影響に関する記述から構成されている。本稿では第23回および第24回SIAM（SIAM 23、SIAM 24）で合意に至った化学物質名および日本担当物質の評価文書の概要を紹介する。

2　SIAM 23およびSIAM 24で合意された化学物質名と日本担当物質の初期評価内容

2006年10月に済州（韓国）で開催されたSIAM 23において、10物質および12物質カテゴリーの初期評価文書が審議され、表1に示す化学物質の初期評価結果および勧告が合意された。

また、2007年4月にパリ（フランス）で開催されたSIAM 24において、15物質および4物質カテゴリーの初期評価文書が審議され、表2に示す化学物質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 23について

（1）2-Ethylbutyric acid (88-09-5)（日本政府）

1）曝露状況

本物質は潤滑剤の中間体や香料添加剤として使用されている。職業曝露の主要経路は吸入および経皮と考えられる。本物質自体の使用は香料添加剤に制限されているので、消費者曝露のレベルは低い。

2）環境影響

本物質が環境に放出された場合、主に土壌（64.7%）および水圏（30.6%）に分布し、残りは大気（4.55%）に分布する。本物質は容易に生分解し、魚類における濃縮性は低い（生物濃縮係数BCF : 3.16 [計算値]）。

水生生物に対する急性毒性では、魚類の半数致死濃度（LC_{50}）は> 50 mg/L（96時間、OECD TG 203）、甲殻類の半数影響濃度（EC_{50}）は70 mg/L（48時間、遊泳阻害：OECD TG 202）、藻類の50%生長阻害濃度（EC_{50}）は> 63 mg/L（72時間、生長速度法：OECD TG 201）であった。慢性毒性では、甲殻類の最大無影響濃度（NOEC）は49 mg/L（21日間、繁殖阻害：OECD TG

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211）、藻類の NOEC は 39 mg/L（72時間、生長速度法：OECD TG 201）であった。本物質は弱酸性であるが、これらの試験において pH は無調整であったため、試験結果は低 pH の影響を受けていた。低 pH の影響については、HPV Programme における Hydrogen chloride（7647-01-0）の評価文書でレビューされている。

３）健康影響

本物質をウサギやラットに投与（経口、皮下）するとグルクロン酸抱合体として尿中に主に排出される。イヌでは、β酸化や脱炭酸反応が起こり、2-pentanone が生じる。

ラットの単回経口投与毒性試験（OECD TG 401）での LD_{50} は> 2,000 mg/kg bw であった。

ラットに交配前2週間および交配期間を含め、雄では計42日間、雌では分娩後哺育4日まで、0、10、50または250 mg/kg bw/day を強制経口投与した反復投与毒性・生殖発生毒性併合試験（OECD TG 422）において、250 mg/kg bw/day で投与後一過性の流涎が雌雄1例ずつに認められた。血液学検査では、50 mg/kg bw/day 以上で雄の白血球数が軽度に減少し、250 mg/kg bw/day で雄の血小板数が減少した。臓器重量では、250 mg/kg bw/day で雄の腎臓の相対重量、雌の腎臓の絶対・相対重量が増加した。これらの結果から、反復投与毒性の無毒性量（NOAEL）は雄で 10 mg/kg bw/day、雌で 50 mg/kg bw/day とされた。また、生殖毒性については、50 mg/kg bw/day 以上で少数例の母動物に産児を集める行動や胎盤を処理する行動の欠如、あるいは分娩遅延等が認められたが、用量依存的では無かった。発生毒性については、250 mg/kg bw/day で出産生児数が減少し、生児出産率および出生率が減少し、哺育4日における生存児数も減少した。これらの結果から、生殖毒性の NOAEL は 250 mg/kg bw/day（最高用量）、発生毒性の NOAEL は 50 mg/kg bw/day とされた。

細菌を用いる復帰突然変異試験では S9mix の存在/非存在下にかかわらず陰性であったが、染色体異常試験では S9mix 非存在下で陽性であった。*In vivo* 小核試験では陰性であった。

４）結論と勧告

本物質は健康に対して有害性（反復投与毒性、発生毒性）を示すが、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（藻類・魚類・甲殻類への急性毒性）を示すが、容易に生分解し、魚類における濃縮性は低いので、環境影響について LP と勧告された。

（２）2-(2-Aminoethylamino) ethanol (111-41-1)（原案作成：ICCA 日本企業）

１）曝露状況

本物質は主に界面活性剤の原料として使用されている。職業曝露の経路としては吸入と経皮が考えられるが、通常閉鎖系で製造されるので、曝露の可能性はわずかしかない。製造過程で未反応な本物質を含む（5 ppm 以内）製品の使用により、消費者曝露の可能性はあるが、その程度は低い。

