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# Developmental Toxicity of Nitrophenolic Herbicide Dinoseb, 2-sec-butyl-4,6-dinitrophenol

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## 1. Introduction

Dinoseb (2-*sec*-butyl-4,6-dinitrophenol; CAS No. 88-85-7), a dark reddish-brown solid or dark orange viscous liquid, depending on the temperature (melting point: 32-42 °C) (Kidd & James, 1991), was approved for sale in the US in 1948 as a nitrophenolic herbicide in soybeans, vegetables, fruits, nuts, citrus and other field crops for the selective control of grass and broadleaf weeds (EXTOXNET, 1996; Schneider, 1986). Dinoseb is also used as an insecticide for grapes and as a seed crop drying agent (EXTOXNET, 1996). Dinoseb is one of the chemicals available on the market on the basis of safety tests conducted by Industrial Bio-Test Laboratory, a concern later found to have submitted many flawed and even fraudulent reports on its procedures and results (Shabecoff, 1986). Subsequently, several studies showed that dinoseb has the potential to produce developmental toxicity including teratogenicity in rats, mice and rabbits (Giavini et al., 1986; Johnson, 1988; Preache & Gibson, 1975a; Preache & Gibson, 1975b).

Dinoseb as a pesticide was banned in the US in 1986 and the EU in 1991 owing to the potential risk of adverse health effects in humans (EXTOXNET, 1996; Rotterdam Convention, 2006), but dinoseb and its salts are still widely used as other agricultural products (PAN, 2006). Dinoseb as a pesticide is also banned in Japan, but its import is permitted (PAN, 2006). The volumes of dinoseb imported into Japan were estimated to be 615 tons in fiscal year 2008 and 726 tons in fiscal year 2009 (NITE, 2009). Dinoseb is a high-volume chemical with production or importation exceeding 1,000 tons per year in Organisation for Economic Co-operation and Development (OECD) member countries (OECD, 2004).

Dinoseb is well absorbed from the gastrointestinal tract by the oral route and can pass through the placenta into the fetus in mice (Gibson & Rao, 1973). A dermal study showed that in six hours young and adult female rats absorbed about 44% of the dose, while at 120 hours 75.9% was absorbed in young and 92.5% in adults (Hall et al., 1992). Dinoseb shows relatively strong acute toxicity with an oral $LD_{50}$ of 5-50 mg/kg in female rats (MHLW, 2005), an intraperitoneal $LD_{50}$ of 14.1-22.5 mg/kg in mice (US EPA, 2003b) and a dermal $LD_{50}$ of 40 mg/kg in rabbits (US EPA, 2003b). Inhalation $LC_{50}$ is 33-290 mg/m$^3$ for 4-hour exposure in rats (US EPA, 2003b). The basic mechanism of toxicity is thought to be

stimulation of oxidative metabolism in cell mitochondria by the uncoupling of oxidative phosphorylation (Leftwich et al., 1982). Toxicity of dinoseb is enhanced by physical activity and high ambient temperature such as in an outdoor agricultural environment (Leftwich et al., 1982; US EPA, 2007). Early symptoms of dinoseb exposure include hyperthermia, sweating, headache and confusion. Severe exposure may result in restlessness, seizures, coma and death (Leftwich et al., 1982; US EPA, 2006; US EPA, 2007).

Exposure to dinoseb may occur by direct contact, ingestion or inhalation by users and producers, but indirect exposure to dinoseb via the environment is also anticipated. The microbial breakdown of dinoseb has been demonstrated in soils, but dinoseb persists for about two to four weeks after application (Health Canada, 1991). A soil persistence of 24 to 42 months was also observed in potato fields in Canada (O'Neill et al., 1989). It has been reported that dinoseb was detected in water supplies in Canada and the US, and dinoseb residues were found in a cotton meal sample (Health Canada, 1991).

Developmentally toxic effects of chemicals are influenced by the susceptibility of animal species and strains, the developmental stages of offspring and administration doses (Schardein, 2000; Wilson & Warkany, 1965). Teratogenicity is governed by dose-effect relations, but there are many variable factors such as the duration of chemical treatment (Wilson, 1966), frequency of dosing (Isaacson & Chaudhry, 1962), routes or modes of administration (Hansen & Billings, 1986; Kavlock et al., 1982; Kimmel, 1977; Staples et al., 1976), the vehicle/suspending agent (Anderson & Morse, 1966) or a combination of chemicals (Wilson, 1964). Dinoseb is one of the chemicals that show differences in developmental toxicity according to these variable factors. We have already reviewed studies on the developmental effects of dinoseb exposed prenatally in experimental animals (Matsumoto et al., 2008c). In the following sections, available literature including new information concerning the developmental toxicity of dinoseb is introduced by focusing on the variable factors for risk assessment of dinoseb. It should be noted that the term dinoseb has been used in the literature to refer to several related chemicals based on 2-*sec*-butyl-4,6-dinitrophenol (CAS: 88-85-7). In this chapter, dinoseb refers to the parent molecule only.

## 2. Developmental toxicity in rabbits

Table 1 shows the results of developmental toxicity of dinoseb in rabbits. There are gavage and dermal dose studies. The data are shown by routes of administration, in order of the most likely route of human intake. Only statistically significant effects are summarized unless noted otherwise.

### 2.1 Gavage study in rabbits
In a teratology study, 16 Chinchilla rabbits were exposed by gavage to dinoseb at 0 (vehicle: corn oil), 1, 3 or 10 mg/kg bw/day on gestation days **(GDs)** 6-18 (Research and Consulting Company, 1986). There were no differences in fetal body weight and sex ratio between the dinoseb-treated and control groups. In the highest dose group, there were neural tube defects including dyscrania associated with hydrocephaly, scoliosis, kyphosis, malformed or fused caudal or sacral vertebrae and/or encephalocele in a total of 40 fetuses in 11/16 litters. Eleven fetuses showed only hydrocephalus and/or micro- or anophthalmia, and 4 fetuses showed only skeletal abnormalities. No maternal death occurred. Body weight gain and food consumption in dams and number of implantations were not affected.

