

DINOCAP

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Explanation

Dinocap was evaluated by the JMPR in 1969, 1974, and 1989 (Annex 1, references 12, 22, 1nd 56). An ADI of 0–0.001 mg/kg bw was allocated in 1989 on the basis of a NOAEL of 0.5 mg/kg bw per day in a study of developmental toxicity in rabbits and a safety factor of 500. At the present Meeting recent data on material of greater purity than that tested previously were evaluated. Dinocap consists of 2,4- and 2,6-dinitro-octylphenyl crotonates in which the octyl moiety is either 1-methylheptyl, 1-ethylhexyl, or 1-propylpentyl. A number of the studies that were reviewed were performed with the methylheptyl isomer used as a model for dinocap.

Evaluation for Acceptable Daily Intake

1. Biological data

(a) Absorption, distribution, and excretion

In a comparison of the absorption, distribution, and excretion of the hethylheptyl isomer of dinocap (DNHPC) in mice after oral or dermal administration, ¹⁴C-phenyl-labelled material (specific activity, 18.6 mCi/g; radioactive purity, 95%) was administered at 25 mg/kg bw to female CD-1 mice as a single oral or single dermal dose or as repeated dermal doses for 4 h/day up to 10 days. For oral administration, corn oil was used as the vehicle; for dermal administration, Karathane formulation blank solvents were used. Blood samples were collected from subgroups of three mice at appropriate times after dosing. The dermal application site was not occluded, but a collar was applied to prevent grooming. For repeated dermal administration, the application site was changed on successive days. A further study was conducted to measure total systemic absorption over 4 h after a single dermal dose of 10 or 25 mg/kg bw in groups of four mice. There are no guidelines for the objectives of this study, but the method was appropriate to the purpose.

The results are shown in Table 1. The peak blood concentrations were about four times lower and the time to peak concentrations slightly delayed after dermal dosing in comparison with oral administration, and the area under the curve was about seven times smaller than after oral administration. The peak blood levels after repeated dermal administration were no higher than that after a single dose, suggesting that clearance of dinocap was sufficiently rapid that no accumulation occurred under these conditions, as reported previously. No material balance was attempted in this part of the study. In the study of dermal penetration, 97–99% of the radiolabel was recovered; recovery from the faeces and urine was 16% at the low dose and 25% at the high dose, with, in each case, slightly more in urine than in faeces. Less than 1% remained at the skin application site. The half-life for elimination of radiolabel from plasma was about 6 h after either oral or dermal administration. These results are consistent with relatively rapid absorption of 25% of the administered dose of dinocap through the skin of mice within 4 h (Evans & Hazelton, 1995)

When compared with the pharmacokinetic data described for rabbits in the 1989 JMPR monograph (Annex 1, reference 58), the peak plasma concentrations were approximately similar after a 25-mg/kg oral dose (15 ppm in rabbits and 20 ppm in mice), and the T_{max} was slightly achieved slightly later in mice (6 h) than in rabbits (3 h).

Penetration of ^{14}C -labelled DNHPC through samples of human and mouse skin was compared *in vitro*. Cryopreserved human skin from the thigh, back, and lateral torso of three donors, respectively, was stripped of fat and sliced at 350 μm ; skin was also derived from eight female CD-1 mice, shaved, and stripped of fat. Discs of skin were inserted into flow-through diffusion cells, and the barrier integrity was verified with tritiated water. ^{14}C -DNHPC was made up in Karathane formulation blank and applied to the skin surface at a rate reported to be 548 $\mu g/cm^2$. The receptor chamber contained Hanks' balanced salt solution and 4% albumin at a flow-through rate of 1.5 ml/min. The skin samples were exposed to the Karathane test material for 24 h then swabbed with a mild detergent. The human skin samples were separated into layers which were analysed individually; mouse skin was analysed intact. As radiolabel recovery was not determined, the results are expressed only in terms of the proportion of radiolabel actually recovered.

Of the radiolabel recovered, only 0.2% was recovered from receptor fluid after penetration through mouse skin and only 0.3% after penetration through human skin; 68% of radiolabel was recovered from within mouse skin, but only 10% from within human skin. Slightly less than 90% of the material recovered from human skin was in washes of the skin surface. Steady-state flux rates through mouse skin were approximately twice those through human skin. A total of 68% of the recovered dose penetrated the mouse stratum corneum, and 10.5% penetrated the human stratum corneum. The finding that only 0.2% DNHPC penetrated into receptor fluid from mouse skin differs from that in mice *in vivo*, described above, which showed 25% penetration of a 25 mg/kg dose of ^{14}C -DNHPC through mouse skin within 4 h and only 1% remaining in skin. No comparison of skin concentrations was presented, but, assuming a dermal application area of 3 cm^2 for a 30-g mouse *in vivo*, the surface concentration is approximately 250 $\mu g/cm^2$. As this value is comparable with the 548 $\mu g/cm^2$ found *in vitro*, dermal surface concentrations are unlikely to account for the difference. A comparison of the penetration of DNHPC through human and mouse skin was not given; however, human skin is generally less penetrable than that of mice (Ruegg, 1996).

In a study reported only in summary, 55% of an undefined dose of radiolabelled dinocap was eliminated in the urine of four adult rhesus monkeys after intramuscular injection; 52% was excreted within 24 h of injection (Maibach, 1985).

Table 1. Absorption of the methylheptyl isomer of dinocap in mice after oral and dermal administration

Treatment	Dose (mg/kg bw)	T_{max} (h)	C_{max} (ppm)	AUC (ppm-h)	$T_{1/2}$ (h)
Single oral	25	2–6	18–21	345	6
Single dermal	25	6–8	4	52	6.5
Repeated dermal	25	NA	2–4	NA	NA

In a further study, dermal absorption of ^{14}C -DNHPC was evaluated by treating groups of four female rhesus monkeys with a dose of 1.6 mg (approximately 0.2 mg/kg bw) as a 1:3 v/v solution in acetone on 40 or 0.64 cm² of abdominal skin. When the skin of one of two groups that received the dose over 40 cm² was washed only with water, the total recovery of radiolabel was poor (approximately 30%). The recoveries were slightly better (50%) in the group in which the skin was washed with aqueous ethanol, and only the results for this group are considered further for this concentration. Occlusion of the application sites was not reported, but the animals were restrained in metabolic chairs for the 6-h exposure. An additional group received a single intravenous dose of 0.1 mg/kg bw DNHPC in dimethyl sulfoxide. After 6 h, the application site was washed, and the animals were transferred to metabolism cages. Samples of blood, faeces, and urine were collected at frequent intervals over a four-day elimination period. Dermal absorption was calculated by correcting measured urinary or faecal radiolabel elimination for the proportions of the total administered dose eliminated by these routes after intravenous injection, a method which fails to address dermal depot formation. The design of the study is not closely compliant with any guideline.

