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 9 mg/kg	GDs 7-19, 6 h/day	Hydrocephaly, anophthalmia 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	GDs 9-11	Delayed ossification
	15.8 mg/kg		External malformations
	17.7 mg/kg		Hydronephrosis
	18.8 mg/kg (combination with phenobarbital)		↓Fetal body weight, resorption
	14.1 mg/kg	GDs 9-11	Delayed ossification, ↓fetal body weight
	15.8 mg/kg (24 h deprivation)		Hydronephrosis, ectopic kidney, internal hydrocephalus, External and skeletal malformations
	14.1 mg/kg	GDs 9-11	↓Fetal body weight,
	15.8 mg/kg (48 h deprivation)		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	GDs 9-11	↓Fetal body weight
	17.7 mg/kg (Room temp; dry)		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
	15 mg/kg (in NaOH)	GDs 5-12	Skeletal variations, delayed ossification ↓fetal body weight
(Giavini et al., 1986)			
Crl:CD rat	15 mg/kg (with diet B)	GDs 5-13	↓Fetal body weight, microphthalmia
	15 mg/kg (with diet A)	GDs 5-13	↓ Fetal body weight
(Giavini et al., 1989)			
Wistar/Han rat [a]	3 mg/kg 10 mg/kg	GDs 6-15	Absence of thoracic vertebrae, Absence of thoracic vertebrae, skeletal variations
(Health Canada, 1991)			
SPF Crl:CD (SD) rat [b]	8.0 mg/kg	GDs 6-15	↓Fetal body weight, skeletal variations, delayed ossifications
	10 mg/kg		↓ Fetal body weight, skeletal variations, delayed ossifications, microphthalmia
(Matsumoto et al., 2010)			

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

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).

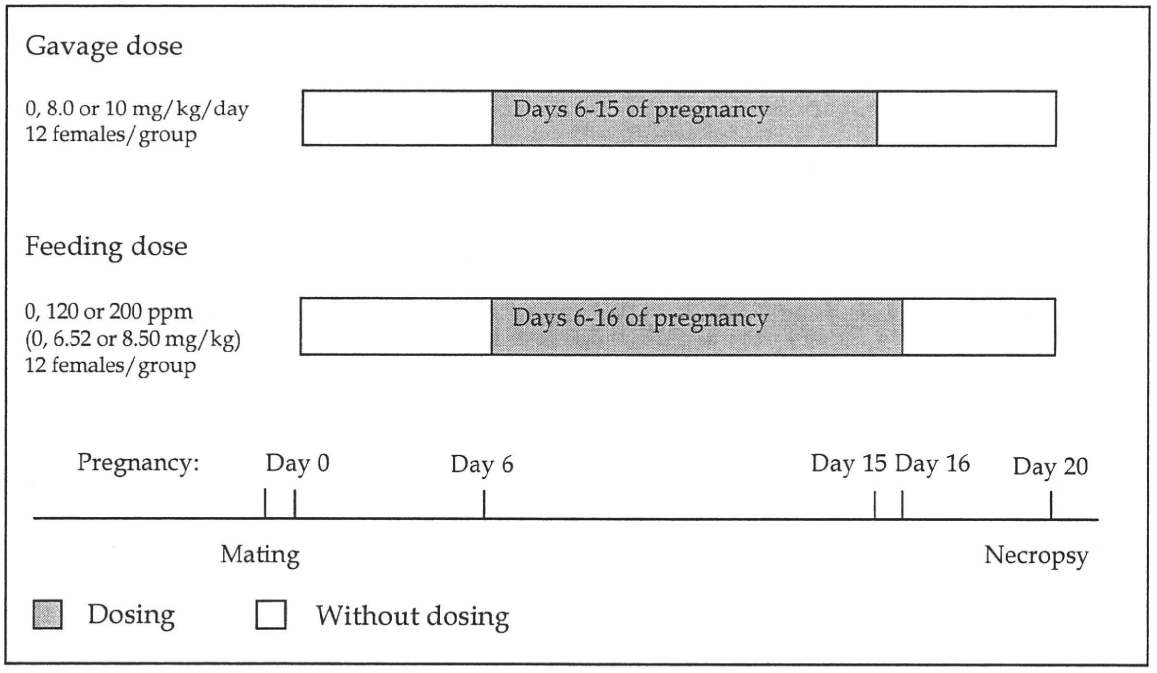

Fig. 1. A study design of prenatal developmental toxicity of gavage or feeding doses of dinoseb in rats (Matsumoto et al., 2010)