## 2.2 Dermal study in rabbits

In a New Zealand white rabbit study, 16-17 pregnant rabbits were dermally given dinoseb at 0, 1, 3, 9 or 18 mg/kg bw/day on GDs 7-19 (Johnson, 1988). The dinoseb (no vehicle was used) was dermally applied to rabbits wearing Elizabethan collars for 6 hours, and the application site was wiped and then dried. Because overt maternal toxicity was observed at 18 mg/kg bw/day and animals were also dying in the 9 mg/kg bw/day group, animals treated with dinoseb at the high dose were reassigned to the 9 mg/kg bw/day dose group and did not contribute to the evaluation. There were increased incidences of anophthalmia and hydrocephaly at 3 and 9 mg/kg bw/day. Dead and resorbed fetuses and fetuses with cleft palate, microphthalmia and microcephaly were increased at 9 mg/kg bw/day. At 3 mg/kg bw/day and higher, hyperthermia and reduced body weight were observed in maternal rabbits.

| Species (Reference) | Dose | Exposure time | Developmental effect |
|---|---|---|---|
| **Gavage** | | | |
| Chinchilla rabbit (Research and Consulting Company, 1986) | 10 mg/kg | GDs 6-18 | External, internal and skeletal defects |
| **Dermal** | | | |
| NZ white rabbit | 3 mg/kg | GDs 7-19, | Hydrocephaly, anophthalmia |
| | 9 mg/kg | 6 h/day | Dead and resorbed fetuses, cleft palate, microcephaly, microphthalmia |
| (Johnson, 1988) | | | |

Table 1. Developmental toxicity of dinoseb in rabbits
GDs: gestation days

# 3. Developmental toxicity of dinoseb in mice

Tables 2.1-2.3 show the results of developmental toxicity studies of dinoseb in mice. There are gavage, intraperitoneal (i.p.) and subcutaneous (s.c.) administration studies. The data are shown by routes of administration, in order of the most likely route of human intake. Only statistically significant effects are summarized unless noted otherwise.

## 3.1 Gavage studies in mice

Pregnant CD-1 mice were administered dinoseb in corn oil on GDs 8-12 at 15 mg/kg bw/day, the expected maximum tolerated dose level of dinoseb. No effects were observed in reproductive and developmental parameters (Chernoff & Kavlock, 1982). Pregnant CD-1 mice were given dinoseb in corn oil by gavage at 26 or 33 mg/kg bw on GD 7. Two out of 40 pregnant animals died at 33 mg/kg bw, but percent mortality and body weight of pregnant mice were not changed. An increased incidence of supernumerary ribs was observed in both dinoseb-treated groups. The authors noted that increased incidence of supernumerary ribs may be a response to a non-specific disruption in maternal status (Kavlock et al., 1985).

Administration of dinoseb to pregnant CD-1 mice by gavage on GDs 7-8 at 50 mg/kg bw/day in NaOH produced reduced fetal weight and increased incidence of fetuses with supernumerary ribs (71% in litters) without maternal death. The authors suggested that

supernumerary ribs are indicative of basic alterations in the development of the axial skeleton (Branch et al., 1996). A similar study conducted by Rogers et al. (2004) revealed a dose-related increased incidence of mouse fetuses with supernumerary ribs following maternal administration of dinoseb in NaOH at 50 mg/kg bw/day on GDs 7-8 and suggested that increased incidence of supernumerary ribs in fetuses is toxicologically significant. Skeletal anomalies such as sternum or vertebral centrum defects and fused ribs were also detected in fetuses of mice given dinoseb on GDs 7-8 at 50 mg/kg bw/day in NaOH. Although the treatment regimes of Branch et al. (1996) and Rogers et al. (2004) were essentially the same, they obtained different developmental effects in fetuses of mice given dinoseb at 50 mg/kg bw/day. Rogers et al. (2004) used 25 pregnant mice. On the other hand, Branch et al. (1996) used only two pregnant mice, which is too few to evaluate the developmental toxicity. Therefore, it appears that a gavage dosing of dinoseb on GDs 7-8 at 50 mg/kg bw/day can induce teratogenic effects without maternal toxicity in CD-1 mice.

Dinoseb was administered to pregnant Swiss-Webster mice during GDs 7-15, GDs 9-11 or GDs 13-15 by gavage up to 50 mg/kg bw/day in NaOH. Gavage dosing of dinoseb produced no increased incidence of gross or soft-tissue anomalies. When dinoseb was given by gavage on GDs 9-11, six out of eight pregnant animals died at 50 mg/kg bw/day, but no effects were observed on developmental parameters. Skeletal variations such as supernumerary ribs and vertebrae were observed after doses of 20 and/or 32 mg/kg bw/day during GDs 7-15. The fetal crown-rump length (CRL) was also reduced at 32 mg/kg bw/day after administration of dinoseb on GDs 7-15. A dose of 32 mg/kg bw/day dinoseb during GDs 13-15 induced absent or not ossified sternebrae. The dose levels that caused these adverse effects in fetuses were also lethal to some dams (Gibson, 1973).

| Species (Reference) | Dose | Exposure time | Developmental effect |
|---|---|---|---|
| CD-1 mouse (Chernoff & Kavlock, 1982) | 15 mg/kg | GDs 8-12 | No effects |
| CD-1 mouse (Kavlock et al., 1985) | 26, 33 mg/kg | GD 7 | Supernumerary ribs |
| CD-1 mouse (Branch et al., 1996) | 50 mg/kg | GDs 7-8 | Supernumerary ribs, ↓fetal weight |
| CD-1 mouse (Rogers et al., 2004) | 50 mg/kg | GDs 7-8 | Supernumerary ribs, sternum and vertebral centrum defects, fused ribs |
| SW mouse | 50 mg/kg | GDs 9-11 | No effects (6/8 dams died) |
| | 20 mg/kg 32 mg/kg | GDs 7-15 | Supernumerary ribs and vertebrae ↓fetal crown-rump length |
| (Gibson, 1973) | 32 mg/kg | GDs 13-15 | Absent or not ossified sternebrae |

Table 2.1. Developmental toxicity of dinoseb administered by gavage in mice
GDs: gestation days

### 3.2 Intraperitoneal studies in mice

No adverse effects were observed in reproductive and developmental parameters after an i.p. administration of dinoseb on GDs 7-15 at 5 mg/kg bw/day in Swiss-Webster mice; however, teratogenicity was obtained after i.p. administration of dinoseb on GDs 13-15 and GDs 9-11 (Gibson, 1973). An increased incidence of soft tissue malformation such as internal hydrocephalus was observed at 10-15.8 mg/kg bw/day in NaOH after i.p. treatment of dinoseb on GDs 9-11. At these doses, no maternal toxicity was observed. Increased incidences of defects in the limbs, tail, ribs, sternebrae and vertebrae, internal hydrocephaly and hydronephrosis were also induced at 17.7 mg/kg bw/day. Fetal body weight and number of fetuses were decreased at 18.8 mg/kg bw/day, and fetal CRL was decreased at 20.0 mg/kg bw/day. At 17.7-20.0 mg/kg bw/day, dinoseb produced hyperthermia and death in dams. Dinoseb at 12.5 and 17.7 mg/kg bw/day on GDs 13-15 caused increased resorptions and decreased fetal body weight, but not maternal toxicity. Unlike administration of dinoseb on GDs 9-11, teratogenicity was not observed after administration of dinoseb on GDs 13-15 up to 17.7 mg/kg bw/day.

