

MEPTYLDINOCAP

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Explanation

Meptyldinocap is the International Organization for Standardization (ISO)–approved name for 2-(1-methylheptyl)-4,6-dinitrophenyl crotonate (International Union of Pure and Applied Chemistry [IUPAC]), with Chemical Abstracts Service (CAS) No. 131-72-6. Meptyldinocap is a new dinitrophenolic fungicidal compound, which acts by uncoupling mitochondrial oxidative phosphorylation.

Meptyldinocap was reviewed for the first time by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) at the request of the Codex Committee on Pesticide Residues (CCPR).

Meptyldinocap is one of the six structural analogues present in the existing active substance dinocap. Dinocap was evaluated previously by the JMPR in 1969, 1974, 1989, 1998 and 2000. In 1998, the acceptable daily intake (ADI) and the acute reference dose (ARfD) for dinocap were established at 0–0.008 mg/kg body weight (bw) and 0.008 mg/kg bw, respectively. In 2000, two ARfDs were established for dinocap, one for women of childbearing age, at 0.008 mg/kg bw, and another for the general population, at 0.03 mg/kg bw. Dinocap contains approximately 22% meptyldinocap.

The database supporting meptyldinocap consists of some new studies performed with meptyldinocap together with earlier studies performed with dinocap. A number of the kinetic and metabolism studies that used dinocap had the radiolabel in the methylheptyl isomer that was subsequently developed as meptyldinocap (see Table 1). Studies with meptyldinocap have been summarized in detail in this monograph, whereas previously evaluated studies with dinocap are summarized in [Appendix 1](#), with only the conclusions presented in the main part of the monograph. Most of the pivotal studies met the basic requirements of the relevant Organisation for Economic Co-operation and Development (OECD) or national test guidelines, although the level of detail in some of the older reports of studies performed with dinocap did not always meet current requirements. A number of studies using dinocap did not contain certificates of compliance with good laboratory practice (GLP).

Meptyldinocap has been known under the development codes DE 126, Dinocap II and RH-23-163. Dinocap was known as Karathane.

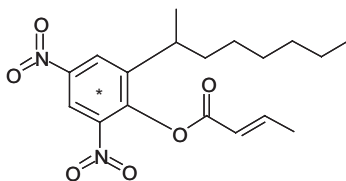
Table 1. Composition of dinocap

Isomer	Abbreviation	Nominal % in dinocap
2,4-dinitro-6-(1-methylheptyl)phenyl crotonate	2,4-DNHPC (meptyldinocap)	22
2,6-dinitro-4-(1-methylheptyl)phenyl crotonate	2,6-DNHPC	11
2,4-dinitro-6-(1-ethylhexyl)phenyl crotonate	2,4-DNEHPC	22
2,6-dinitro-4-(1-ethylhexyl)phenyl crotonate	2,6-DNEHPC	11
2,4-dinitro-6-(1-propylpentyl)phenyl crotonate	2,4-DNPPC	22
2,6-dinitro-4-(1-propylpentyl)phenyl crotonate	2,6-DNPPC	11

Evaluation for acceptable daily intake

The structure of meptyldinocap is shown in Figure 1.

Figure 1. Structure of meptyldinocap with position of radiolabel (*)



1. Biochemical aspects

No new absorption, distribution, metabolism and excretion (ADME) studies on meptyldinocap in mammals have been conducted. However, in a number of the ADME studies with dinocap, the radiolabel was present on the methylheptyl analogue, which is the primary component of meptyldinocap.

Table 2. Recovery of ^{14}C label from male mice ($n = 4$) following a single oral dose of [^{14}C]dinocap (mean $\pm$ standard deviation)

^{14}C compound	^{14}C dose (mg/kg bw)	Recovery (% of administered dose)					
		Urine	Urine funnel wash	Faeces	Cage wash	Tissues	Total
2,4-DNHPC	0.5	32 $\pm$ 19	13 $\pm$ 10	40 $\pm$ 16	7.2 $\pm$ 3	3.9 $\pm$ 0.6	96 $\pm$ 20
2,4-DNHPC	3	20 $\pm$ 9	39 $\pm$ 6	37 $\pm$ 9	3.5 $\pm$ 1	0.9 $\pm$ 0.2	100 $\pm$ 9
2,4-DNHPC	25	23 $\pm$ 8	16 $\pm$ 14	32 $\pm$ 5	12 $\pm$ 11	0.2 $\pm$ 0.02	83 $\pm$ 0.9
2,6-DNHPC	25	23 $\pm$ 17	17 $\pm$ 9	43 $\pm$ 16	6 $\pm$ 3	0.16 $\pm$ 0.1	90 $\pm$ 13

From DiDonato et al. (1986)

1.1 Absorption, distribution and excretion

(a) Oral route

Mice

Groups of male CD-1 mice received gavage doses of [phenyl- ^{14}C]dinocap (with the radiolabel present as either [^{14}C]2,4-dinitro-6-(1-methylheptyl)phenyl crotonate [2,4-DNHPC; meptyldinocap] or [^{14}C]2,6-dinitro-4-(1-methylheptyl)phenyl crotonate [2,6-DNHPC]). The vehicle was gum tragacanth, and the dose levels of dinocap were 0.5, 3 and 25 mg/kg bw. Blood samples were taken 6 times in the first 24 hours and then every 24 hours until 96 hours. Urine and faecal samples were taken at 6, 24, 48, 72 and 96 hours. At sacrifice (96 hours all doses; 6, 24, 48 and 72 hours at 25 mg/kg bw), tissue samples were taken and analysed for ^{14}C . Excretion was approximately equally divided between urine and faeces, but interpretation is confounded by the high residue in the urine funnel (Table 2), with no marked differences between analogues or among administered doses. The absorption half-life was calculated to be less than 1 hour. Elimination half-lives were longest for brain and eyes with 2,4-DNHPC and for liver and eyes with 2,6-DNHPC (DiDonato et al., 1986).

The rapid absorption and excretion of dinocap (containing [^{14}C]DNHPC) by male CD-1 mice were reported by Potter (1996), with 93% of the total urinary excretion occurring within 24 hours of gavage dosing at 25 mg/kg bw in corn oil. Urinary excretion (+ funnel wash) at 96 hours accounted for approximately 58% of the administered dose.