２）環境影響

本物質が環境に放出された場合、99.99%が水圏に分布する。本物質は容易に生分解し、魚類における濃縮性は低い（BCF：≦3.7）。

水生生物に対する急性毒性では、魚類の LC_{50} は 640 mg/L（96時間）、ミジンコの EC_{50} は 22 mg/L（48時間、遊泳阻害：OECD TG 202）、藻類の EC_{50} は 354 mg/L（72時間、生長速度法）であった。

３）健康影響

雌ラットへの ^{14}C 標識体を用いた単回経口投与（0.5, 50 mg/kg bw）では、本物質は速やかに吸収され、0.5時間以内に血中レベルは最高値となった。投与した量の大部分（85-98%）が尿中に投与後48時間以内に排出された。血漿中には本物質のみが認められ、その消失半減期は二相性（1-6時間の計算値：1.6-1.8時間、8-48時間の計算値：16.7-17.3時間）を示した。尿中

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には主に未変化体が排出され、その他、未変化体を含む 4 種の代謝産物うち 2 物質は未知の構造であった。妊娠ラットへの単回経口投与（50 mg/kg bw）の結果は、非妊娠ラットと同様であった。雌ラットへの経皮投与（480 mg/kg bw、8 時間）において、血漿濃度は検知できなかったが、尿中へ排出され、生物学的利用能は経口投与の約 10%であった。これらの全ての試験において、諸器官への分布は同程度に少なく、また、投与量、曝露経路、妊娠の有無による差異は認められなかった。

OECD ガイドライン試験（OECD TG 401）を含む、ラットにおける数種類の単回経口投与毒性試験での LD_{50} は> 2,000 mg/kg bw であった。毒性症状（呼吸困難、無関心、よろめき歩行）は> 2,000 mg/kg bw でみられた。また、直接刺激により胃炎を引き起こした。単回吸入毒性試験（OECD TG 403）において本物質の飽和空気（濃度不明）にラットを 6 または 8 時間曝露させたところ、毒性は認められなかった。ラットおよびウサギを用いた単回経皮投与毒性試験（OECD TG 402）において、LD_{50} は> 2,000 mg/kg bw、また、毒性症状は塗布部位における皮膚の炎症および壊死であった。ウサギの皮膚と眼に対して腐食性が認められた。

本物質には皮膚感作性（モルモット Maximization 試験およびマウス LLNA 試験）があり、Diethyleneamine、Triethyleneamine、Ethylenediamine および Piperazine との交差反応が認められた。また、ヒトにアレルギー性接触皮膚炎を起こす可能性がある。そして、本物質を含む接着溶剤の蒸気を吸入した作業者に重篤な遅発性アレルギー性喘息の生じた報告があるが、おそらくは労働衛生環境の改善により、この 30 年間はこのような報告はない。

ラットに 0、60、250 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、血液学検査では 250 mg/kg bw/day 以上で雄に GOT 活性の上昇、1,000 mg/kg bw/day で雌に総コレステロールの減少が認められ、尿検査では雌雄にタンパクの増加、250 mg/kg bw/day 以上の雌で比重の上昇、1,000 mg/kg bw/day の雌で尿量の減少が認められた。1,000 mg/kg bw/day の雄の腎臓で絶対・相対重量の増加、雌の腎臓で相対重量の増加が認められ、組織学的には 250 mg/kg bw/day 以上の雄および 1,000 mg/kg bw/day の雌に腎臓の皮髄境界部における近位尿細管の腫大と両染性小体の沈着がみられた。また、250 mg/kg bw/day の雌雄に胃の境界縁粘膜の肥厚がみられた。これらの結果から、NOAEL は雌雄ともに 60 mg/kg bw/day とされた。

ラットに 0、100、300 または 1,000 mg/kg bw/day を 28 日間経皮投与した試験において、一般的な毒性は認められなかった。本物質には皮膚刺激性／腐食性があるので、塗布部位にのみ局所的影響がみとめられた。一般毒性の NOAEL は 1,000 mg/kg bw/day とされた。

ラットに 0、50、250 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、50 および 250 mg/kg bw/day の児において、大動脈や頸・肺動脈に動脈瘤が認められた。複数の追跡試験によると、これらの病変は出生後早期に現れ、生後 60 日までに動脈瘤は治癒していた。0、0.2、1、5 および 50 mg/kg bw/day を投与した追加試験（OECD TG 421 に類似）における動脈瘤の発生に基づき、LOAEL が 0.2 mg/kg bw/day とされた。また、1,000 mg/kg bw/day では児の分娩は認められず、発生毒性あるいは受胎率の低下によるのかは不明であるが、生殖への影響の可能性から、生殖毒性の NOAEL は 250 mg/kg bw/day とされた。