In a later review study for perinatal nephropathies, Gibson (1976) stated that an incidence of 30-40% of fetuses with hydronephrosis was observed at cesarean section owing to i.p. administration of dinoseb on GDs 9-11; however, no grossly observable hydronephrosis was evident in pups at 1 or 2 weeks of age. Renal alteration observed in offspring of mice given dinoseb seems to be a transient dilatation of the renal pelvis, which is also suggested by studies in rats (Daston et al., 1988; McCormack et al., 1980), as described in 4.3. On the other hand, i.p. treatment of dinoseb on GDs 9-11 at 15.8 mg/kg bw/day caused a impairment in p-aminophippuric acid (PAH) uptake into renal cortical slices of offspring at one and two weeks of age, and this effect was also evident at seven weeks of age (Gibson, 1976).

Effects of food deprivation, phenobarbital (an inducer of chemical metabolism) and 2-diethylaminoethyl-2,2-diphenylvalerate hydrochloride (SKF-525A; an inhibitor of chemical metabolism) on the developmental toxicity of dinoseb were evaluated in Swiss-Webster mice (Preache & Gibson, 1975a). Pregnant mice were treated i.p. with dinoseb at doses of 0-18.8 mg/kg bw/day on GDs 9-11. These treatments were preceded by 24 or 48 h food deprivation or by pretreatment with phenobarbital or SKF-525A. Dinoseb-induced external and skeletal anomalies were increased by 24 h food deprivation. Effects of phenobarbital pretreatments on dinoseb-induced developmental toxicity were inconsistent at 17.7 and 18.8 mg/kg bw/day. At these doses, maternal death was not observed. Pretreatment with SKF-525A in combination with dinoseb at 15.8 mg/kg bw/day caused fetal anomalies, potentiated dinoseb-induced resorptions and produced maternal mortality. SKF-525A in combination with dinoseb at 17.7 mg/kg bw/day was markedly lethal maternally; however, developmental parameters could not be analyzed because of the small number of litters surviving. Therefore, it is likely that the proximate toxicant for maternal toxicity was dinoseb itself.

Swiss-Webster mice were treated with dinoseb on GDs 9-11 and maintained at an increased environmental temperature (32 °C) for 24 h or a decreased temperature (0-6 °C) for 1.5-4 h (Preache & Gibson, 1975b). Exposure to 32 °C enhanced adverse effects of dinoseb; it increased maternal mortality, decreased fetal body weight and increased the incidence of fetal anomalies at 7.5 mg/kg bw/day. Fetal body weight and the frequency of malformations were generally the same in groups exposed to low temperature and maintained at room temperature at 15.8-17.7 mg/kg bw/day. Maternal mortality was observed at doses that caused fetal toxicity. On the basis of these results, higher temperature enhanced the maternal and developmental toxicity of dinoseb.

| Species (Reference) | Dose | Exposure time | Developmental effect |
|---|---|---|---|
| SW mouse | 10 mg/kg | GDs 9-11 | Soft-tissue malformation |
|  | 17.7 mg/kg |  | Gross and skeletal malformations |
|  | 18.8 mg/kg |  | ↓Fetal body weight, no. of fetuses, resorption |
|  | 20 mg/kg |  | ↓Fetal crown-rump length |
|  | 12.5-17.7 mg/kg | GDs 13-15 | ↓Fetal body weight, ↑resorption |
|  | 5 mg/kg | GDs 7-15 | No effects |
| (Gibson, 1973) |  |  |  |
| SW mouse (Gibson, 1976) | 15.8 mg/kg | GDs 9-11 | ↓PAH uptake by renal cortical slices |
| SW mouse | 15.8 mg/kg | GDs 9-11 | Hydronephrosis, ectrodactyly, resorption |
|  | 17.7, 18.8 mg/kg (combination with SKF-525A) | GDs 9-11 | Hydronephrosis |
|  | 14.1 mg/kg 15.8 mg/kg 17.7 mg/kg 18.8 mg/kg (combination with phenobarbital) | GDs 9-11 | Delayed ossification External malformations Hydronephrosis ↓Fetal body weight, resorption |
|  | 14.1 mg/kg 15.8 mg/kg (24 h deprivation) | GDs 9-11 | Delayed ossification, ↓fetal body weight Hydronephrosis, ectopic kidney, internal hydrocephalus, External and skeletal malformations |
|  | 14.1 mg/kg 15.8 mg/kg (48 h deprivation) | GDs 9-11 | ↓Fetal body weight, External and skeletal malformations |
| (Preache & Gibson, 1975a) |  |  |  |
| SW mouse | 7.5 mg/kg (32°C) | GDs 9-11 | ↓Fetal body weight, external, soft-tissue and skeletal malformations, delayed ossification |
|  | 15.8 mg/kg (Room temp; wet) | GDs 9-11 | ↓Fetal body weight, external and soft-tissue malformations, resorption |
|  | 15.8 mg/kg (6°C; wet) | GDs 9-11 | ↓Fetal body weight, external and soft-tissue malformations |
|  | 15.8 mg/kg 17.7 mg/kg (Room temp; dry) | GDs 9-11 | ↓Fetal body weight External and soft-tissue malformations, skeletal retardation, variation and malformation |
|  | 17.7 mg/kg (6°C; dry) | GDs 9-11 | ↓Fetal body weight, external and soft-tissue malformations, skeletal retardation, variation and malformation |
| (Preache & Gibson, 1975b) |  |  |  |

Table 2.2. Developmental toxicity of dinoseb administered by intraperitoneally in mice
GDs: gestation days