Rats

In an initial study, albino rats (one per sex) received 62 mg/kg bw per day of [^{14}C]dinocap (phenyl ring labelled) by oral gavage for 7 days. The [^{14}C]dinocap was a mixture of 2,4-dinitro-6-(2,3,4-octyl)phenyl crotonate (2,4-DNOPC) and 2,6-dinitro-4-(2,3,4-octyl)phenyl crotonate (2,6-DNOPC) (specific activity 20 MBq/g). Urine, faeces, exhaled carbon dioxide and cage wash were collected daily over the course of the study. After a 4-day withdrawal period, animals were sacrificed, and tissues were analysed for radioactivity. Quantification of the radioactive material was performed by liquid scintillation counting. In males, excretion was evenly split between urine and faeces; in females, faecal excretion predominated (about 65% versus 35% in urine). At sacrifice, tissue levels were low ($< 0.5\%$ of the administered dose), highest levels of ^{14}C being found in kidney, liver and skin (Graham, 1970).

Groups of male Sprague-Dawley rats received gavage doses of dinocap containing [^{14}C]2,4-DNHPC (meptyldinocap). The vehicle was gum tragacanth, and dose levels were 3, 25 or 100 mg/kg bw. Blood samples were taken 6 times in the first 24 hours and then every 24 hours until 96 hours.

Table 3. Recovery of ^{14}C label from male rats ($n = 4$) following a single oral dose of [^{14}C]dinocap (mean $\pm$ standard deviation)

^{14}C dose (mg/kg bw)	Recovery (% of administered dose)					
	Urine	Urine funnel wash	Faeces	Cage wash	Tissues	Total
3	38 $\pm$ 4.5	12 $\pm$ 9	61 $\pm$ 17	1.1 $\pm$ 1	0.3 $\pm$ 0.1	113 $\pm$ 12
25	29 $\pm$ 11	4.0 $\pm$ 2	57 $\pm$ 17	0.4 $\pm$ 0.2	0.13 $\pm$ 0.03	90 $\pm$ 29
100	35 $\pm$ 9	5.4 $\pm$ 2	64 $\pm$ 4	0.9 $\pm$ 0.3	0.15 $\pm$ 0.04	105 $\pm$ 12

From DiDonato et al. (1986)

Table 4. Findings in female rabbits ($n = 4$) treated orally with [^{14}C]2,4-DNHPC (means)

[^{14}C]2,4-DNHPC dose (mg/kg bw)	Number of animals	Specific activity (MBq/g) ^a	Peak plasma concentration at 6 h (ppm)	% of administered dose in urine + funnel wash	% of administered dose in faeces	% of dose absorbed ^a
0.5	4	205	0.29	45	72	69
3	4	146	1.3	31	67	60
25	4	18	15	44	57	64

From DiDonato & Longacre (1985)

ppm, parts per million

^a % of administered dose in urine versus % of intravenous dose in urine.

Urine and faecal samples were taken at 6, 24, 48, 72 and 96 hours. At sacrifice (96 hours all doses; 6, 24, 48 and 72 hours at 25 mg/kg bw), tissue samples were taken and analysed for ^{14}C . Excretion was primarily via faeces (about 60% versus 30–40% in urine) (Table 3), with no marked differences among administered doses. The absorption half-life was calculated to be less than 1 hour. Elimination half-lives were longest for brain and eyes (DiDonato et al., 1986).

The rapid absorption and excretion of dinocap (containing [^{14}C]DNHPC) by male Sprague-Dawley rats were reported by Potter (1996), with 95% of the total urinary excretion occurring within 24 hours of gavage dosing at 100 mg/kg bw in corn oil. Urinary excretion (+ funnel wash) at 96 hours accounted for approximately 31% of the administered dose.

Rabbits

Female New Zealand White rabbits (four per group) received [^{14}C]2,4-DNHPC (meptyldinocap), uniformly radiolabelled in the phenyl ring, by gavage at 0.5, 3 or 25 mg/kg bw (Table 4). Peak plasma levels increased with dose, whereas the proportion of ^{14}C in faeces and urine remained unchanged. In the region of 60–70% of the oral dose was calculated to be absorbed when related to the urinary excretion of an intravenous dose of 3 mg/kg bw (52%) and corrected for recovery (DiDonato & Longacre, 1985).

(b) Dermal route

Rabbits

In a study to complement a dermal developmental study with dinocap, female New Zealand White rabbits (four per group) were exposed to [^{14}C]2,4-DNHPC (meptyldinocap) uniformly radiolabelled in the phenyl ring. A number of doses and vehicles were used (Table 5). The proportion of the applied dose that was absorbed varied between 3.7% and 13%. Peak plasma concentrations determined at 6 hours increased with applied dose, indicating little, if any, saturation, and were 50–100 times lower than those from an equivalent oral dose (Table 4) (DiDonato & Longacre, 1985).

Table 5. Findings in female rabbits treated dermally with [^{14}C]2,4-DNHPC

[^{14}C]2,4-DNHPC dose (mg/kg bw)	Application rate (mg/cm ²)	Vehicle	Number of animals	Specific activity (MBq/g)	Peak plasma concentration at 6 h (ppm)	% of dose absorbed ^a
25	2.2	Neat	4	33.5	0.15	3.7 ± 1.8
100	8.3	Neat	4	8.2	0.47	5.1 ± 2.5
220	18	Neat	4	3.8	1.5	4.8 ± 3.8
25 (7 days)	2.1	Neat	4	32.7	0.24	13.1 ± 6.1
25	2.4	Powder	4	32.7	0.19	9.2 ± 3.2
25	2.5	Liquid concentrate	4	32.7	0.24	4.0 ± 1.3
25	2.2	Acetone	4	32.7	0.28	7.3 ± 2.2
25 (× 13)	—	Neat	4	32.7	—	6.2 ± 0.9

From DiDonato & Longacre (1985)

ppm, parts per million

^a % of administered dose in urine versus % of intravenous dose in urine.

Monkeys

Groups of four female Rhesus monkeys were exposed to [^{14}C]dinocap at dose levels of 0.2 mg/kg bw (6-hour exposures of abdominal skin). Plasma and urine samples were taken pretest and then repeatedly up to 96 hours. No ^{14}C was detected in plasma above the limit of quantification. Based on urinary excretion, corrected for approximately 49% urinary excretion of an intramuscular dose, the dermal absorption was estimated to be approximately 16% at an application rate of 40 $\mu\text{g}/\text{cm}^2$ and 5% at an application rate of 2500 $\mu\text{g}/\text{cm}^2$ (Wester & Maibach, 1985).

(c) Intravenous/intramuscular route

Rabbits

Female New Zealand White rabbits (four per group) received a single intravenous dose of [^{14}C]2,4-DNHPC (meptyldinocap) uniformly radiolabelled in the phenyl ring; the vehicle was dimethyl sulfoxide (DMSO). Total recovery was low (mean 76% ± 25%), with approximately 52% of the dose excreted in the urine, 11% in the urine funnel wash and 14% in the faeces (DiDonato & Longacre, 1985).