The plasma levels after dermal administration were low. Total recovery of the application of 0.2 mg/kg to 0.64 cm², equivalent to 2500 µg/cm², was approximately 80%, and absorption was calculated to be about 5% on the basis of both faecal and urinary elimination. Recovery of the more dilute application (0.2 mg/kg bw to 40 cm², equivalent to 40 µg/cm²) was approximately 50%, and absorption was calculated as 10% from urinary elimination and 20% from faecal excretion (Wester & Maibach, 1985).

(b) *Biotransformation*

In a study conducted to characterize urinary metabolites, a mixture of ^{13}C - and ^{14}C -2,4-DNHPC was administered by gavage in corn oil to three Sprague-Dawley rats at 100 mg/kg bw and to 15 CD-1 mice at 25 mg/kg bw. Pooled urine samples collected during the first 24 h after administration, which contained 90% of the radiolabel eliminated by the urinary route, were analysed for metabolites by high-performance liquid chromatography and mass spectroscopy.

Approximately 30% of the radiolabel administered to rats and 58% of that administered to mice was recovered in urine, and 94% of the radiolabel in rat urine and 82% of that in mouse urine could be allocated to identified metabolites. Structures were assigned to 12 metabolites in rats and 13 in mice. In both species, the pattern of metabolites was consistent with a metabolic pathway involving extensive initial hydrolysis of the croton ester, resulting in loss of the crotonate group (which was not found in any urinary metabolite), leaving dinitrooctylphenol. Subsequent metabolites appeared to be formed by β - or α -oxidation of the methylheptyl group. Small proportions of radiolabel were eliminated as conjugates: 4.5% in rats as acetyl conjugates and 7.7% in mice as unidentified conjugates but including sulfates. Seven metabolites, accounting for 12% of the total recovered dose, were identified as occurring in mice but not in rats; 6.5% of the administered dose was attributable to unidentified conjugates, and the remainder were chain-shortened alcohols and aldehydes attributed to intermediates of α -oxidation with a common end-product, as found in rats. The metabolites were allocated to α -oxidation products on the basis that they showed shortening of the heptyl moiety, but they were not obviously consistent with β -oxidation. Overall, 85% of metabolites found in rats were also found in mice, and 70% of those found in mice were also found in rats (Potter, 1996).

The proposed metabolic pathways of dinocap in rats and mice are shown in Figures 1 and 2.

2. Toxicological studies

(a) *Acute toxicity*

The results of studies of the acute toxicity of various formulations of dinocap are shown in Table 2. Purified technical-grade dinocap appeared to be marginally less acutely toxic than the less highly purified material described in the 1989 report (Annex 1, reference 58). The purified material