### 3.3 Subcutaneous study of dinoseb

Dinoseb was subcutaneously administered to pregnant Swiss-Webster mice during GDs 8-16, 10-12 or 14-16 at 0, 10 or 17.7 mg/kg bw/day (Gibson, 1973). Adverse effects were observed only at 17.7 mg/kg bw/day. Dinoseb on GDs 14-16 induced increases in resorption rate and the incidence of cleft palate and decreases in the number of live fetuses, fetal CRL and fetal body weight. At this dose, one out of eight dams died. Dinoseb on GDs 10-12 induced an increase in the incidence of fused ribs/vertebrae and absent or not ossified sternebrae, and on GDs 8-16 induced supernumerary ribs/vertebrae, absent or not ossified sternebrae, decreased fetal body weight and decreased fetal CRL without maternal toxicity. The authors concluded that an s.c. dose of dinoseb was not teratogenic and cleft palate induced by treatment of dinoseb was not considered as a toxicological response because this anomaly was not found in any i.p. treated groups, as described in 3.2, or in other s.c. treatment groups given 17.7 mg/kg bw/day. However, this anomaly can be considered as a toxic effect because the incidence of cleft palate was statistically significant, and other later studies showed that i.p. dose of dinoseb induced cleft palate in mice (Preache & Gibson, 1975b) and in rabbits (Johnson, 1988). Moreover, a recent survey by international experts in the field of reproductive/developmental toxicology resulted in strong agreement that fused ribs and vertebrae can be considered as malformations (Solecki et al., 2001). Therefore, it can be concluded that an s.c. dosing of dinoseb in mice may have the potential to produce teratogenic effects in the same way as i.p. dosing of dinoseb.

| Species (Reference) | Dose | Exposure time | Developmental effect |
|---|---|---|---|
| SW mouse | 17.7 mg/kg | GDs 10-12 | Fused ribs and vertebrae, absent or not ossified sternebrae |
| | 17.7 mg/kg | GDs 14-16 | Cleft palate, ↑resorption, ↓no. of fetuses, ↓fetal body weight, ↓fetal crown-rump length |
| (Gibson, 1973) | 17.7 mg/kg | GDs 8-16 | Skeletal variations, ↓fetal body weight, ↓fetal crown-rump length, absent or not ossified sternebrae |

Table 2.3. Developmental toxicity of dinoseb administered by subcutaneously in mice
GDs: gestation days

## 4. Developmental toxicity in rats

Tables 3.1-3.3 show the results of developmental toxicity studies of dinoseb in rats. There are oral (gavage and diet) and i.p. administration studies. The data are shown by routes of administration, in order of the most likely route of human intake. Only statistically significant effects are summarized unless noted otherwise.

### 4.1 Gavage studies in rats

In our previous study, male Crj:CD(SD)IGS rats were administered dinoseb by gavage for a total of 42 days beginning 14 days before mating and females underwent this treatment for a

total of 44-48 days beginning 14 days before mating to day 6 of lactation at 0 (vehicle: corn oil) , 0.78, 2.33 or 7.0 mg/kg bw/day (Matsumoto et al., 2008a). As for the developmental parameters, no changes attributable to the chemical were noted in the 0.78 and 2.33 mg/kg bw/day dose groups. Eight of twelve females died and two animals were moribund during late pregnancy at 7.0 mg/kg bw/day. Developmental toxicity of dinoseb was not precisely estimated because only one dam with live pups was obtained at the highest dose, and newborn rats were only examined externally. No increased incidence of pups with an external malformation was noted in the dinoseb-treated groups.

In teratology studies in rats, skeletal variation, delayed ossification and/or decreased fetal body weight was commonly observed in fetuses of dams treated with dinoseb. Giavini et al. (1986) administered dinoseb to pregnant CD rats by gavage in corn oil either once a day on GDs 5-14 at 0, 2.5, 5, 10 or 15 mg/kg bw/day or twice a day on GDs 5-12 at 15 (7.5 x 2) or 20 (10 x 2) mg/kg bw/day. Dinoseb was also administered to pregnant rats on GDs 5-12 at 15 mg/kg bw/day in NaOH. This vehicle was selected to conform to a vehicle used in a study by Gibson (1973) in which dinoseb in NaOH showed teratogenicity in mice when administered i.p. but not by gavage. An increased incidence of supernumerary ribs was observed at 10 mg/kg bw/day and higher, and fetal weight was decreased at 15 and 20 mg/kg bw/day regardless of frequency of dosing or vehicle. Delayed ossification of caudal vertebrae, metacarpals or sternebrae was observed at a single dose of 15 mg/kg bw/day (in both corn oil and NaOH). These doses also caused maternal toxicities such as mortality and decrease in body weight gain. No malformations were observed in fetuses of dams treated with dinoseb under the test condition regardless of the dosing regimen or vehicle used in the experiment.

Fetal body weight was decreased when pregnant Crl:CD rats were given dinoseb at 15 mg/kg bw/day with diet A (protein 21%, fat 3.5%, fiber 6.5%, ash 7.5% and N-free extractives 61.5%; Italiana Mangimi, Settimo Milanese, Italy) and diet B (protein 21%, fat 4.8%, fiber 4.2%, ash 8.5% and N-free extractives 61.5%; Mangimi Piccioni, Gessate, Italy) on GDs 5-13 (Giavini et al., 1989). Dinoseb induced microphthalmia in fetuses of animals fed diet B but did not induce maternal toxicity. Maternal mortality and decreased maternal body weight gain were observed when dinoseb was given with diet A. Although developmental toxicity was different according to the type of diet, there were no differences in dinoseb concentrations in maternal plasma and in embryos between the two dietary groups.

Wistar/Han rats were administered dinoseb by gavage on GDs 6-15 at 0, 1, 3 or 10 mg/kg bw/day (Health Canada, 1991). No information on the vehicle was presented in this study. Only slight depressions were observed in food consumption and body weight gain of dams at 10 mg/kg bw/day. Fetuses at the highest dose showed a slight decrease in body weight, and increases in the incidence of delayed ossification and incidence of skeletal variations, especially supernumerary ribs. At 3 mg/kg bw/day and higher, absence of thoracic vertebrae was observed. No further information is available for this study, but the result indicates that dosing of dinoseb by gavage is hazardous in Wistar/Han rats.

The details of our new findings (Matsumoto et al., 2010) shown in Table 3.1 are described below (see 4.4).