Monkeys

Four female Rhesus monkeys received a single dose of 670 μl (368 kBq) of ^{14}C -labelled dinocap by intramuscular injection. Urinary excretion from 0 to 4 hours accounted for 27% of the administered dose, with 52% excreted in urine by 24 hours (Maibach, 1985). Similar results were reported by Wester & Maibach (1985), with 49% of an intravenous dose of 200 $\mu\text{g}/\text{kg}$ bw being excreted in urine in 96 hours.

1.2 Biotransformation

Mice

The hydrolysis of [^{14}C]2,4-DNHPC (meptyldinocap) to the free phenol (2,4-dinitro-6-(1-methylheptyl)phenol) [2,4-DNHP] was investigated using CD-1 mouse plasma and liver microsomes. Incubations were performed at 37 °C. For plasma, a 2.5-minute reaction time and substrate concentration range of 10–100 $\mu\text{mol}/\text{l}$ were used; for liver microsomes, the incubation period was 2.5 minutes, with a substrate concentration range of 5–100 $\mu\text{mol}/\text{l}$. The results, presented in Table 6, show

Table 6. Kinetic parameters for the hydrolysis of 2,4-DNHPC by rat and mouse plasma and liver microsomes

	Mouse plasma	Rat plasma	Mouse liver	Rat liver
$V_{\max}$ (nmol/min per milligram protein)	5.6	12.5	1.7	3.3
K_m ($\mu\text{mol/l}$)	14	20	5.9	33

From Anderson & Longacre (1986)

K_m , Michaelis-Menten constant; $V_{\max}$, maximum rate

that while there are differences between rats (see next section) and mice, these do not appear to be significant in terms of the potential capacity to hydrolyse the crotonate bond (Anderson & Longacre, 1986).

An investigation of the metabolism of dinocap was performed by Udinsky, Tran & Frederick (1986). Groups of male CD-1 mice received a single dose of dinocap (containing [^{14}C]2,4-DNHPC [meptyldinocap]) at 0.5, 3 or 25 mg/kg bw by gavage in gum tragacanth. Excreta samples were taken over 24 hours and analysed for metabolites using liquid scintillation counting, high-performance liquid chromatography (HPLC) and ultraviolet (UV) absorption. There was a dose-related decrease in the proportion of the administered dose excreted in the urine (60% at 0.5 mg/kg bw, 24% at 25 mg/kg bw). Conjugation was found to be of limited significance following incubations with glucuronidases or arylsulfatases. In urine, there were three major metabolites accounting for over 60% of the urinary radioactivity. These were tentatively identified as being formed after hydrolytic cleavage of the crotonate moiety. In faeces, the main component was parent compound (approximately 30% of faecal radioactivity), with the hydrolysis product 2,4-DNHP representing about 20% (Udinsky, Tran & Frederick, 1986).

Fifteen male CD-1 mice received single doses of 25 mg/kg bw of an isotope mixture of [^{14}C]2,4-DNHPC (lot No. 742.0211; specific activity 500 MBq/g) and [^{13}C]2,4-DNHPC (lot No. 4133-0) dispersed in corn oil (total 2,4-DNHPC dose 2.5 mg/ml) by oral gavage. The isotope ratio of [^{14}C]2,4-DNHPC and [^{13}C]2,4-DNHPC administered to mice was 1:1 (no [^{12}C]2,4-DNHPC isotope was present). The isotopes were uniformly incorporated in the phenyl ring. Urine was collected over dry ice at 24-, 48-, 72- and 96-hour intervals after dosing. Aliquots (1 ml) of urine from 24-hour collection samples were pooled and used for identification of metabolites by HPLC and mass spectrometry and for quantification of metabolites by HPLC. Quantification of the radioactive material was performed by liquid scintillation counting. Identification was aided by comparison with synthetic standards.

Over 80% of the urinary radioactivity was eluted as 13 discrete HPLC peaks. The results are summarized in Table 7. None of the urinary metabolites contained the crotonate moiety, indicating that the initial reaction is probably hydrolysis of this component. Subsequent reactions include oxidation of the octyl chain, with β -oxidation the predominant metabolic pathway (approximately 47% of the urinary metabolites), whereas α -oxidation metabolites represent approximately 33%. Approximately 7% of the urinary metabolites were conjugated, but these were only partially characterized (Potter, 1996).

Rats

The hydrolysis of [^{14}C]2,4-DNHPC (meptyldinocap) to the free phenol (2,4-DNHP) was investigated using Sprague-Dawley rat plasma and liver microsomes. Incubations were performed at 37 °C. For plasma, a 2.5-minute reaction time and substrate concentration range of 10–100 $\mu\text{mol/l}$ were used; for liver microsomes, the incubation time was 5 minutes, with concentrations of 10–100 $\mu\text{mol/l}$. The results are presented in Table 6 above and show that whereas there are differences between rats

Table 7. Main metabolites found in urine of mice and rats administered meptyldinocap

Chemical name	% urinary radioactivity in mice	% urinary radioactivity in rats
2-Acetamino-4-nitro-6-(1-methyl- α,β -pentenoate)phenol	< 1%	2
2,4-Diacetamino-6-(1-methylpentanoate)phenol	< 1%	2
2,4-Dinitro-6-(1-methyl- β -hydroxypentanoate)phenol	8	4
2,4-Dinitro-6-(1-methylpropanoate)phenol	6	2
2,4-Dinitro-6-(1-methylbutanoate)phenol	24	18
2,4-Dinitro-6-(1-methyl- α,β -pentenoate)phenol	9	3
2,4-Dinitro-6-(1-methylpentanoate)phenol	22	58
2,4-Dinitro-6-(1-methyl- α,β -buten-4-ol)phenol	3	< 1%

From Potter (1996)

and mice, these do not appear to be significant in terms of the potential capacity to hydrolyse the crotonate bond (Anderson & Longacre, 1986).

Male and female Wistar rats were treated with daily doses of 11.7 mg/kg bw per day of [^{14}C]2,4-DNOPC (isomeric mixture of 95% *trans*-[^{14}C]2,4-DNOPC and 5% *cis*-[^{14}C]2,4-DNOPC; specific activity 65 MBq/g; 95% radiochemical purity) by oral gavage (vehicle: methylcellulose–water solution) for 7 days. Samples of excreta were analysed for metabolites using liquid scintillation counting, gas chromatography, mass spectrometry and nuclear magnetic resonance. Excretion was predominantly in faeces (52–58%), with 15–20% in urine. Over 20 metabolites were detected, but only 3 of these were identified. There were clear differences between the metabolite patterns in males and females. The main metabolites in male rat excreta were identified as 2,4-dinitro-6-(1-methyl-5-hydroxy-heptyl)phenol (37% of urinary excretion; 4% of faecal excretion), 2,4-dinitro-6-(1-methyl-7-carboxy-heptyl)phenol (27% of urinary excretion) and 2,4-dinitro-6-(2-octyl)phenol (5% of faecal excretion) (Honeycutt & Garska, 1976a,b).