妊娠 9-19 日の妊娠ラットに 0、0.5、2、10 または 50 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、上述の試験（OECD TG 421）において 50 mg/kg bw/day でみられた顕著な毒性はみられず、母体毒性や胚や胎児への毒性は認められなかった。

細菌を用いる復帰突然変異試験およびチャイニーズ・ハムスター培養細胞を用いる染色体異常試験はともに陰性であり、また、*in vivo* 小核試験も陰性であった。その他、種々の遺伝毒性試験が行われたが、遺伝毒性の可能性を示す結果は認められなかった。

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4）結論と勧告

本物質は健康に対して有害性（皮膚や眼への刺激性、皮膚感作性、血管の発生毒性）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、環境に対して有害性（甲殻類への急性毒性）を示すが、容易に生分解し、魚類における濃縮性は低いので、環境影響については LP と勧告された。

2－2　SIAM 24 について

（1）2-sec-Butyl-4,6-dinitrophenol (88-85-7)（日本政府）

1）曝露状況

本物質は重合開始剤として使用されているが、日本では 2006 年以降生産されていない（2004 年には 215 トン、2005 年には 110 トン生産されていた）。農薬として使用されたことが過去にあり、本物質は容易に生分解しないので環境中に残留している可能性がある。概ね閉鎖系で使用されるので、職業/消費者曝露の可能性は低い。

2）環境影響

本物質が大気に放出された場合は土壌（約 60%）、大気（約 30%）、水圏（約 10%）に分布し、水圏に放出された場合は水圏（約 92%）と沈殿物（約 8%）に分布し、土壌に放出された場合は土壌（約 100%）に分布し、大気・土壌・水圏に放出された場合は土壌（約 80%）と水圏（約 17%）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：0.3～2.5）。

水生生物に対する急性毒性では、魚類の LC_{50} は 0.032-0.54 mg/L（48 時間または 96 時間）、ミジンコの EC_{50} は 0.24-0.74 mg/L（48 時間）、無脊椎動物（ヨコエビ）の LC_{50} は 1.8 mg/L（96 時間）、藻類の EC_{50} は 0.49-1.4 mg/L（72 時間、生長速度法）であった。慢性毒性では、魚類の NOEC は<0.0005 mg/L、ミジンコの NOEC は 0.062 mg/L（21 日間、繁殖阻害：OECD TG 211）、藻類の NOEC は 0.36 mg/L（72 時間、生長速度法：OECD TG 201）であった。

陸生高等植物への急性毒性が実地試験において認められたが、土壌中の曝露量の測定が困難であり、毒性評価は難しい。また、3 種の鳥類に 5 日間混餌投与して回復期間を 3 日間とした鳥類摂餌毒性試験（OECD TG 205）の結果、LC_{50} は 410- > 540 ppm であった。

3）健康影響

^{14}C 標識体を用いた試験について、本物質の経皮吸収は若齢／成熟雌ラットにおいて二相性を示し、その吸収量は 6 時間後には投与量の約 44%であったが、120 時間後では 75.9%（若齢）／92.5%（成熟）であった。120 時間後に成熟ラットで総回収量の約 70%が尿中に、約 16%が糞中に排出され、約 7%が体内に残留していた。24 時間後に成熟ラットの尿を分析したところ、親物質はほぼ代謝されていた。本物質由来の ^{14}C 濃度は血中において最高であった。

マウスの妊娠 11 日に ^{14}C を含む本物質を腹腔内投与または強制経口投与したところ、胎盤を通して胚に移行した（母体の血漿中濃度の 2.5%以下）。胚における ^{14}C の最高値は腹腔内と経口ともに同程度であったが、最高値に達するのは腹腔内投与のほうが著しく速い。母体では ^{14}C は全ての組織に移行した。薬物動態試験の消失速度定数は経口投与で 0.02/hr、腹腔内投与では 0.09/hr であった。投与経路にかかわらず、単回投与の 64 時間以内に、投与量の 67-78%が尿と糞で回収された。

単回投与における LD_{50} または LC_{50} は、吸入で 35-130 mg/m³（4 時間、ラット）、経皮で 40-146 mg/kg bw（ウサギ）、経口では 5-50 mg/kg bw（ラット）であった。

本物質にはウサギの眼に対する強い刺激性が認められた。

ラットに交配前 2 週間および交配期間を含め、雄では計 42 日間、雌では分娩後哺育 6 日まで、0、0.78、2.33 または 7 mg/kg bw/day を強制経口投与した反復投与毒性・生殖発生毒性併合試

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