## 4.2 Feeding studies in rats

Feeding of dinoseb to CD rats on GDs 5-14 produced a specific teratogenic effect, increased incidence of fetuses with microphthalmia, reduced fetal weight and increased incidence of fetuses with supernumerary ribs at 200 ppm (15 mg/kg bw/day) accompanied by decreased maternal body weight gain (Giavini et al., 1986). An increased incidence of fetuses

| Species (Reference) | Dose | Exposure time | Developmental effect |
|---|---|---|---|
| Crj:CD(SD) IGS rat (Matsumoto et al., 2008a) | 7 mg/kg | 44-48 days | ↓ No. of dams delivered, ↓no. of dams with live pups at delivery |
| CD rat | 10 mg/kg 15 mg/kg (in corn oil) | GDs 5-14 | Skeletal variations Delayed ossification, ↓fetal body weight |
| | 7.5, 10 mg/kg (twice/day) | GDs 5-12 | Skeletal variations, ↓fetal body weight |
| (Giavini et al., 1986) | 15 mg/kg (in NaOH) | GDs 5-12 | Skeletal variations, delayed ossification ↓fetal body weight |
| Crl:CD rat | 15 mg/kg (with diet B) | GDs 5-13 | ↓Fetal body weight, microphthalmia |
| (Giavini et al., 1989) | 15 mg/kg (with diet A) | GDs 5-13 | ↓ Fetal body weight |
| Wistar/Han rat [a] (Health Canada, 1991) | 3 mg/kg 10 mg/kg | GDs 6-15 | Absence of thoracic vertebrae, Absence of thoracic vertebrae, skeletal variations |
| SPF Crl:CD (SD) rat [b] | 8.0 mg/kg | GDs 6-15 | ↓Fetal body weight, skeletal variations, delayed ossifications |
| (Matsumoto et al., 2010) | 10 mg/kg | | ↓ Fetal body weight, skeletal variations, delayed ossifications, microphthalmia |

Table 3.1. Developmental toxicity of dinoseb administered by gavage in rats
a: only secondary literature or abstract is available.
b: the details are described in 4.4
GDs: gestation days

with microphthalmia and reduced fetal weight were also observed when pregnant Crl:CD rats were given dinoseb in diet B at 200 ppm on GDs 5-13. At this dose, maternal food consumption and body weight gain were decreased compared with those of control groups (Giavini et al., 1989). When dinoseb was fed with diet A, maternal food consumption and body weight gain were reduced, but no effects were found in fetuses (Giavini et al., 1989). These findings indicate that the developmental toxicity, including teratogenicity, of dinoseb in rats was influenced by diet composition (see 4.1 for the compositions of diet A and diet B).

Following feeding of dinoseb on GDs 5-14 at 0, 50, 100, 150, 200, 250, 300 and 350 ppm (0, 3.26, 6.9, 9.23, 10.86, 9.38, 9.49 and 8.6 mg/kg bw/day) in SD rats, the number of resorptions at 200-350 ppm, early embryo loss at 200-350 ppm, and total intra-uterine loss at 150-350 ppm were increased in a dose-related manner (Spencer & Sing, 1982; US EPA, 2003b). Body weight gain in dams was decreased at 150-350 ppm. At 200 ppm, hypoplastic tail was observed in 8 out of 62 fetuses and fetal weight was decreased. In decidualized females given dinoseb on days 7-10 of pseudopregnancy, uterine protein and glycogen concentrations were decreased at 200 ppm and higher in a dose-related manner. The authors suggested a toxic role of dinoseb in the uterine environment.

Hall et al. (1978) provided a brief summary of a subchronic feeding study in which Sherman male and female rats were fed a diet containing dinoseb at 0, 50, 100, 150, 200, 300, 400 and 500 ppm for 153 days. The 300, 400 and 500 ppm groups were terminated at day 21 of administration owing to mortality of 14, 100 and 100%, respectively, and only animals fed dinoseb up to 200 ppm were evaluated. Fertility, fecundity, neonate survival, weight gain, viability and lactation were depressed. No further details are available.

In an unpublished five-generation study, decreased body weight gains were observed in parents during the pre-mating period (F0, F1 and F2) at 10 mg/kg bw/day dinoseb in the diet and in pups on postnatal day **(PND)** 21 (F1, F2 and F3) at 1, 3 and 10 mg/kg bw/day, but weights at birth were similar to the controls. Body weight gain in F4 and F5 pups was increased and absolute and relative gonadal weights in F4 pups were decreased at all dose levels. A low viability index was obtained (from F4 to F5) at all dose levels. No detailed information is available for this study (Health Canada, 1991; US EPA, 2003a).

The details of our new findings (Matsumoto et al., 2010) shown in Table 3.2 are described below (see 4.4).

### 4.3 Intraperitoneal studies in rats

Two i.p. studies in rats showed similar results on developmental toxicity. When dinoseb was given to SD rats on GDs 9-11 at doses up to 15.8 mg/kg bw/day in NaOH, all pregnant rats given dinoseb at 11.2 mg/kg bw/day and higher and three of the 16 pregnant rats at 9.0 mg/kg bw/day died. There were dilated renal pelvis and ureters in fetuses, decreased body weight in fetuses, and pathological changes in the liver and kidney in both fetal and neonatal rats at 8.0 mg/kg bw/day without maternal toxicity. At 9.0 mg/kg bw/day, fetal CRL was decreased, and neonatal body weight was decreased on PNDs 1 and 7 but not on PND 42. In surviving dams, dinoseb did not affect the number of live fetuses or the resorption rate in surviving dams (McCormack et al., 1980).

When dinoseb was administered i.p. to pregnant SD rats on GDs 9-11 or 10-12 at 0-18.0 mg/kg bw/day in NaOH, fetal body weight was decreased at 7.5 mg/kg bw/day and higher, but weights at birth and on PND 6 were not affected. Maternal death was observed at 8.0 mg/kg bw/day and higher, and 10.5 mg/kg bw/day was an approximate $LD_{50}$ in pregnant rats. Postnatal observation on PND 30 revealed that there was a body weight reduction and an increase in relative kidney weight at 10.5 mg/kg bw/day. On PND 6, there were a deficit in urinary concentrating ability in pups of dams given dinoseb on GDs 9-11 at 10.5 mg/kg bw/day (Daston et al., 1988).