Groups of male Sprague-Dawley rats received a single dose of [^{14}C]2,4-DNHPC (meptyldinocap) at 3, 25 or 100 mg/kg bw by gavage in gum tragacanth. Excreta samples were taken over 24 hours and analysed for metabolites using liquid scintillation counting, HPLC and UV absorption. There was no evidence of any decrease in the proportion of the administered dose excreted in the urine with increasing dose level. Conjugation was found to be of limited significance following incubations with glucuronidases or arylsulfatases. In urine, there were two major metabolites accounting for over 80% of the urinary radioactivity. These were tentatively identified as having been formed after hydrolytic cleavage of the crotonate moiety. UV spectra indicated that the rat metabolites were not substituted on the phenolic oxygen, whereas mouse metabolites were. In faeces, the main component was parent compound (approximately 30–40% of faecal radioactivity), with the hydrolysis product 2,4-DNHP representing about 13–30% (Udinsky, Tran & Frederick, 1986).

Five male Sprague-Dawley rats received single doses of 25 mg/kg bw of an isotope mixture of [^{14}C]2,4-DNHPC (lot No. 742.0211; specific activity 500 MBq/g) and [^{13}C]2,4-DNHPC (lot No. 4133-0) dispersed in corn oil (total 2,4-DNHPC dose 2.5 mg/ml; meptyldinocap) by oral gavage. The isotope ratio of [^{14}C]2,4-DNHPC and [^{13}C]2,4-DNHPC administered was 1:1 (no [^{12}C]2,4-DNHPC isotope was present). The isotopes were uniformly labelled in the phenyl ring position. Urine was collected over dry ice at 24-, 48-, 72- and 96-hour intervals after dosing. Aliquots (1 ml) of urine from 24-hour collection samples were pooled and used for identification of metabolites by HPLC and mass spectrometry and for quantification of metabolites by HPLC. Quantification of the radioactive

Table 8. Acute toxicity studies with meptyldinocap

Test substance	Species	Strain	Sex	Route	LD ₅₀ (mg/kg bw)	LC ₅₀ (mg/l)	Purity (%); batch	Vehicle	Reference
Meptyldinocap	Rat	F344	F	Oral	> 2000		92.9; SL1088R301	None	Merkel (2005a)
Meptyldinocap	Rat	F344	F	Dermal	> 5000		92.9; SL1088R301	None	Merkel (2005b)
Meptyldinocap	Rat	F344	M & F	Inhalation (nose only) ^a		1.2 (M) 1.6 (F)	91.5; SL1088R301	None	Krieger & Garlinghouse (2009)

F, female; LC₅₀, median lethal concentration; LD₅₀, median lethal dose; M, male

^a Mass median aerodynamic diameter 2.0–3.6 µm.

material was performed by liquid scintillation counting. Identification was aided by comparison with synthetic standards.

Over 90% of the urinary radioactivity was eluted as 12 discrete HPLC peaks. The results are summarized in Table 7 above. None of the urinary metabolites contained the crotonate moiety, indicating that the initial reaction is probably hydrolysis of this component. Subsequent reactions include oxidation of the octyl chain, with β -oxidation the predominant metabolic pathway (approximately 72% of the urinary metabolites), whereas α -oxidation metabolites represent approximately 18% (Potter, 1996).

The proposed metabolic pathway for rats, based primarily on the data of Potter (1996), is shown in Figure 2.

2. Toxicological studies

2.1 Acute toxicity

(a) Lethal doses

Meptyldinocap was of low acute toxicity via the oral and dermal routes and of moderate acute toxicity via the inhalation route (Table 8).

(b) Dermal and ocular irritation and dermal sensitization

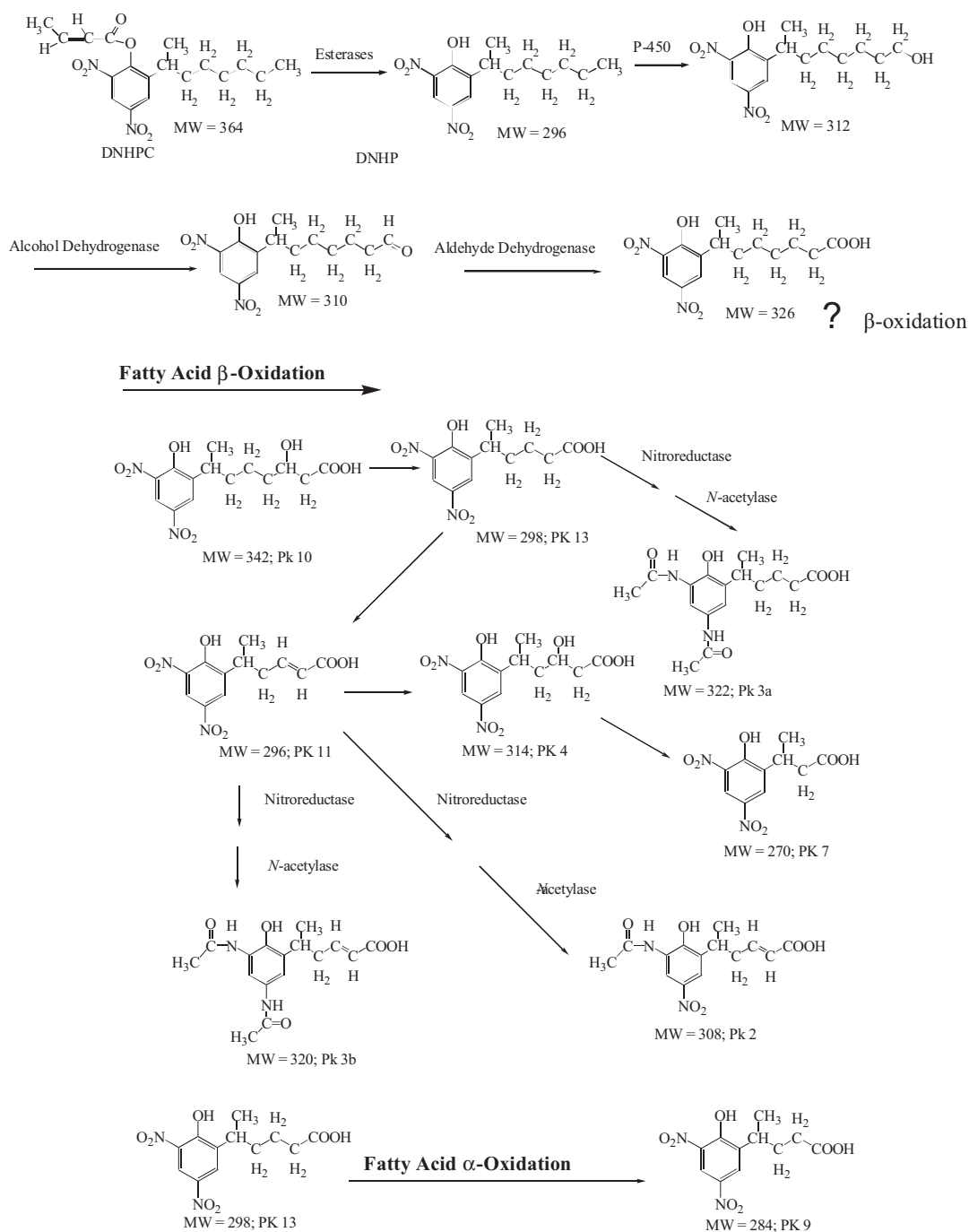
Meptyldinocap was slightly irritating to rabbit skin (Merkel, 2005c), was a moderate eye irritant (Merkel, 2005d) and gave positive results in a local lymph node assay for skin sensitization (Woolhiser & Wiescinski, 2005).