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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3）構造活性相関による遺伝毒性の予測

本間正充

Structural Activity Relationship Approaches for Assessing Genotoxicity

Masamitsu Honma

The focus of the latest legislative and governmental efforts is to establish simple screening tools for identifying those chemicals most likely to cause adverse effects without experimental testing of all chemicals of regulatory concern. The use of structure-activity relationship (SAR) models is a powerful *in silico* technique that should be considered for prioritizing chemicals for subsequent experimental verification. Because carcinogenicity and genotoxicity are among the toxicological endpoints that pose the highest concern for human health, efforts in SAR models for them have been much more pronounced than for any of the other human health end points. This review paper overviews the historical background of SAR models for predicting carcinogenicity and genotoxicity, the current status of capacity and usefulness of some *in vitro* genotoxicity SAR models, and their perspective.

Keywords; Structural activity relationship (SAR), Genotoxicity, Ames tests, Sensitivity, Specificity

はじめに

化学物質の規制に関わる国際機関や，各国規制当局の最近の関心の焦点は，規制の対象となるすべての化学物質を実験的に試験することなく，有害作用を引き起こす化学物質を同定するための単純なスクリーニングツールを確立することにある．構造活性相関（Structure Activity Relationship; SAR）は，コンピュータトキシコロジーの重要な研究分野であり，有害作用を引き起こす可能性が高い化学物質を，その化学構造から*in silico*で予測する手法である．SARは統合型毒性評価システムの重要な構成要素の１つであり，安全性評価が必要とされる化学物質の優先順位付けや絞り込みに有用である．また，動物実験の代替，もしくは最小化にも貢献できる．SARは，創薬における探索試験段階での医薬品候補化合物の選択や，実際の試験が困難な不純物の安全性評価にも利用されている．現在，工業化学物質，農薬，食品添加物，化粧品材料，医薬品候補化合物の毒性予測のため多くのエキスパートシステムやSARツールが開発されている．

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発がん性，遺伝毒性予測とSAR

発がん性は，ヒトの健康にとって最も関心の高い毒性の一つであり，日常生活において暴露する可能性のある発がん性物質に関するSARモデル化の試みは，その他のヒトにおける健康関連のエンドポイントのいずれに対する試みよりも多大な努力が払われている．化学物質の構造から発がん性を予測する研究の歴史も古い．すでに1930年頃には強力な発がん物質として知られているベンツピレンの物性と発がん性との関連を明らかにするための研究が行われている．当初は，吸収，蛍光，赤外，NMRスペクトル，イオン化ポテンシャル，電位，磁気異方性，化学反応性などと，発がん性との関係が詳細に調べられたが，発がん性を規定する性質を見つけることはできなかった．1940年代にSchmidtおよびPullmanらはベンツピレン分子内の電子分布と発がん性の関係に注目し，一部の芳香族炭化水素の発がん性を合理的に説明することに成功した[1,2]．これらの研究が発端となり，いわゆるベンツピレン分子のK領域や，Bay領域と言われる部分構造と，発がん性との相関が明らかとなった．

1970年代，James & Elizabeth Millerらは発がん性アルキル化剤の求電子性に注目し，多くの発がん性化学物質は，求電子性誘導体か，もしくは生体内でそれらに代謝されて，発がん標的組織においてDNAやタンパク質などの求核性基と結合し，がんを引き起こすという理論を唱えた[3]．それ以降，化学発がんに関する研究は急速

に進展した．重要な進歩の一つは，化学発がん研究の重要なツールであるげっ歯類を用いた発がん性試験に対して，より安価で短期間の代替バイオアッセイが確立されたことである．Amesは発がん性化学物質（アルキル化剤，インターカレーターなど）に感受性をもつ一連のサルモネラ変異株を開発し，いわゆるエームス試験を確立した[4]．エームス試験は，発がん性化学物質を検出する*in vitro*モデルといえる．当時の既知の発がん物質の多くは遺伝毒性機序によるものでありエームス試験で陽性を示す化学物質の作用は，ほとんどMillers仮説の範疇で妥当と考えられた．現在では作用機序の観点から，発がん性物質は以下の二つに分類される．1)遺伝毒性発がん物質であり，DNAに直接損傷を与え，突然変異を誘発し，これが発がんの第1ステップになりうる．2)エピジェネテックな発がん物質であり，これはDNAとは共有結合はせず，直接的にDNA損傷を起こすことは無い．またエームス試験のような標準的な遺伝毒性試験では通常陰性を示す．1)のカテゴリーに属す遺伝毒性発がん物質がMiller仮説に従った特徴を持ち，それ自体が求電子性であるか，その代謝産物や代謝中間体が求電子性を持つ．