As described above, dinoseb produced suggestive renal damage in rat offspring following maternal administration. However, pathological changes in the kidney observed in prenatal rats were reduced in incidence or not detected at 42-day postpartum in the study of

| Species (Reference) | Dose | Exposure time | Developmental effect |
|---|---|---|---|
| CD rat (Giavini et al., 1986) | 200 ppm (15 mg/kg) | GDs 5-14 | ↓Fetal body weight, microphthalmia, skeletal variations |
| Crl:CD rat | 200 ppm (diet B) | GDs 5-13 | ↓Fetal body weight, microphthalmia |
| (Giavini et al., 1989) | 200 ppm (diet A) | GDs 5-13 | No effects |
| SD rat | 150 ppm (9.23 mg/kg) | GDs 5-14 | ↑Total intra-uterine loss |
| (Spencer & Sing, 1982; US EPA, 2003b) | 200 ppm (10.86 mg/kg) | | ↑Early embryonic loss, resorptions, ↓fetal body weight, hypoplastic tail |
| Sherman rat [a] (Hall et al., 1978) | < 200 ppm | 153 days | ↓ Fertility, ↓fecundity, ↓neonate survival, ↓body weight gain, ↓viability, ↓ lactation |
| CD (SD) rat [a] | 1, 3, 10 mg/kg | 3-generation | ↓Body weight gain in pups (F1, F2, F3) |
| (Health Canada, 1991; US EPA, 2003a) | | Next 2-generation | ↓Body weight gain in pups (F4, F5), ↓absolute/relative gonadal weight (F4), ↓viability index (F5) |
| SPF Crl:CD (SD) rat [b] | 120 ppm (6.52 mg/kg) | GDs 6-16 | ↓Fetal body weight |
| (Matsumoto et al., 2010) | 10 mg/kg (8.0 mg/kg) | | ↓Fetal body weight, skeletal variations, delayed ossifications |

Table 3.2. Developmental toxicity of dinoseb administered in diet in rats
a: only secondary literature or abstract is available
b: the details are described in 4.4
GDs: gestation days

McCormack et al. (1980). In the study of Daston et al. (1988) a deficit in urinary concentrating ability observed during postnatal development also disappeared after functional maturation (PND 30). Prenatal incidence of dilated renal pelvis was not dose-dependent. Moreover, Woo and Hoar (1972) noted that the renal parenchyma increased in weight rapidly, but that the renal papilla increased in length solely during late pregnancy, and they suggested that this discrepancy in growth rate frequently resulted in the kidney with an enlarged renal pelvis. Taken together, these renal effects appear to be a developmental delay, but not a permanent functional impairment.

| Species (Reference) | Dose | Exposure time | Developmental effect |
|---|---|---|---|
| SD rat | 8.0 mg/kg | GDs 9-11 | ↓Fetal body weight, dilated renal pelvis and ureters in fetuses Pathological changes in liver and kidney in fetuses and neonates |
| | 9.0 mg/kg | | ↓Fetal crown-rump length, ↓ neonatal body weight |
| (McCormack et al., 1980) | | | |
| SD rat | 7.5 mg/kg 10.5 mg/kg | GDs 9-11, 10-12 | ↓Fetal body weight Functional defect of kidney (PND 6), ↓body weight in pups (PND 30), ↑relative weight of kidney (PND 30) |
| (Daston et al., 1988) | | | |

Table 3.3. Developmental toxicity of dinoseb administered by intraperitoneally in rats
GDs: gestation days
PND: postnatal day

## 4.4 Further clarification of teratogenicity in rats

Several studies including our previous study did not demonstrate the teratogenicity of dinoseb in rats (Daston et al., 1988; Matsumoto et al., 2008a; McCormack et al., 1980), but we considered that the teratogenic potential of dinoseb in rats was unclear because of the influence of variable factors. Because detailed test conditions were not described in the studies of Giavini et al. (Giavini et al., 1989; Giavini et al., 1986), adequate experimental conditions for the production of fetal malformations by the administration of dinoseb to pregnant rats remained unknown. Therefore, we recently conducted gavage and feeding studies to clarify the experimental conditions that produce fetal malformations when dinoseb is given to pregnant rats (Matsumoto et al., 2010).

Pregnant rats (12 animals/group) were given dinoseb by gavage at 0, 8.0 or 10 mg/kg bw/day on GDs 6-15 or in the diet (CRF-1; protein 22%, fat 5.7%, fiber 2.9%, ash 6.3% and N-free extractives 55.3%; Oriental Yeast Co., Ltd., Tokyo, Japan) at 0, 120 or 200 ppm on GDs 6-16 (Figure 1). The feeding dose groups were expected to consume similar amounts of dinoseb to those in the gavage groups. Dinoseb induced dose-dependent decreases in maternal body weight gain and food consumption during pregnancy in all the dinoseb-treated groups. The decrease in food consumption was greater in the feeding dose groups than the gavage dose groups; therefore, the decreased food consumption may be related to a reduced palatability of the diet in the feeding groups. Intakes of dinoseb by feeding dose were estimated to be 0, 6.52 and 8.50 mg/kg bw/day (0, 120 and 200 ppm).