Figure 1. Proposed metabolic pathway of dinocap in rats

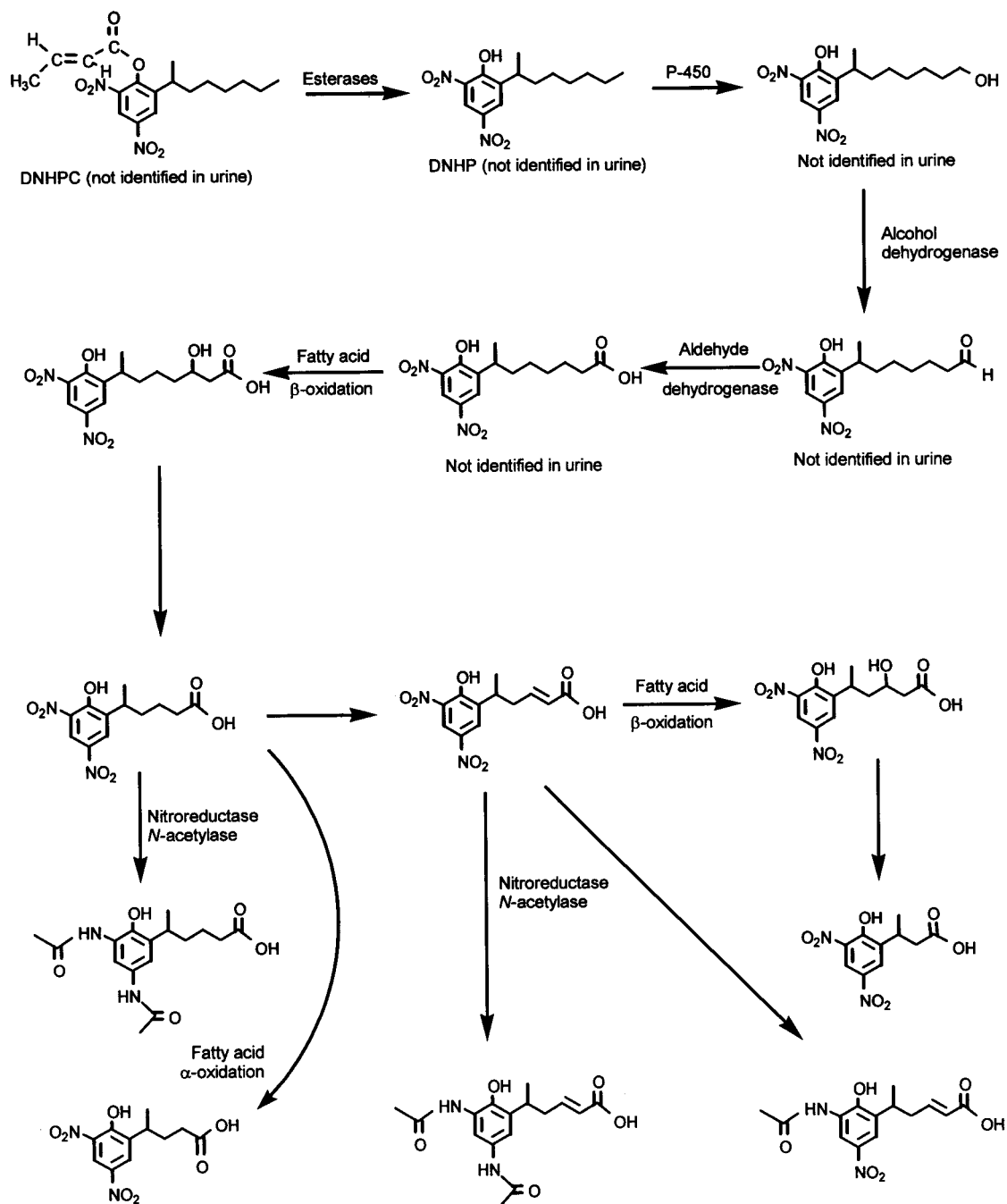
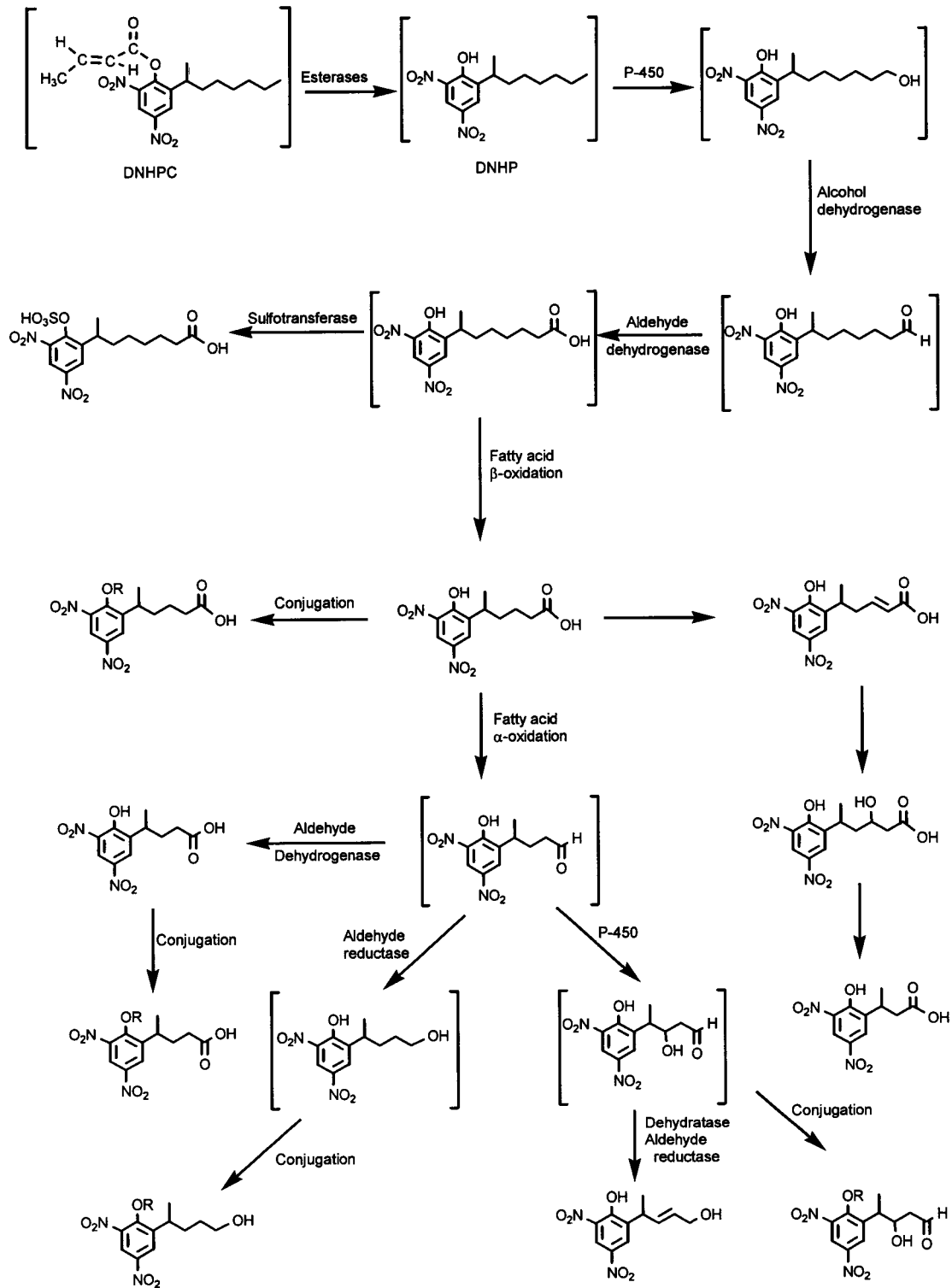


Figure 2. Proposed metabolic pathway of dinocap in mice



The compounds in brackets were not identified in urine.