2.2 Short-term studies of toxicity

(a) Oral administration

Mice

In a GLP-compliant study, groups of CD-1 mice (five of each sex per dose) received meptyldinocap (purity 92.9%; lot No. SL1088R301) in the diet at 0, 100, 200 or 750 parts per million (ppm) for 28 days. Parameters evaluated included daily cage-side observations, weekly detailed clinical observations, ophthalmic examinations, fundic photographs, body weights, feed consumption, clinical chemistry, haematology (including prothrombin time), urinalysis and selected organ weights,

Figure 2. Proposed metabolic pathway of meptyldinocap in rats

with gross examinations of all animals and histopathological examinations (about 40 tissues) of control and top-dose animals plus any gross findings.

Achieved dietary concentrations and homogeneity were confirmed analytically; achieved mean intakes are given in Table 9. In the meptyldinocap groups, there were no deaths or clinical signs of note other than yellow urine staining in all treatment groups. Body weight gain was unaffected by treatment. Feed consumption was reduced in all treated male groups at the start of the study, but this is possibly related to unusually high consumption in control males; in females, feed consumption was

Table 9. Findings in mice (*n* = 5) receiving meptyldinocap in the diet for 28 days

	Males				Females			
	Dietary concentration (ppm)							
	0	100	200	750	0	100	200	750
Achieved intakes (mg/kg bw per day)	0	17	33	126	0	21	44	173
Body weight, week 4 (g)	39	37	38	38	29	27	28	27
Body weight gain, days 0–4 (g) (mean ± SD)	3.0 ± 2.1	1.2 ± 0.4	2.0 ± 0.5	2.0 ± 0.5	1.1 ± 0.8	0.9 ± 0.1	1.1 ± 0.6	1.2 ± 0.9
Feed consumption, days 0–4 (g/day) (mean ± SD)	7.0 ± 0.4	6.2 ± 0.1	5.9 ± 0.4	6.1 ± 0.7	5.3 ± 0.2	5.4 ± 0.7	5.4 ± 0.6	7.0 ± 1.1
Urine pH	7.5	7.0	7.0	6.5	6.0	6.5	7.0	6.0
Liver weight, absolute (g)	2.22	2.09	2.29	2.56	1.58	1.49	1.57	1.59
Liver weight, relative to body weight (%)	5.9	5.8	6.0	6.9*	5.5	5.5	5.6	6.0*
Aggregates of mononuclear cells, focal	0	0	0	1	0	0	0	0
Aggregates of mononuclear cells, multifocal	1	0	0	0	3	0	0	1
Necrosis, focal	0	0	0	0	0	0	1	0
Necrosis, with accompanying inflammation, focal	0	0	0	1	0	0	0	1

From Thomas, Yano & Dryzga (2005)

SD, standard deviation; * $P < 0.05$, both sexes combined due to small group size

increased in top-dose animals at the start of the study. There were no notable findings in ophthalmoscopy, clinical chemistry or haematology (although total white blood cell counts showed great variation across all groups). Urinary pH fluctuated between test groups; however, with no other indications of renal impairment, this finding is considered to be related to the production of acidic metabolites and not adverse. One top-dose male had a unilateral preputial abscess, and a top-dose female had a cloudy cornea, both of which are considered to be spontaneous; there were no other notable gross pathological findings. Liver weights were increased at 750 ppm, together with indications of very slight focal necrosis accompanied by inflammation (Table 9). No information is available on the mode of action behind the liver effects.

The no-observed-adverse-effect level (NOAEL) is 200 ppm (equal to 33 mg/kg bw per day), based on increased liver weights at 750 ppm (equal to 126 mg/kg bw per day) (Thomas, Yano & Dryzga, 2005).

Rats

In a GLP-compliant study, groups of Sprague-Dawley rats (10 of each sex per group) were given meptyldinocap (purity 92.9%; lot No. SL1088R301) in the diet at 0, 200, 650 or 2000 ppm for 90 days. Parameters evaluated were daily observations, detailed clinical observations, ophthalmological examinations, body weight, feed consumption, haematology (including prothrombin time), clinical chemistry, urinalysis and selected organ weights, with gross examinations of all animals plus histopathological examinations in controls and top-dose animals (about 40 tissues, including additional nervous system samples) plus all gross abnormalities. In addition to the above routine

Table 10. Findings in rats (*n* = 10) receiving meptyldinocap in the diet for 90 days

	Males				Females			
	Dietary concentration (ppm)							
	0	200	650	2000	0	200	650	2000
Achieved intakes (mg/kg bw per day)	0	12	40	122	0	14	44	137
Body weight, day 8 (g)	307	315	302	297	216	213	213	207
Body weight, day 92 (g)	527	563	503	485	298	298	293	279
Body weight gain, days 1–8 (g) (mean ± SD)	55 ± 4.6	58 ± 8.8	52 ± 6.4	45 ± 5.3	22 ± 8.3	22 ± 4.7	20 ± 9.0	15 ± 7.3
Feed consumption, days 1–8 (g/day) (mean ± SD)	24 ± 2.1	26 ± 2.2	25 ± 1.5	23 ± 1.1	17 ± 1.6	17 ± 0.9	17 ± 1.2	15* ± 0.9
Total protein (g/dl)	6.9	6.8	6.8	6.9	7.3	7.5	7.6	7.9*
Albumin (g/dl)	3.7	3.6	3.7	3.8	4.1	4.2	4.3	4.5*
Cholesterol (mg/dl)	52	54	46	64	61	72	71	82*
Liver weight, absolute (g)	12.9	14.7	12.9	13.6	7.0	7.2	7.2	7.8
Kidney weight, absolute (g)	3.5	3.6	3.3	3.4	1.9	1.9	1.9	2.0
Thyroid weight, absolute (g)	0.022	0.025	0.024	0.024	0.014	0.014	0.014	0.016
Hepatocyte hypertrophy, centrilobular/midzonal (very slight)	1/10	0/0	1/10	7/10*	0/10	0/0	0/10	3/10
Lacrimal glands, mononuclear cell infiltrate (very slight)	2/10	0/10	1/1	2/10	3/10	0/0	0/0	7/10*

From Johnson & Andrus (2005)

SD, standard deviation; * $P < 0.05$

parameters, potential neurotoxicity was assessed by an automated test of motor activity and a functional observational battery (FOB), including assessment of grip performance, landing foot splay and rectal temperature conducted pre-exposure and at the end of the study.