Significantly decreased body weights of fetuses were observed in all the dinoseb-treated groups, except for the group fed dinoseb at 120 ppm. Skeletal examinations of fetuses revealed an increased incidence of fetuses with skeletal variations in all the dinoseb-treated groups and delayed ossification at 8.0 and 10 mg/kg bw/day and at 200 ppm. An increased incidence of fetuses with microphthalmia was observed at 10 mg/kg bw/day, but there was no increased incidence of fetuses with external, internal or skeletal malformations in the groups given dinoseb at 8.0 mg/kg bw/day by gavage or 120 or 200 ppm by feeding (Table 3.1 and 3.2).
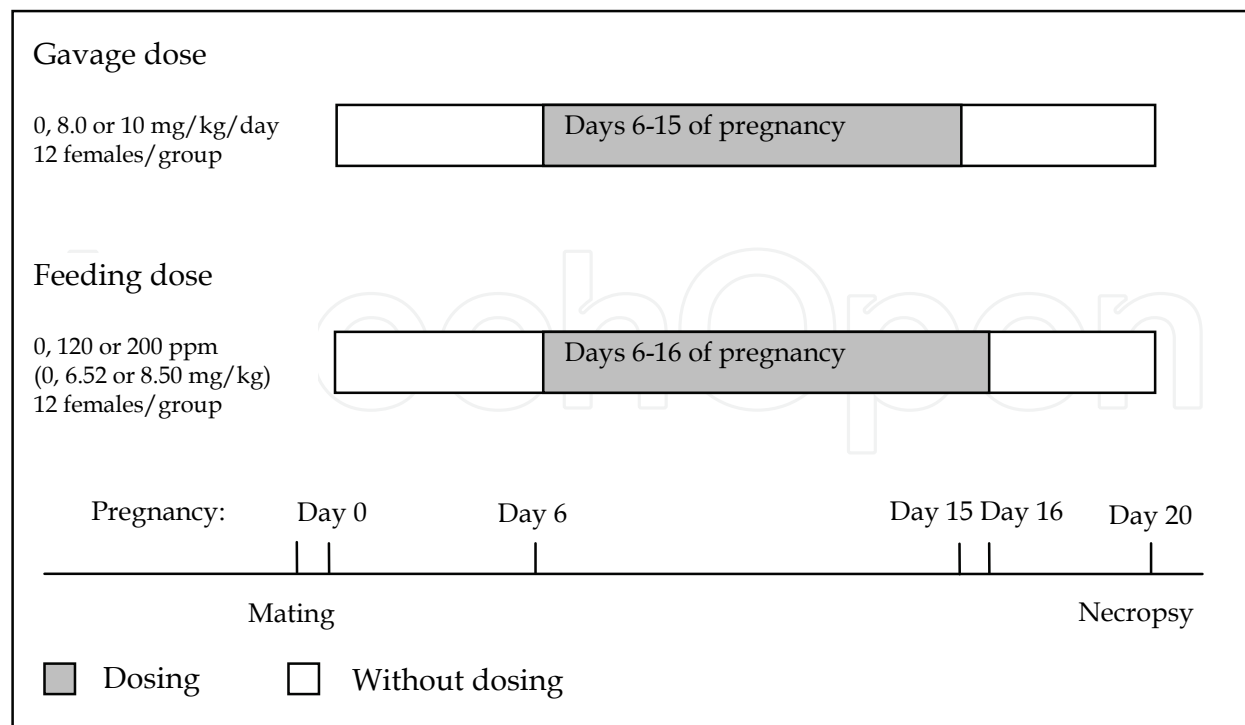
Fig. 1. A study design of prenatal developmental toxicity of gavage or feeding doses of dinoseb in rats (Matsumoto et al., 2010)

Although the feeding dose of dinoseb at 200 ppm (15 mg/kg bw/day) was previously reported to be teratogenic in rats (Giavini et al., 1986), the feeding dose of dinoseb up to 200 ppm (8.5 mg/kg bw/day) did not induce teratogenicity in our study. The diets used in the studies of Giaini et al. did not meet the current nutrient requirement of rats for fat (more than 5%) (ILAR, 1995; Suckow et al., 2005) while the diet used in our study is a standard rat diet; however, fat concentration seems unrelated to dinoseb-induced teratogenicity, and it seemed impossible to identify the definitive dietetic factor involved. Dose levels of dinoseb in our study might not have been sufficiently high to induce teratogenicity; however, pregnant rats did not consume sufficiently high amounts of dinoseb to produce fetal malformations because food consumption was reduced in the feeding groups. It seems unlikely that a feeding study is appropriate to evaluate the toxicity of dinoseb.

Microphthalmia, which was found in rats after exposure to dinoseb by gavage or feeding (Giavini et al., 1989; Giavini et al., 1986) and in rabbits by gavage (Research & Consulting Company, 1986) or dermal application (Johnson, 1988), was predominantly observed after administration of dinoseb at 10 mg/kg bw/day by gavage. As a rule, the administration of a suitable dosage of a teratogen generally results in the production of some normal offspring, some malformed offspring and some dead or resorbed offspring (Schardein, 2000). In our study, the increased incidence of malformed fetuses was not accompanied by an increased incidence of intrauterine deaths of offspring after the administration of dinoseb. This phenomenon was also observed in the previous studies of Giavini et al. (Giavini et al., 1989; Giavini et al., 1986). One possible explanation for this is that microphthalmia itself is not lethal in utero. Because maternal death was observed after the gavage dose of dinoseb at 10 mg/kg bw/day, the exposure range of dinoseb where malformations are observed seems to be narrow in rats. The findings of our study confirmed the experimental condition that could induce malformation in rats fed a standard diet.

## 5. Discussion and conclusions

A difficulty lies in the risk assessment of chemical compounds for developmental toxicity because there are many variable factors in the manifestation of developmental toxicity of chemicals. The administration route is one of the definitive factors for risk assessment of chemicals. The data obtained from animal experiments by oral administration are the most important for risk assessment of chemicals because the oral route is the most relevant route for human exposure to dinoseb.

Gavage dosing of dinoseb during organogenesis in rabbits produced external, internal and skeletal malformations in fetuses without maternal toxicity at 10 mg/kg bw/day (Health Canada, 1991; US EPA, 2003a). In mice, gavage dosing of dinoseb during organogenesis induced skeletal variations and growth retardation at or above maternally toxic levels (26-50 mg/kg bw/day) (Branch et al., 1996; Kavlock et al., 1985). Teratogenic effects were observed without maternal toxicity at 50 mg/kg bw/day by gavage in CD-1 mice (Rogers et al., 2004). Doses of dinoseb in rats during organogenesis induced skeletal variations and growth retardation at maternally toxic levels (8.0-20 mg/kg bw/day) by gavage and (6.52-15 mg/kg bw/day) by feeding (Giavini et al., 1986; Matsumoto et al., 2010). Malformations such as microphthalmia or hypoplastic tail were observed when dinoseb was given in the diet (10.86-15 mg/kg bw/day) with maternal toxicity (Giavini et al., 1989; Giavini et al., 1986; Spencer & Sing, 1982), but not in our study (Matsumoto et al., 2010). Microphthalmia was also observed when dinoseb was given by gavage (8.0-15 mg/kg bw/day) in CD rats with maternal toxicity (Giavini et al., 1989; Giavini et al., 1986; Matsumoto et al., 2010). In Wistar/Han rats, absence of thoracic vertebrae was observed by gavage dose of dinoseb at 3 mg/kg bw/day and higher without maternal toxicity. No detailed test condition is available for this study, but genetic difference in strains of rats may also influence the teratogenic potential of dinoseb. Although there are differences in susceptibility of developmental toxicity by the oral route among rabbits, mice and rats, namely susceptibility to developmental toxicity caused by dinoseb was greater in rabbits than in rats and mice, teratogenicity was noted at some doses without maternal toxicity in these animal species. More precisely, dinoseb can be a selective teratogen in these animal species.