Table 2. Acute toxicity of formulations of dinocap

Species	Strain	Sex	Route	Purity (%)	LD ₅₀ or LC ₅₀ (mg/kg bw or mg/L)	Reference
<i>Technical-grade dinocap</i>						
Mouse	CD-1	M	Oral	95	292	Morrison et al. (1987)
Rat	Crl:CD	M	Oral	95	3100	Morrison et al. (1987)
Rat	Crl:CD	M	Oral	95	> 500	Romanello et al. (1987a)
		F			< 5000	
Rat	Crl:CD	M	Inhalation	95	> 3	Ferguson (1997)
		F			3	
Rabbit	New Zealand white	M/F	Dermal	95	> 5000	Romanello et al. (1987b)
<i>Karathane LC fungicide-miticide</i>						
Mouse	CD-1	M	Oral	40.4	517	Onishi (1989)
		F			413	
Mouse	CD-1	M/F	Dermal	35.6	1020	Procopio & Parno (1995)
Rat	Crl:CD	M/F	Oral	37.6	> 2000	Bernacki & Hamilton (1992a)
Rat	Crl:CD	M	Oral	39	> 500	Lampe et al. (1987a)
		F			< 5000	
Rat	Crl:CD	M/F	Dermal	37.6	> 2000	Bernacki & Hamilton (1992b)
Rabbit	New Zealand white	M/F	Dermal	39	> 5000	Lampe et al. (1987b)
Rat	Crl:CD	M/F	Inhalation	50	0.9	Blair & Cavender (1979)
<i>Karathane FN-57 fungicide-miticide</i>						
Mouse	CD-1	M/F	Oral	20	1401	Morrison & Hamilton (1991)
Rat	Crl:CD	M	Oral	20	> 500	Romanello et al. (1987c)
		F			< 5000	
Rat	Crl:CD	M/F	Inhalation	20	> 4.9	Wanner & Hagan (1991)
Rabbit	New Zealand white	M/F	Dermal	20	> 5000	Krajewski et al. (1987a)

M, male; F, female

was moderately irritating to the skin (Romanello et al., 1987d) and eye (Romanello et al., 1987d) and was sensitizing to the skin when tested by the Buehler method (Anderson & Baldwin, 1990a); the previous conclusions of the JMPR remain unaltered by these data. Products containing dinocap were also shown to be irritating to the skin and eye of rabbits (Bernacki & Hamilton, 1992c; Krajewski et al., 1987b,c; Morrison & Hamilton, 1992; Romanello et al., 1987e,f,g) and to have skin sensitizing potential (Anderson & Baldwin, 1990b,c).

(b) *Short-term studies of toxicity*

Mice

In a dose range-finding study reported only in brief, purified technical-grade dinocap (purity, 95.7%) was administered in the diet of CD-1 mice (age at start of study unspecified) at concentrations of 25, 75, 225, 500, 1000, 2000, or 4000 ppm for 28 days. The full extent of histological examination was not reported. Concentrations of 500 ppm and higher caused deaths, with total loss of males at 2000 or 4000 ppm and of females at 1000 ppm and higher. The results are shown in Table 3. The liver appeared to be the principal target organ, showing hepatocellular necrosis that appeared to be of only mild to moderate severity when given at doses that caused deaths. The amount of detail provided was insufficient to identify a NOAEL (Bernacki & Baldwin, 1987).

(c) *Long-term studies of toxicity and carcinogenicity*

Mice

Groups of 60 male and 60 female CD-1 mice were fed diets containing 0 (control), 15, 100, or 200 ppm dinocap (purity, 93.2–96.2% at intervals during the study) for 78 weeks. The mice were seven to eight weeks old at the start of treatment and thus two weeks older than required by the guideline; much of the most rapid phase of growth was thus missed. The study design included

Table 3. Results of a dose range-finding study in mice

Outcome	Dose (ppm in diet)							
	0	25	75	225	500	1000	2000	4000
Mortality, males	0/10	0/10	0/10	0/10	0/10	5/10	10/10	10/10
Mortality, females	0/10	0/10	0/10	0/10	7/10	10/10	10/10	10/10
Final body weight, males (g)	34	33	33	32	29	25		
Final body weight, females (g)	27	28	29	26	20			
Liver weight, males (g)	2.0	2.0	2.1	1.9	1.8	1.7*		
Liver weight, females (g)	1.5	1.6	1.7*	1.5	1.2*			
Liver weight, males (% bw)	5.8	5.9	6.1	6.0	6.4*	6.8*		
Liver weight, females (% bw)	5.5	5.7	6.0*	5.9*	5.9			
Hepatocellular necrosis, males	NR	NR	NR	NR	NR	3/10	9/10	6/10
Hepatocellular necrosis, females	NR	NR	NR	NR	5/10	6/10	7/10	3/10

NR, not reported

From Bernacki & Hamilton (1987)

* Statistically significant at $0.05 > p \geq 0.01$

ophthalmological examination. After the deaths of 14 females at the highest dose during week 1, that dose was reduced to 150 ppm from the start of week 2 for females and week 3 for males. With the exception of the deaths in week 1, there was no treatment-related effect on survival, which was about 80% in controls and 72% at the highest dose (group with highest mortality) at 78 weeks. There were no overt treatment-related symptoms of toxicity. Body-weight gain and food consumption were reduced in males at 150 ppm and in females at 100 ppm (20% less than that of controls) and 150 ppm. The efficiency of food use appeared to be impaired only in males at the highest dose and not in females, thus suggesting that the effect in females was at least partly a consequence of palatability. Mean testis weight was reduced in males at 150 ppm, and histopathological examination revealed a background incidence of unilateral and bilateral testicular atrophy in all groups, including controls, with incidences of 11/60, 4/60, 11/59, and 25/60 at 0, 15, 100, and 150 ppm, respectively. A moderate incidence of individual cell necrosis was reported in mice in all groups, possibly associated with mouse hepatitis virus infection indicated by serological markers at 18 months, but the incidence of necrosis seen in the 28-day study described previously was not reproduced. There was no evidence of carcinogenicity in any tissue. The NOAEL was 15 ppm, equivalent to 2.7 mg/kg bw per day, on the basis of reduced body-weight gain with a corresponding reduction in food consumption in females at 100 ppm (Moore, 1991).

(d) Genotoxicity

Studies of the genotoxicity of dinocap of lower purity than that used in the studies reported in the previous monograph are summarized in Table 4. The results are consistent with the previous conclusions of the JMPR.