Achieved dietary concentrations and homogeneity were confirmed analytically; achieved mean intakes are given in Table 10. One animal from the 650 ppm group died the night before scheduled necropsy; the cause of death could not be ascertained. There were no clinical signs of note other than yellow urine staining. Body weight gain was consistently reduced at 2000 ppm; although the magnitude was relatively small, the study authors considered it to be treatment related. Feed consumption was reduced in top-dose females at most time points. There were no notable findings in the FOB, haematology, ophthalmoscopy or urinalysis investigations other than yellow coloration of the urine. Top-dose females had increased levels of total protein, albumin and cholesterol. Absolute liver, kidney and thyroid weights were increased (approximately 10%) in top-dose females, with marked increases in relative organ weights consequent to the low body weights. Histopathological findings were limited to very slight hepatocyte hypertrophy and mononuclear cell infiltrate of the lacrimal glands in top-dose animals (Table 10).

The NOAEL is 650 ppm (equal to 40 mg/kg bw per day), based on reduced feed consumption, altered clinical chemistry parameters and histopathological findings in the lacrimal glands at 2000 ppm (equal to 122 mg/kg bw per day) (Johnson & Andrus, 2005).

Dogs

Studies in dogs with dinocap had found marked ocular lesions at dose levels of 60 ppm and above (see [Annex 1](#), references 58 and 85, and [Appendix 1](#)).

Table 11. Acclimatization protocol for dietary incorporation of meptyldinocap

Day -7	Day -6	Day -5	Day -4	Day -3	Day -2	Day -1	Day +1
—	—	—	—	—	—	—	0 ppm
—	—	—	—	—	—	—	15 ppm
—	—	—	15 ppm	15 ppm	30 ppm	30 ppm	60 ppm ^a
15 ppm	15 ppm	30 ppm	30 ppm	60 ppm	60 ppm	60 ppm	120 ppm

From Stebbins, Brooks & Lacher (2005)

^a For dinocap and meptyldinocap.

In a GLP-compliant study, groups of Beagle dogs (four of each sex per group or eight of each sex at 120 ppm) received meptyldinocap (purity 92.9%; lot No. SL1088R301) in the diet at 0, 15, 60 or 120 ppm for 90 days; another group received dinocap (purity 93.4%; lot No. SB03088R301) at 60 ppm. The dogs were introduced to test material gradually to try to overcome palatability problems (Table 11). Parameters evaluated included daily cage-side observations, weekly detailed clinical observations, ophthalmic examinations, fundic photographs, body weights, feed consumption, clinical chemistry, haematology, prothrombin time, urinalysis, selected organ weights and gross and histopathological examinations (about 40 tissues) of all animals.

Achieved dietary concentrations and homogeneity were confirmed analytically. The achieved mean intakes are given in Table 12. In the meptyldinocap groups, there were no deaths or clinical signs of note other than yellow urine staining at 60 and 120 ppm. Reduced body weight gain was seen in males but not in females after the first week of dosing with 120 ppm (equal to 3.9 mg/kg bw per day), following the gradual introduction to the treated diets. The company proposed that the reduced body weight gain was due to reduced feed consumption, but this was not supported by data from individual animals or overall patterns of feed consumption (Table 13). Terminal body weights were not affected in the meptyldinocap groups, but interpretation is confounded by the large difference in initial body weights in males (8.9 kg in controls, 10.6 kg at 120 ppm). The overall body weight gain in top-dose males was reduced by 41% over the duration of the study. There were no notable findings in ophthalmoscopy, clinical chemistry or haematology. Fluctuations were seen in a number of clinical chemistry parameters, but there were no consistent patterns across or within test groups, and concurrent control values were often outside typical ranges. Uterine weights were increased in 15 ppm females, but this was attributed to estrous cycle stages. There were no treatment-related gross pathological findings. Very slight kidney tubule degeneration was present in a small number of animals (Table 12) receiving meptyldinocap, but it is not considered to be toxicologically adverse, given the low severity and absence of effects on renal function. One top-dose female had very slight degeneration of individual tibial nerve fibres.

In the animals receiving diet containing dinocap at 60 ppm, a range of findings was seen, including body weight loss, markedly reduced feed consumption, eye lesions and tibial nerve fibre degeneration (Table 12).

The NOAEL for meptyldinocap is 60 ppm (equal to 1.6 mg/kg bw per day), based on reduced body weight gain at 120 ppm (equal to 3.9 mg/kg bw per day) in males (Stebbins, Brooks & Lacher, 2005).

In an extension of Stebbins, Brooks & Lacher (2005), groups of Beagle dogs (four of each sex) received meptyldinocap (purity 92.9%; lot No. SL1088R301) in the diet at 120 ppm for 1 year. The dogs were introduced to meptyldinocap gradually (see Table 11 above) to overcome palatability issues. Parameters evaluated, which were relatively limited, included daily cage-side observations, weekly detailed clinical observations, monthly ophthalmic examinations, fundic photography, body weights, feed consumption and complete gross and selected histopathological examinations (eyes, tibial nerve and heart).

Table 12. Findings in dogs exposed to meptyldinocap and dinocap for 90 days.