Dermal exposure is the next most likely route of exposure to dinoseb in humans, especially in users and producers. A dermal teratology study in rabbits showed a markedly increased incidence of dead and resorbed fetuses (Johnson, 1988). The survivors exhibited a high incidence of external and soft tissue malformations at application levels of dinoseb, but these dose levels were also maternally toxic.

Prenatal i.p. and s.c. doses of dinoseb induced growth retardation, embryolethality and/or teratogenicity at or over the maternally toxic dose levels (10-20 mg/kg bw/day) in Swiss-Webster mice (Gibson, 1973; Preache & Gibson, 1975a; Preache & Gibson, 1975b). Prenatal i.p. dose of dinoseb did not induce teratogenicity but induced growth retardation at or above the maternally toxic level in rats (Daston et al., 1988; McCormack et al., 1980). The teratogenic effects were observed with or without maternal toxicity in rats and mice, but the maternal toxicity of dinoseb seems greater in rats than in mice because dinoseb treatment (i.p.) during GDs 10-12 at 9.0 mg/kg bw/day caused 3/16 maternal deaths in rats (McCormack et al., 1980) while no maternal toxicity was observed at 15.8 mg/kg bw/day after i.p. dosing of dinoseb during GDs 10-12 in mice (Gibson, 1973). This may explain why teratogenicity was induced in mice, but not in rats, after i.p. dosing of dinoseb. It can be considered that maternal mice were tolerant to dose levels that can produce fetal malformations. Prenatal i.p. and s.c. doses of dinoseb also showed teratogenic potentials; however, these exposure routes are not likely to be relevant to human exposure to dinoseb and may not be important for risk assessment of dinoseb.

The developmental toxicity of dinoseb was also influenced by administration methods. These effects are considered to be related to differences in absorption due to the concentration of the chemical, duration of exposure and rate of release or to differences in metabolic fate and the nature of the metabolites reaching the embryo (Kalter, 1968). In fact, food deprivation for 24 h that enhanced external, soft-tissue and skeletal malformations slowed the disappearance of dinoseb from the plasma, but phenobarbital, which reduced developmental toxicity, hastened the disappearance of dinoseb from the plasma. SKF-525A pretreatment, which enhanced both maternal and developmental toxicity, decreased the rate of disappearance from the liver (Preache & Gibson, 1975a). When pregnant mice were administered dinoseb, either i.p. at 17.7 mg/kg bw or by gavage at 32 mg/kg bw, the amount of dinoseb and its metabolites present in the embryo was greater after i.p. than oral administration, and peak levels were reached much earlier after i.p. administration (8 min vs. 12 h for oral) (Gibson & Rao, 1973). Developmental effects of i.p. dosing of dinoseb in mice can be related to rapid and relatively extensive uptake of the compound or its metabolites by the embryo.

Over the years, many investigations have been conducted using laboratory animals to assess the risk to humans. We here reiterate the importance of the administration method to extrapolate laboratory results to humans. We showed that fetal malformations by dinoseb were produced by the anticipated routes of human exposure (oral and dermal exposure) in laboratory animals. These results for routes/modes of administration relevant to human intake should be used for human risk assessment.

There is no clear understanding of the fundamental mechanism of developmental toxicity of dinoseb, although an energy-deficient intrauterine environment due to uncoupling of cellular oxidative phosphorylation may explain dinoseb-induced developmental toxicity. A prenatal dose of thiabendazole, an ATP-synthesis inhibitor, induced a deformity involving reduced limb size in mice fetuses (Ogata et al., 1984), and ATP levels in fore- and hindlimb buds of fetuses were related to the incidence of this deformity (Tsuchiya & Tanaka, 1985). Dinoseb-induced teratogenicity may be related to the degree of reduction in ATP expression influenced by variable factors.

Recent studies have investigated the role that mitochondria play in mediating apoptotic signals (Green & Kroemer, 2004; Linsinger et al., 1999; Little & Mirkes, 2002). Programmed cell death **(PCD)** is an essential component of normal physiological processes such as embryogenesis and normal tissue development (Vaux & Korsmeyer, 1999). Altering normal patterns of PCD could be teratogenic because areas of the body with a high incidence of malformations coincide with areas where PCD occurs (Knudsen, 1997; Sulik et al., 1988). Some studies showed a positive correlation between mitochondrial uncoupling activity and PCD (Maccarrone et al., 2001; Maccarrone et al., 2003), and 2,4-dinitrophenol, an uncoupling agent, enhanced the Fas apoptotic signal in Jurkat Bcl-2 cells (Linsinger et al., 1999). These findings imply that the enhanced uncoupling of oxidative phosphorylation in mitochondria may alter normal patterns of PCD. However, the link between malformations and mitochondrial uncoupling activity is still poorly understood. In addition, we previously showed that these apoptotic activities could also involve in testicular toxicity of dinoseb in rats and mice (Matsumoto et al., 2008b). Further mechanistic studies are necessary to clarify the toxicity of dinoseb.

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**Herbicides and Environment**

Edited by Dr Andreas Kortekamp

Herbicides are much more than just weed killers. They may exhibit beneficial or adverse effects on other organisms. Given their toxicological, environmental but also agricultural relevance, herbicides are an interesting field of activity not only for scientists working in the field of agriculture. It seems that the investigation of herbicide-induced effects on weeds, crop plants, ecosystems, microorganisms, and higher organism requires a multidisciplinary approach. Some important aspects regarding the multisided impacts of herbicides on the living world are highlighted in this book. I am sure that the readers will find a lot of helpful information, even if they are only slightly interested in the topic.

**How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Mariko Matsumoto, Akihiko Hirose and Makoto Ema (2011). Developmental Toxity of Nitrophenolic Herbicide Dinoseb, 2-Sec-Butyl-4,6-Dinitrophenol, Herbicides and Environment, Dr Andreas Kortekamp (Ed.), ISBN: 978-953-307-476-4, InTech, Available from: http://www.intechopen.com/books/herbicides-and-environment/developmental-toxicity-of-nitrophenolic-herbicide-dinoseb-2-sec-butyl-4-6-dinitrophenol

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