(e) Reproductive toxicity

(i) Multigeneration reproductive toxicity

Rats

Groups of 26 Crl:CD rats of each sex received dinocap (purity, 96%) in the diet at concentrations of 40, 200, or 1000 ppm for two generations. At weaning of the F₁ animals, the highest dose was reduced to 400 ppm because of a high rate of mortality in these pups. The litters were culled to eight pups at day 4 *post partum*. In addition to the normal guideline requirements, selected F₁ males at 0, 40, or 200 ppm were used in a study of gonadal function (sperm motility, sperm count, weight of cauda epididymis). Body-weight gain and food consumption were retarded at 1000 ppm during the F₀ pre-mating period and at 400 ppm during the F₁ pre-mating period. There was increased pup mortality at weaning among litters of the group at 1000 ppm, until this dose was reduced to 400 ppm; the cause of death could not be determined but the presence of yellow or red

Table 4. Results of studies of the genotoxicity of dinocap

End-point	Test object	Dose	Purity (%)	Results	Reference
<i>In vitro</i>					
Gene mutation	Chinese hamster ovary cells, <i>hprt</i> locus	3–10 µg/ml ^a 15–25 µg/ml ^b	83.9	Negative Negative	Foxall (1985)
Metaphase analysis	Chinese hamster ovary cells	1–10 µg/ml ^a 5–20 µg/ml ^b	NR	Negative Negative	Ivett & Myhr (1986)
<i>In vivo</i>					
Metaphase analysis	CD-1 mouse bone marrow	12.6–126 mg/kg bw	83.9	Negative	Sames et al. (1986)

^a Without an exogenous metabolic system

^b With an exogenous metabolic system

discolouration of the contents of the gastrointestinal tract and bladder suggested the presence of test material or its metabolites. There were no specific effects on reproductive function or the ability to rear young, and there was no effect on gonadal function. Increased liver weights, with no evidence of a histological correlate, were found among rats receiving 400 or 1000 ppm. The NOAEL was 200 ppm, equal to 13 mg/kg bw per day (Morseth, 1990).

(ii) *Developmental toxicity*

Mice

Groups of 24 CD-1 mice presumed to be pregnant were given dinocap (purity, 94.4%) in aqueous 1% tragacanth gum at doses of 0 (control), 4, 10, or 25 mg/kg bw per day by gavage on days 6–15 of gestation. On day 18 of gestation, about half of the mice in each group were killed and the fetuses were examined; the remaining dams were allowed to deliver and rear their litters, which were culled on day 4 *post partum* to two pups per litter. On day 43, these mice were evaluated for swimming performance.

The results of this study are shown in Table 5. There were no maternal deaths during the study and no overt signs of toxicity. The body-weight gain of the pregnant mice was minimally impaired at 25 mg/kg bw per day on days 12–16 of gestation, although the weight difference may have been a consequence of the reduced litter size. Gross examination showed no treatment-related findings. In animals killed on day 18 of gestation (the normal end of a study of teratogenicity), the average number of resorptions was increased in dams at 25 mg/kg bw per day, with an associated decrease in litter size. The incidence of resorptions was slightly increased in dams at 10 mg/kg bw per day, but the increase was within the range of historical controls (0–1.6). All treated animals that delivered naturally had a statistically significant increase in the duration of gestation, although this was not considered to be toxicologically significant.

The mean fetal weight was reduced, and there was an increased incidence of fetuses with cleft palate and open eyelids at 10 and 25 mg/kg bw per day. There was an increased incidence of stillborn pups and decreased pup body weight on days 7–21 *post partum* at 25 mg/kg bw per day, and pup survival to day 4 *post partum* was reduced. The incidence of litters with pups with head tilt and the incidence of pups with cleft palate were increased at 25 mg/kg bw per day, although all nine pups with cleft palate were from the same litter. Among the pups that were allowed to survive until day 43 *post partum*, there was an increased incidence of mice with head tilt, and ungroomed coats, corneal opacity, and ptosis were seen in one or two males at the highest dose. Body-weight gain and average body weights were decreased in mice at this dose, and there was an increased incidence of mice with altered swimming postures or ability. Of the 11 pups with altered swimming posture, 10 had head tilt. A further three pups with head tilt did not have altered swimming posture. The NOAEL for developmental toxicity was 4 mg/kg bw per day on the basis of the increased incidence of open eyelids and cleft palate in pups at 10 mg/kg bw per day. The NOAEL for maternal toxicity was 10 mg/kg bw per day on the basis of minimally retarded weight gain during days 12–16 of gestation in dams at 25 mg/kg bw per day. Dinocap was teratogenic, causing malformations at doses that had no effect on the dam (Lochry, 1989).

Table 5. Results of a study of developmental toxicity in mice

Parameter	Dose (mg/kg bw per day)			
	0	4	10	25
Maternal weight gain (g), days 6–16	14.8	16.0	16.5	13.8
days 12–16	8.2	8.7	8.8	6.4
days 0–18	26.3	27.0	27.3	23.3
<i>Caesarian-derived pups</i>				
No. of litters evaluated	12	12	12	9
Resorptions per litter	1.0	0.5	1.2*	1.8*
Litter size	11.2	11.2	10.8	8.7
Mean fetal weight (g)	1.4	1.35	1.30*	1.10**
Open eyelids, litters (total no. fetuses)	0	0	1 (1)	2 (3**)
Cleft palate, litters (total no. fetuses)	0	0	3 (4)	7 ** (65**)
<i>Naturally-delivered pups</i>				
No. of litters evaluated	11	12	12	10
Gestation duration (days)	19.4	19.8*	19.8*	19.9**
Litter size, day 1 <i>post partum</i>	11.4	11.3	11.8	11.8
Head tilt (no. of litters)	0	0	0	3**
Cleft palate/pups dying days 0–21 <i>post partum</i>	0/3	0/1	0/0	9/16
Abnormal swimming ability, day 43, litters (no. fetuses)	0/11	0/11	0/12	5/9** (11/39)**

From Lochry (1989)

* Statistically significant at $0.05 > p \geq 0.01$; ** statistically significant at $0.01 > p$

A study was conducted to establish suitable doses for a study of teratogenicity after dermal application to CD-1 mice. Fewer animals were used than recommended in guidelines. Dinocap was applied as the formulation Karathane LC XF, considered to be appropriate for estimating the risk due to occupational exposure, but the doses used were corrected for content and expressed as dinocap. Appropriate dilutions were obtained with the formulation blank. In phase 1 of the study, both untreated and vehicle control groups were used; in phase 2, only a vehicle control group was used. The dose volume of 290 $\mu\text{l}/\text{kg}$ bw was applied to one to five areas of shaven dorsal skin for 4 h each day on days 6–15 of presumed gestation; the site was changed each day, recommencing at the first site on the sixth day. Occlusion of the application site was not mentioned, but a collar was placed to prevent ingestion; no mention is made of how the mice were restrained during application. After completion of the 4-h exposure each day, the test material was washed off gently with soap and water.