	Control	Meptyldinocap			Dinocap
		Dietary concentration (ppm)			
		15	60	120	60
Males					
Compound intake (mg/kg bw per day)	0	0.5	1.6	3.9	1.9
Terminal body weight (kg)	10.6	11.1	11.4	11.6	10.4
Body weight gain, days 0–90 (kg)	1.7	1.7	1.3	1.0	0.5
Body weight gain, days 0–8 (g)	395	373	332	156*	–6
Eye, hyper-reflective fundus	0/4	0/4	0/4	0/4	4/4
Eye, blood vessel attenuation	0/4	0/4	0/4	0/4	3/4
Eye, hyperpigmentation associated with retinopathy	0	0	0	0	0
Kidney, tubule degeneration, focal (very slight)	0/4	0/4	0/4	0/4	1/4
Kidney, tubule degeneration, multifocal (very slight)	0/4	0/4	0/4	1/4	0/4
Tibial nerve fibre degeneration, focal (very slight)	0/4	0/4	0/4	0/4	3/4
Females					
Compound intake (mg/kg bw per day)	0	0.5	2.3	4.2	2.1
Body weight gain, days 0–90 (kg)	0.87	0.87	1.1	1.3	0.3
Body weight gain, days 0–8 (g)	144	254	334	193	–124
Eye, hyper-reflective fundus	0/4	0/4	0/4	0/4	3/4
Eye, blood vessel attenuation	0/4	0/4	0/4	0/4	2/4
Eye, hyperpigmentation associated with retinopathy	0	0	0	0	2/4
Kidney, tubule degeneration, focal (very slight)	0/4	0/4	0/4	1/4	0/4
Kidney, tubule degeneration, multifocal (very slight)	0/4	0/4	1/4	0/4	0/4
Tibial nerve fibre degeneration, focal (very slight)	0/4	0/4	0/4	1/4	3/4

From Stebbins, Brooks & Lacher (2005)

* $P < 0.05$

Achieved dietary concentrations and homogeneity were confirmed analytically. The achieved mean intake over the 12-month study was 3.5 mg/kg bw per day. There were no deaths or clinical signs other than yellow urine staining. Body weight gains in males were reduced for the first 90 days, but after that, the dogs maintained body weights consistent with controls in other 1-year studies. The only gross pathological or histopathological findings of note were very slight, unilateral degeneration of the tibial nerve fibres in one male and one female and slight inflammation of the skin/subcutis in two females and one male. The authors noted that spontaneous degeneration of peripheral nerves in young dogs has been reported at an incidence of less than 3%, and affected dogs were without clinical evidence of neuromuscular disease. A number of incidental ophthalmoscopic changes were reported: clear periocular soiling, well-defined lenticular nucleus, retinal folds and unilateral prolapse of the nictitans gland. Prolapse of the nictitans gland was considered to be genetic in origin. The original supplier of the Beagle dogs used in this study has indicated that the Beagle colony has a 5% incidence rate of spontaneous prolapse of the nictitans gland. Hypopigmented areas of the fundus were also noted in some dogs. This finding, when not associated with other retinal changes such as hyper-reflectivity, was considered by the examining ophthalmologist to be incidental pigment variation.

This study shows that the ocular effects seen in dogs administered 60 ppm dinocap for 90 days are not produced with higher doses of meptyldinocap (120 ppm for 1 year) (Stebbins, Brooks & Lacher, 2006a,b).

Table 13. Body weight and feed consumption data for individual dogs fed meptyldinocap for 90 days

Group	Animal number	Body weight (kg)		Body weight gain over days 1–8 (kg)	Feed consumption, days 1–8 (kg/day)	Pretest feed consumption, days –7 to –1 (kg/day)
		Test day 1	Test day 8			
0 ppm	264	7.542	7.841	0.299	0.307	—
	265	9.696	10.090	0.394	0.385	—
	266	9.359	9.879	0.520	0.361	—
	267	8.938	9.304	0.366	0.372	—
	Mean	8.884	9.279	0.395	0.356	—
	SD	0.947	1.014	0.093	0.034	—
120 ppm	280	9.524	9.766	0.242	0.354	0.319
	281	9.995	10.301	0.306	0.405	0.393
	282	10.899	11.161	0.262	0.351	0.320
	283	10.091	10.251	0.160	0.344	0.351
	284	10.727	10.683	–0.044	0.367	0.411
	285	10.297	10.099	–0.198	0.329	0.404
	286	11.840	12.045	0.205	0.427	0.465
	287	11.643	11.951	0.318	0.437	0.413
	Mean	10.626	10.782	0.156	0.377	—
	SD	0.809	0.856	0.183	0.041	—

From Stebbins, Brooks & Lacher (2005)

SD, standard deviation

(b) Dermal application

No data were submitted.

(c) Exposure by inhalation

No data were submitted.

2.3 Long-term studies of toxicity and carcinogenicity

All studies were performed with dinocap. Dinocap contains approximately 22% of the analogue present in meptyldinocap. The weight of evidence from short-term and developmental studies is that meptyldinocap is of lower toxicity than dinocap. It is considered that the carcinogenic potential of meptyldinocap, in terms of exposures from pesticidal use, can be based on the existing studies performed with dinocap.

Mice

Groups of CD-1 rats (60 of each sex per group) received dinocap (purity 96%; lot No. 2893) incorporated into the diet at levels of 0, 15, 100 or 200 ppm for 78 weeks. The NOAEL for general toxicity is 15 ppm (equal to 2.8 mg/kg bw per day for females), based on body weight deficits at 100 ppm (equal to 18 mg/kg bw per day). The NOAEL for carcinogenicity is 150 ppm (equal to 23 mg/kg bw per day), the highest dose tested (Moore, 1991) (see [Appendix 1](#) for further details).

Rats

Groups of CrI:CD®BR rats (20 of each sex per group) received dinocap (purity 97%; batch No. 632 6C21) in the diet at 0, 20, 200 or 2000 ppm for 52 weeks. The NOAEL is 200 ppm (equal to 11.4 mg/kg bw per day), based on reduced body weight gain and feed consumption, liver and thyroid changes and increased blood urea nitrogen at 2000 ppm (equal to 121 mg/kg bw per day) (Ferguson, Morrison & Bernacki, 1999) (see [Appendix 1](#) for further details).

Groups of Wistar rats (80 of each sex per group) received dinocap technical (purity 77.5%; batch not specified) incorporated into the diet at a level of 0, 20, 200 or 2000 ppm for 30 months (130 weeks). Interim kills were performed after 13, 26 and 52 weeks. The NOAEL for general toxicity is 200 ppm (equal to 6.4 mg/kg bw per day for males and 8.05 mg/kg bw per day for females) based on body weight deficits at 2000 ppm (equal to 71 mg/kg bw per day). The NOAEL for carcinogenicity is 2000 ppm (equal to 71 mg/kg bw per day), the highest dose tested (Maita et al., 1981) (see [Appendix 1](#) for further details).

Dogs

In a pre-GLP study, groups of Beagle dogs (four of each sex per dose) received dinocap technical; purity 78%; lot No. 2-0375) in the diet at 0, 15, 60 or 240 ppm for up to 2 years. The NOAEL is 15 ppm (equivalent to 0.4 mg/kg bw per day), based on retinal atrophy seen at 60 ppm (equivalent to 1.5 mg/kg bw per day) (Weatherholtz et al., 1979) (see [Appendix 1](#) for further details). These ocular lesions were not evident in the 1-year dog study with meptyldinocap described above (Stebbins, Brooks & Lacher, 2006a,b).