Millerの求電子理論に続いて，AshbyとTennantは発がん化学物質に対する構造アラート（Structural Alert; SA）と，発がん性予測のコンパイル（SARモデル）を開発した[5]．発がん性に対するSAは，化学物質の発がん性活性に関連した分子官能基，または部分構造と定義され，同時に発がんの主要なステップであるDNAへの損傷や，突然変異の誘発をもたらす遺伝毒性のSAとも考えられた．Ashbyは米国National Toxicology Programの222の化学物質の中からげっ歯類発がん性試験陽性と強い相関性を示す18種類のSAを同定した．Fig. 1に"Ashby's polycarcinogen"と呼ばれる18の全てのSAを持つ仮想の究極発がん物質を示す[5]．Toxnet公開データベース，Gold/ZeigerのCarcinogenic Potency Database，イタリアのInstituto Superiore di Santaの動物がん原性ISSCAデータベースに存在する698のエームス試験データと，878のげっ歯類発がん性試験データを用いてAshbyのSARモデルの検証を行ったところ，発がん性試験結果とは65%の一致に留まったのに対して，エームス試験結果とは78%が一致した[6]．このことは，エームス試験用サルモネラ菌株はDNA応答性に対して正しくデザインされており，Millerの提唱する発がん性物質の求電子理論を適切に反映することを示している．一方，他のバイオマーカーについてはSARモデルとの相関性が低い．このような事実から，SARモデルの予測率の検証にはエームス試験が使われることが多い．

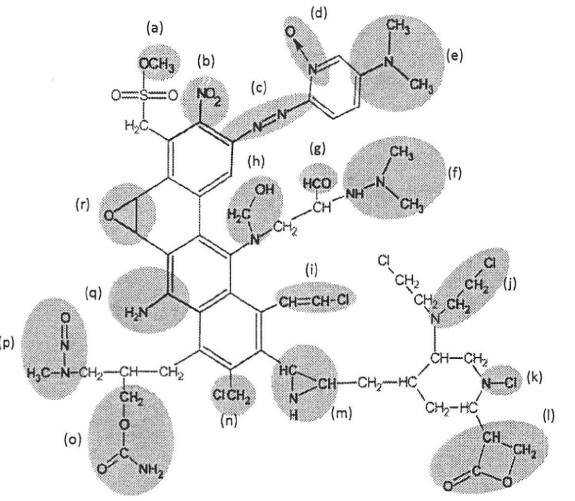

Fig. 1 "Ashby's poly-carcinogen" : Major structural units which are classified as structure-active positive in the Ames tests. The structures are as follows: (a) alkyl ester of either phosphonic or sulphonic acids; (b) aromatic nitro group; (c) aromatic azo group; (d) aromatic ring N-oxides; (e) aromatic mono-and dialkylamino group; (f) alkyl hydrazines; (g) alkyl aldehyde; (h) N-methylol derivatives; (i) monohaloalkanes; (j) a large family of N and S mustards; (k) N-chloramines; (l) propiolactones and propiosultones; (m) aromatic and aliphatic aziridinyl derivatives; (n) both aromatic and aliphatic substituted primary alkyl halides; (o) derivative of urethane; (p) alkyl N-nitrosoamines; (q) aromatic amines; (r) aliphatic and aromatic epoxide.

代表的なSARモデルによるエームス試験の予測

先に述べたようにエームス試験の予測に関しては多くのSARモデルが開発されている．そのモデルはアプローチ法により2つに大別される．1つは，知識ベース，規則ベースのエキスパートシステムで，Ashbyらが行ったように，既知データから陽性をもたらす特徴的な部分構造を定義し，ルール化された経験則から，定性的にエームス試験結果の予測を行うものである．もう一つは，化学物質の構造をフラグメントに分解後，パラメータ（数値データ）に変換し，エームス試験陽性と相関性の高いパラメータを用いて，多変量解析，パターン認識により試験結果を予測する人工知能型アプローチである．数値データから定量的な毒性の予測が可能であり，こちらはQSAR（Quantitative SAR）モデルである．前者としては英国ラーサ社のDEREK，後者のQSARとしては富士通が開発したADMEWORKSなどが代表的である．また，その中間型として，化学物質の構造と特徴を表す構造記述子と，多数の部分的構造（Biophore）を機械的に検出し，統計理論からエームス試験陽性と相関する構造記述子を選別し，予測を行うMultiCASEがある．3つのSARモデルの一般的な特徴に関しては本特論集の