Initially (phase 1), groups of eight presumed-pregnant mice were given doses of 0 (control), 50, 80, or 100 mg/kg bw per day. Because of excessive toxicity, similar groups of eight mice were given doses of 0, 1, 4, 10, or 25 mg/kg bw per day by a similar protocol. The mice were sacrificed on day 18 of presumed gestation; half of the fetuses were used for skeletal examination, and the remaining half for specific examination of otoliths and of the remaining skeleton. For mice weighing 20–40 g, the dose volumes would have been 6–12 μl : the practical difficulty of administering such small volumes may have resulted in variations in accuracy. One, three, and four deaths occurred during treatment at 50, 80, and 100 mg/kg bw per day, respectively. Death was usually briefly (up to 24 h) preceded by signs of toxicity, including dermal erythema, ataxia, red or tan vaginal discharge, weight loss, and cold to touch. These doses all reduced weight gain, with weight loss at the highest dose. The numbers of live young were reduced at 50 and 80 mg/kg bw per day, and the litters of the three surviving dams at 100 mg/kg bw per day contained only resorbed conceptuses. Gross malformations were seen in 88% of fetuses at 50 mg/kg bw per day and 100% of those at 80 mg/kg bw per day.

The principal treatment-related effects seen in phase 2 are shown in Table 6. Little maternal toxicity occurred, and body-weight gain was apparently unaffected by treatment. One mouse at the highest dose was found dead on day 15 of gestation having been found stuporous with an impaired righting reflex after dosing the day before. Death was attributed to trauma, although no clear injury was detected at necropsy. There was a slight incidence of skin irritation at the two highest doses. The litter sizes, numbers of live fetuses per litter, and fetal body weights were unaffected by treatment. There was no change in the degree of skeletal ossification, but three fetuses of one litter at the highest dose had cleft palate, and two fetuses of another litter had open eyelids. Also at the

highest dose, otolith development was clearly impaired. These findings are consistent with those of previous studies of the teratogenicity of dinocap in mice after oral administration. In view of these results, no further study by the dermal route was conducted. The study involved too few animals to determine a NOAEL for teratogenicity in mice treated dermally, particularly with respect to cleft palate; however, the quality of the data on mean otolith scores in 49 fetuses may provide some degree of confidence that the NOAEL for these effects is 10 mg/kg bw per day (Foss, 1995).

2,4-DNHPC and 2,6-DNHPC were not teratogenic in mice. Samples of each isomer (purity, 95%) were tested separately and in combination, and the results were compared with those in mice receiving technical-grade dinocap (purity, 84%), each group at a dose of 25 mg/kg bw per day in corn oil. Fewer pregnant animals were used than recommended in the guidelines. Although litters born to dinocap-treated mice showed a pattern of developmental defects typical of those seen in the studies described above, the two isomers were inactive at the same dose, both alone and in combination (Rogers et al., 1987). In a separate study by the same group, otolith formation was compared in the pups of mice and hamsters treated with dinocap during gestation. Otolith formation was impaired in mice. Dinocap affected otolith formation in hamsters, but only at a dose associated with severe maternal and fetal toxicity (Rogers et al., 1989).

Rats

Groups of 25 Sprague-Dawley rats presumed to be pregnant received dinocap (purity, 96%) as a suspension in aqueous methylcellulose at doses of 0 (control), 10, 50, or 150 mg/kg bw per day by gavage on days 6–15 of gestation. There were no deaths, and the only overt sign of toxicity was an increased incidence of soft faeces in rats at 150 mg/kg bw per day. Maternal body-weight gain was impaired at 150 mg/kg bw per day during the first two days of treatment, and food consumption was slightly reduced in this group during treatment. Gross examination revealed no treatment-related changes in pregnant or non-pregnant females. There were no treatment-related changes in pup weight and no increase in the incidence of malformations. There was an apparent increase in the incidence of extra ribs at 150 mg/kg bw per day, but seven of the 10 affected fetuses were from two litters. The NOAEL for maternal and fetal toxicity was 50 mg/kg bw per day. There was no evidence of teratogenicity at the highest dose of 150 mg/kg bw per day (Solomon & Lutz, 1989).

Rabbits

Groups of 20 artificially inseminated New Zealand white rabbits received dinocap (purity, 95.4%) in aqueous gum tragacanth by gavage at doses of 0, 3, 12, 48, or 84 mg/kg bw per day on days 7–19 of presumed pregnancy. Pups were delivered by caesarian section on day 29 and examined for developmental abnormalities in accordance with normal guideline requirements. Two does at the high dose and one at the intermediate dose died between days 24 and 29 of gestation, therefore at least five days after dosing, but the deaths were considered to be related to

Table 6. Results of a study for developmental toxicity in mice

Effect	Dose (mg/kg bw per day)				
	0	1	4	10	25
No. of pregnant dams	8	8	7	8	6
Maternal weight gain on days 6–18 (g)	21.4	26.4	20.8	26.4	25.0
No. of fetuses	77	96	67	93	73
Cleft palate (no. of litters)	0	0	0	0	1
Open eyelids (no. of litters)	0	0	0	0	1
Mean otolith score ^a	9.2	9.6	9.0	9.0	2.2
No. of fetuses examined	37	49	34	49	38

From Foss (1995)

^a Three otoliths were scored for completeness on a scale of 1–4, to give a maximum potential score of 12.

treatment. These animals all showed weight loss and anorexia before death. The doe at the intermediate dose and one at the high dose aborted; both does at the high dose that died had non-viable litters (late resorptions). An additional five deaths were considered unrelated to treatment and were primarily a consequence of intubation errors.