小野の稿を参照されたい．

当研究所，変異遺伝部と総合評価研究室は，我が国の既存化学物質データベースからエームス試験データを有する206化学物質についてDEREK，MultiCASE，ADMEWORKSを用いてその予測性を評価した[7]（Table 1）．予測の評価は感度（Sensitivity；エームス陽性物質を陽性と判定する能力），特異性（Specificity；エームス陰性物質を陰性と判定する能力），および一致性（Concordance；陽性および陰性の一致率）を指標として行った．表が示すように感度が最も高かったものがDEREKとADMEWORKS（73.1%），特異性が最も高かったものがMultiCASE（91.1%），一致率ではDEREK（86.4%）が最も高かった．ADMEWORKSは特異性が69.7%と低く，このことは多くのエームス陰性物質の約3割を間違って陽性と判定する（False positive）ことを意味する．同様の傾向は，英国のKirklandが報告した703のエームス試験を含む*in vitro*遺伝毒性試験データベース（CGX database）での評価結果からも得られた[7,8]．一方，Snyderらは2002年～2004年のPhysicians' Desk Referenceに収載の医薬品からエームス試験データがある394品目を抽出し，DEREK，MultiCASE，TOPKAT（ADMEWORKSと同様の人工知能型QSARモデル）の3種類のSARモデルを用いて試験結果の予測を行ったところ，MultiCASE，TOPKATは比較的高い特異性を示したが，感度は50%以下であった[9]．また，DEREKにおいては感度，特異性とも低く一致率は3つの中で最低であった．医薬品と工業化学物質では化学構造に含まれるSAに特徴があるため異なった結果になったものと予測される．いずれにせよここでのSAR利用の目的は遺伝毒性可能性物質のスクリーニングであり，できるだけ感度を上げ，偽陰性（False negative）を減らすモデルの構築，改良が重要である．

SARモデルの組合せによる予測率の向上と，化学物質の優先付けへの適用

DEREK，MultiCASE，ADMEWORKSの3つのSARモデルはそれぞれ異なる経験的，数学的，統計的アプローチが取り入れられており，そのエームス試験予測結果も時として異なることは先に述べた．Hayashiらは3つのSARモデルを組み合わせることにより，お互いの欠点を相補し，エームス試験の予測率の向上を図ることに成功した[7]．また，彼らはこれまでの化学物質の安全性評価の経験から，分子量が3,000以上の高分子化合物の大部分はバクテリアの細胞壁を通過することができず，一般にエームス試験陰性であること，例外としてエポキシ基を持つ高分子ポリマーにはエームス陽性を示す化合物が存在することなどを，SARアプローチの前に考慮した決定樹を提唱した（Fig. 2）．これは実際の毒性試験を必要とする化学物質の優先付けや，絞り込みに有用である．3つのSARモデルにおいて2つ以上で陽性もしくは陰性の場合，3つ全てにおいて陽性もしくは陰性の

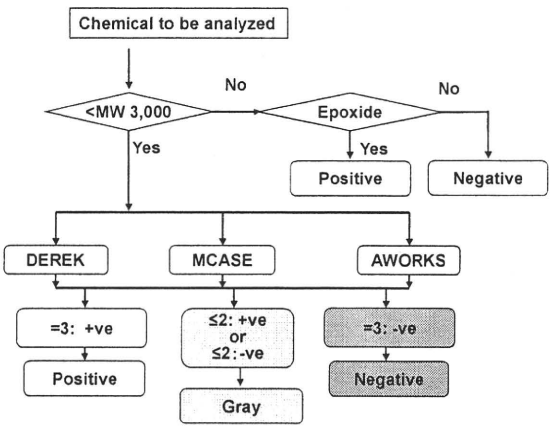

Fig. 2 Decision tree for prioritizing chemicals for consequent experimental verification. MCASE: MultiCASE, AWORKS: ADMEWORKS

Table 1 Performance of SAR models for the Ames test (Hayashi et al., 2005)