The main results are shown in Table 7; statistical analyses were not reported. There was an increased incidence of premature delivery and abortion at 48 and 84 mg/kg bw per day; the premature deliveries at the highest dose were primarily late resorptions. Clinical signs (lack of faeces or dried faeces) and impaired weight gain and food consumption were seen among does receiving these doses, litter sizes and pup weights were reduced, and there was an increased incidence of pups with skeletal malformations (vertebral assymetry and fused or forked ribs). There was also a slight increase in delayed ossification at a few sites in does at these doses. The NOAEL for maternal toxicity was 3 mg/kg bw per day on the basis of weight-gain retardation during treatment. The NOAEL for developmental toxicity was 12 mg/kg bw per day on the basis of increased resorptions and reduced litter sizes at 48 mg/kg bw per day (Hoberman & Christian, 1987).

This conclusion is different from that of the 1989 JMPR (Annex 1, reference 58) but is based on different data. In the latter review, an increased incidence of hydrocephaly and neural tube defects was found at doses ≥ 3 mg/kg bw per day in the studies of Costlow & Kane (1984 a,b). In the earlier studies, however, a less pure form of dinocap (84%) was used, and the neural tube defects found by Costlow and Kane (1984a) at a dose of 3 mg/kg bw per day were not found in the second study (Costlow & Kane, 1984b) at 48 mg/kg bw per day nor in studies by dermal administration in which the increased numbers of resorptions and delayed ossifications were similar to these found by Hoberman & Christian (1987).

(f) *Special studies: Ocular toxicity*

A NOAEL of 15 ppm was observed for ocular toxicity in a two-year study in dogs (Weatherholz et al., 1979) in the 1989 JMPR (Annex 1, reference 38). At 60 ppm, four of eight dogs had slight to moderate discolouration of the tapetum lucidum, although no retinal atrophy and no changes in the vascularity of the retina were noted. The remaining four dogs at this dose showed moderate to marked discolouration of the tapetum, reduced vascularity of the retina, and retinal atrophy (three dogs). At 120/240 ppm, the four surviving dogs had marked discolouration of the tapetum, reduced vascularity of the retina, and retinal atrophy. Retinal atrophy was therefore present only in dogs with severe tapetal changes, and no dog showed retinal effects without effects in the tapetum.

Table 7. Results of a study of developmental toxicity in rabbits

Result	Dose (mg/kg bw per day)				
	0	3	12	48	84
Adult animals					
No. pregnant	17	17	15	15	14
No. with abortions	0	0	0	3	1
No. with premature delivery	1	0	0	1	3
No. with absence of faeces (on any day)	0	0	0	0	4
No. with dried faeces (on any day)	3	0	2	7	17
Weight change, days 7-20 (g)	150	200	90	-330	-550
Food consumption, days 7-20 (g/day)	160	170	152	65	51
No. with gastric ulceration	0	0	1	3	4
Litters					
No. evaluated	12	17	14	10	9
Mean no. of live fetuses	7.2	7.5	8.1	6.3	5.9
Dead or resorbed fetuses per litter (%)	11.8	4.9	7.1	20.3	27.0
Mean fetal weight (g)	40.9	42.7	40.0	37.6	35.8
Vertebral assymetry: no. of litters (pups)	1 (1)	1 (1)	1 (2)	5 (7)	3 (8)
No. of litters (pups) with rib malformation	1 (1)	0	2 (3)	2 (2)	2 (6)
No. of litters (pups) with skeletal malformations	4 (4)	3 (3)	4 (6)	5 (7)	6 (12)

From Hoberman (1987)

A review of the literature for compounds that are toxic to the tapetum lucidum of dogs was submitted (Solomon, 1991). Of six compounds known to affect the tapetum, three (zinc pyridine-thione, SCH 19927, and rosaramicin) had no effect in atapetal dogs, and no ocular effects were reported in other atapetal animals. The other three compounds (dinocap, hydroxy pyridinethione, and diphenyl thiocarbazine) affected the tapetum in dogs (with no information on atapetal dogs) but did not cause ocular effects in atapetal species. The author concluded that the retinal atrophy seen with dinocap was secondary to damage to the tapetum lucidum. Since humans do not have a tapetum, it was concluded that humans would not be susceptible to retinal damage as a consequence of this effect. The review is brief, and the adequacy of the methods used, the sensitivity, and the comparability of the findings were not considered. The conclusions cannot therefore be regarded as definitive, although the data support the hypothesis. The NOAEL in the study of Weatherholz et al. (1979) was therefore identified for other effects. The 1989 JMPR report (Annex 1, reference 58) indicates that significant effects, including deaths, occurred at doses of 180–240 ppm, giving a NOAEL of 60 ppm, equivalent to 1.5 mg/kg bw per day.

Comments

Dinocap is well absorbed after oral exposure. A proportion (5–25%) is absorbed after dermal exposure, varying with species and concentration. No conclusions were drawn about the degree of dermal absorption in humans from the results of a study in which mouse and human skin were compared; however, human skin is generally regarded as being less permeable to toxicants than that of mice.

The urinary metabolites of the methylheptyl isomer in rats and mice have been extensively characterized; characterization of the faecal metabolites was reported by the 1989 JMPR, which concluded that the pattern of metabolites in faeces seen by thin-layer chromatography was similar to that observed in squash and cucumbers.

The new data confirmed the generally low degree of acute toxicity of dinocap in rats; mice, however, appear to be more sensitive than rats to both acute and developmental effects. Dinocap is a skin irritant and sensitizer. The available studies did not address the uncoupling of oxidative phosphorylation, identified by the 1989 JMPR as a potentially significant mode of action.

WHO has classified dinocap as 'slightly hazardous' (WHO, 1996).

In a study of carcinogenicity in mice at doses of 0, 15, 100, or 200 ppm, no evidence of carcinogenicity was found. The NOAEL was 15 ppm, equal to 2.7 mg/kg bw per day. The lack of carcinogenicity in mice is consistent with the absence of carcinogenicity in rats reported by the 1989 JMPR.

The results of tests for genotoxicity (on the less pure form of dinocap) were negative.

A multigeneration study of reproductive toxicity at dietary concentrations of 0, 40, 200, or 1000 ppm in rats showed no specific effect on any reproductive parameters; the NOAEL was 200 ppm, equal to 13 mg/kg bw per day.

In a study of developmental toxicity in mice dosed by gavage at 0, 4, 10, or 25 mg/kg bw per day, impaired otolith formation was seen at 25 mg/kg bw per day. A dose-related increase in the incidence of open eyelids and cleft palate extended down to 10 mg/kg bw per day in the absence of maternal toxicity. The NOAEL was 4 mg/kg bw per day. Dermal application of 50, 80, or 100 mg/kg bw per day to mice proved excessively toxic for an evaluation of developmental toxicity. A further dermal study in mice at 0, 1, 4, 10, or 25 mg/kg bw per day showed malformations, including impaired otolith formation, at 25 mg/kg bw per day in the absence of maternal toxicity. The NOAEL for developmental toxicity after dermal application to mice was 10 mg/kg bw per day. The results of the recent studies of developmental toxicity confirmed the teratogenic potential of purified dinocap in mice, even when applied dermally. Impaired otolith development, characteristic of the teratogenicity of dinocap in mice, was also seen in hamsters at doses that are maternally toxic. Less specific malformations were seen in rabbits at maternally toxic doses. The present Meeting concluded that the NOAEL in the studies in rabbits described by the 1989 JMPR was 3 mg/kg bw per day rather than 0.5 mg/kg bw per day, since the findings on which the putative effect level was established do not appear to be repeatable or clearly dose-

related. The methylheptyl isomer has been shown not to be teratogenic to mice. The reason for the species difference in the teratogenicity of dinocap in rats and mice therefore cannot be deduced from the data on the metabolism of the methylheptyl isomer.

The two-year study in dogs that was evaluated at the 1989 Joint Meeting was also reassessed on the basis that the critical effect (retinal atrophy) was secondary to effects on the tapetum lucidum. Since the tapetum lucidum is not present in humans, or in rats or mice in which no retinal effect was seen, the Meeting concluded that it would be inappropriate to base the evaluation on this effect. The NOAEL was 60 ppm, equivalent to 1.5 mg/kg bw per day.

Teratogenic effects in mice were considered to be the toxicological end-point of greatest concern. Since dinocap was teratogenic in mice after either oral or dermal administration and since malformations were seen in at least three species, the Meeting considered a high safety factor to be appropriate. An ADI of 0–0.008 mg/kg bw was established on the basis of the NOAEL of 4 mg/kg bw per day in the developmental toxicity study in mice and a safety factor of 500.

Establishment of an acute reference dose (RfD) was considered to be appropriate since teratogenicity may occur after a single exposure. An acute RfD was established on the basis of the NOAEL of 4 mg/kg bw per day for teratogenicity in mice and a safety factor of 500, to give an acute RfD of 0.008 mg/kg bw, which is appropriate for women of child-bearing age.

Toxicological evaluation

Levels that cause no toxic effect

Mouse:	15 ppm, equal to 2.7 mg/kg bw per day (toxicity in a study of carcinogenicity) 4 mg/kg bw per day (developmental toxicity) 10 mg/kg bw per day (maternal toxicity in a study of developmental toxicity)
Rat:	200 ppm, equal to 6.4 mg/kg bw per day (toxicity in a study of carcinogenicity) 50 mg/kg bw per day (maternal and developmental toxicity in a study of developmental toxicity)
Rabbit:	3 mg/kg bw per day (maternal toxicity in a study of developmental toxicity)
Dog:	60 ppm, equivalent to 1.5 mg/kg bw per day (study of toxicity)

Estimate of acceptable daily intake for humans

0–0.008 mg/kg bw

Estimate of acute reference dose

0.008 mg/kg bw

Information that would be useful for continued evaluation of the compound

Further observations in humans

List of end-points for setting guidance values for dietary and non-dietary exposure

Absorption, distribution, excretion and metabolism in mammals

Rate and extent of oral absorption	60–69% absorbed, max. concentration at 2–6 h
Dermal absorption	5–25%
Distribution	Widely distributed
Potential for accumulation	Limited, < 0.3% in tissue after 7 days
Rate and extent of excretion	Biphasic; half-life is 3 h for 1st phase, 44 h for 2nd phase, oral administration, rabbit

Metabolism in animals	Extensive; > 96% metabolized		
Toxicologically significant compounds (animals, plants and environment)	Metabolites assumed to be of similar toxicity to parent		
<i>Acute toxicity</i>			
Rat: LD ₅₀ oral	3100 mg/kg bw		
Rat: LD ₅₀ dermal	> 5000 mg/kg bw		
Rat: LC ₅₀ inhalation	3 mg/L		
Skin irritation	Irritating		
Eye irritation	Irritating		
Skin sensitization	Sensitizing		
<i>Short-term toxicity</i>			
Target/critical effect	General toxicity		
Lowest relevant oral NOAEL	Dog: 1.5 mg/kg bw per day		
Lowest relevant dermal NOAEL	Mouse: 10 mg/kg bw per day (teratogenicity)		
Lowest relevant inhalation NOAEL	No data		
<i>Genotoxicity</i>			
	Not genotoxic in an adequate battery of tests		
<i>Long-term toxicity and carcinogenicity</i>			
Target/critical effect:	Impaired weight gain		
Lowest relevant NOAEL	Mouse: 2.7 mg/kg bw per day (carcinogenicity)		
Carcinogenicity	Not carcinogenic		
<i>Reproductive toxicity</i>			
Reproduction target/critical effect	No effect on fertility or ability to rear young		
Lowest relevant reproductive NOAEL	Rat: 13 mg/kg bw per day (multigeneration study)		
Developmental target/critical effect	Malformations		
Lowest relevant developmental NOAEL	Mouse: 4 mg/kg bw per day		
<i>Neurotoxicity/Delayed neurotoxicity</i>			
	No data, but no concern raised in other studies		
<i>Other toxicological studies</i>			
	Inhibits oxidative phosphorylation; methylheptyl isomer not teratogenic		
<i>Medical data</i>			
	No significant dinocap-related effects reported		
Summary	Value	Study	Safety factor
ADI	0–0.008 mg/kg bw	Mouse, developmental toxicity	500
Acute reference dose	0.008 mg/kg bw	Mouse, developmental toxicity	500

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