	Ames Results	+	-	Total	Sensitivity (%)	Specificity (%)	Concordance (%)
DEREK	+	19	7	26			
	-	21	159	180			
	Total	40	166	206	73.1	88.3	86.4
MCASE	+	13	20	20			
	-	13	146	146			
	Total	26	166	166	65.0	91.1	88.0
AWORKS	+	19	26	26			
	-	54	178	178			
	Total	73	204	204	73.1	69.7	70.1

MCASE: MultiCASE; AWORKS: ADMEWORKS

場合にカテゴリー化することにより予測率を向上させることができる．後者の場合，感度87%，特異性95%，一致率94%と予測率は格段に向上した（Table 2）．一方，すべての化学物質は予測結果の違いからこのようなカテゴリーの中に入るわけではない．当然適応される化学物質の割合（Applicability）は低下し，先の場合は約半分程度（55%）となる．しかしながら，現在，年間10トン以上生産されている既存化学物質は我が国で20,000種類以上も存在し，そのうち約90%である18,000種類の化学物質についてはエームス試験さえ実施されていない状況を考えると，9,900種類（18,000×0.55）の化学物質の絞り込みには依然として重要ある．

他の *in vitro* 遺伝毒性の予測

染色体異常試験はエームス試験と同様に化学物質の承認及び登録における安全性確認に重要な*in vitro*遺伝毒性試験項目の一つである．染色体異常試験についてもSARモデルが開発されているが，染色体異常は化学物質とDNAの直接的相互作用に加え，DNA複製に関する酵素（トポイソメラーゼ等）や，染色体分配に関与する核タンパク質（ヒストンタンパク質等）との相互作用などのメカニズムによっても誘発されるため，より複雑である．また，染色体異常自体も，分裂中期における染色体の構造的特性の結果として観察可能となるため，上記の全てのメカニズムが染色体異常として認識されるわけではない．従って，染色体異常を引き起こす化学物質の予測のためのモデル化には多様な計算的アプローチが必要である．また，エームス試験と比較して，SAの抽出に必要な実験データベースは少ない．

知識ベースのSARモデルであるDEREK（バージョン11）には74種類の染色体異常試験陽性のSAが収納されている（エームス試験は87種類）．エームス試験と同様に209の既存化学物質に関する染色体異常試験の予測性を評価した（Table 3）．感度，特異性，一致性とも全てエームス試験に劣り，特に感度は64%であった．このことは36%の染色体異常誘発物質を予測できないことを示す（False negative）．染色体異常試験結果自体が，げっ歯類発がん性試験結果と相関性が低いことも指摘されており，一部の染色体異常陽性結果は遺伝毒性発がん性と無関係であるのかもしれない．染色体異常試験の予測性の向上にはSARモデルの改良と，染色体異常試験自体の改良の両者が必要である．

化学物質は薬物代謝によって活性化され遺伝毒性を発現するものが少なくない．*In vitro*遺伝毒性試験の場合，通常ラット肝臓から調製されたミクロゾーム分画（S9）を試験化合物と同時に加えることにより，予測される肝臓での代謝物の評価を同時に行っている．親化合物の化学構造と遺伝毒性作用との関連性が低い場合，薬物代謝による活性化体の関与が考えられる．代謝に起因する毒性発現の複雑さはSAR研究では厄介である．ブルガス大学のMekenyanらは，ラット肝S9での代謝反応，および非生物的反応の総合的ライブラリーと，代謝による変換確率の推定値を用いて妥当な代謝マップを創り出す帰納的アルゴリズムを開発した[10]．これは組織代謝シミュレータ（TIssue MEtabolite Simulator；TIMES）と呼ばれている．既存の文献データから得られた薬物代謝に関する変換率の情報を用いて，特定の基準条件に対して変換確率を較正することができ，また，データがない場合は組み合わせアルゴリズムを用い，既知の代謝マップを適合性が最も高い変換確率に書き換えることができ

Table 2　Performance of combined SAR model for the Ames test (Hayashi et al., 2005)

Combination of 3 SARs		+++	---	Total	Sensitivity (%)	Specificity (%)	Concordance (%)
Ames	+	13	2	15			
	-	5	94	99			
	Total	18	96	114*	86.7	94.9	55.3

*Among 206 chemicals, 114 chemicals were categorized into +++ or ---.
Applicability 55.3% (114/206).

Table 3　Performance of DEREK for the chromosome aberration test

DEREK		+	-	Total	Sensitivity (%)	Specificity (%)	Concordance (%)
Chrom	+	60	34	94			
Ab	-	29	86	115			
	Total	89	120	209	63.8	74.8	69.9

Chorm Ab: Chromosome aberration test