2.4 Genotoxicity

Testing of the genotoxicity of meptyldinocap has been performed in a range of assays. The overall database is considered adequate to conclude that meptyldinocap is not genotoxic.

Data on the genotoxicity of meptyldinocap are summarized in [Table 14](#).

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

Groups of CD:CrI®BR rats (26 of each sex per group) received dinocap (purity 96%; batch No. 2893) in the diet at 0, 40, 200 or 1000/400 ppm for 70 days before mating and during the mating and gestation periods of two generations. Dinocap produced no adverse effects on reproduction at the highest dose tested (1000 ppm, equal to 65 mg/kg bw per day in the first mating; 400 ppm, equal to 27 mg/kg bw per day in the second mating). The NOAEL for pup development is 200 ppm (equal to 13 mg/kg bw per day), based on reduced body weight and survival in the F₁ generation at 1000 ppm). The NOAEL for parental toxicity is 200 ppm (equal to 13 mg/kg bw per day), based on reduced body weight gains (Morseth, 1990) (see [Appendix 1](#) for further details).

(b) Developmental toxicity

Developmental toxicity was a particular concern with dinocap, with a variety of findings reported in mice, rats, hamsters and rabbits (see [Annex 1](#), references 58 and 85).

Table 14. Results of studies of genotoxicity with meptyldinocap

Test substance (vehicle)	End-point	Test object	Concentration	Purity (%); batch	Result	Reference
In vitro						
Meptyldinocap (DMSO)	Reverse mutation	<i>Salmonella typhimurium</i> strains TA98, TA100, TA1535, TA1537; <i>Escherichia coli</i> (WP2uvrA)	1, 3, 10, 33, 100, ^a 333, (1000, ^{b,c} 5000 ^b) µg/plate, with S9 mix 3, 10, 33, 100, 333, 1000, ^{a,c} 5000 µg/plate, without S9 mix	92.9; SL1088R301	Negative	Mecchi (2005)
Meptyldinocap (DMSO)	Gene mutation (HPRT)	Chinese hamster ovary cells (K ₁ -BH ₄)	0.5, 1, 2, 3, 4, 5, 6, 7, 8, ^a 9, 10, 15, 20 µg/ml without S9 mix 2, 4, 10, 20, 30, 40, 50, 52 ^a µg/ml with S9 mix	92.9; SL1088R301	Negative	Seidel, Schisler & Kleinert (2005)
Meptyldinocap (DMSO)	Chromosomal aberration	Sprague-Dawley rat lymphocytes	1, 3, 10 µg/ml –S9 (4 h) 0.3, 1, 3 µg/ml –S9 (24 h) 20, 30, 40 µg/ml +S9 (4 h)	92.9; SL1088R301	Negative	Charles, Schisler & Kleinert (2005)
In vivo						
Meptyldinocap (0.5% aqueous methylcellulose)	Micronucleus assay	CD-1 mouse bone marrow (gavage)	0, 500, 1000, 2000 mg/kg bw (24 h) 0, 2000 mg/kg bw (48 h)	92.9; SL1088R301	Negative	Charles, Grundy & Schisler (2005)

DMSO, dimethyl sulfoxide; S9, 9000 × g supernatant from livers of rats

^a Cytotoxicity became evident.

^b *E. coli* only.

^c Precipitation became evident.

Mice

In a GLP-compliant study, groups of CD-1 mice (15 mated females per group) received meptyldinocap (purity 98.5%; lot No. 2003-03220-64) by gavage in 0.5% methylcellulose on days 6–17 of gestation. Dose levels were 0, 100, 250 or 500 mg/kg bw per day. The study focused on investigations on the inner ear and cleft palate, which had been identified as targets for dinocap, and included a positive control group that received dinocap at 25 mg/kg bw per day. Dams were subjected to routine in-life observations. On gestation day (GD) 18, they were sacrificed and examined grossly, and the uterine contents were examined. All fetuses were examined for external alterations. Full visceral and skeletal examinations were not performed.

The pregnancy rate was low in the 100 and 500 mg/kg bw per day groups (Table 15), but this is not related to treatment, as pregnancy is determined before the dosing with meptyldinocap started. The only clinical sign of note in the meptyldinocap groups was the presence of bright yellow urine staining from the first day of dosing. One dam in the dinocap positive control group died on GD 18. One dam from the top-dose group delivered on GD 18. Body weights were reduced in the dinocap group but not in animals exposed to meptyldinocap. There were no significant findings in the meptyldinocap-treated groups; marked fetotoxicity was seen in the dinocap-treated group (Table 15). The single cleft palate seen in the low-dose meptyldinocap group is within the historical background range and, as it is not reproduced at higher dose levels, is not considered to be treatment related.

Table 15. Findings in mice exposed to meptyldinocap or dinocap during gestation

	Dose (mg/kg bw per day)				
	0	25 (dinocap)	100 (meptyldinocap)	250 (meptyldinocap)	500 (meptyldinocap)
No. bred	15	15	15	15	15
Pregnant (%)	12/15 (80.0)	13/15 (86.7)	9/15 (60.0)	12/15 (80.0)	8/15 (53.3)
No. of deaths	0	1	0	0	0
No. of litters with viable fetuses	12	10	9	12	7
No. of implantations/dam	13.5 ± 1.4	12.6 ± 1.4	13.2 ± 3.8	13.5 ± 1.8	13.4 ± 2.5
No. of resorptions/litter	0.5 ± 0.8	0.9 ± 1.5	2.4 ± 3.6	1.0 ± 1.1	0.7 ± 0.8
Resorptions/litters with resorptions	1.5 (6/4)	2.2 (11/5)	3.7 (22/6)	1.7 (12/7)	1.3 (5/4)
Mean % postimplantation loss	3.98 ± 6.4	24.20 ± 37.0*	18.3 ± 23.0	8.27 ± 9.20	5.23 ± 5.8
Viable fetuses/litter	13.0 ± 1.9	9.3 ± 4.5*	10.8 ± 4.4	12.4 ± 2.3	12.7 ± 2.4
Fetuses with cleft palate	0	109*	1	0	0
Mean otoconia score	11.8 ± 0.4	0.8* ± 1.0	11.7 ± 0.5	11.7 ± 0.6	11.8 ± 0.4

From Carney & Tornesi (2005a)

* $P < 0.05$

The data show that meptyldinocap does not have the same teratogenic potential in mice as dinocap. The NOAEL for maternal and fetotoxicity is 500 mg/kg bw per day, the highest meptyldinocap dose tested (Carney & Tornesi, 2005a).

In a non-GLP study, the developmental effects of dinocap and some of its component analogues were investigated in small groups of CD-1 mice. In the first phase, groups of 6–9 pregnant mice received dinocap (purity 84%; lot No. 3-83122) or the analogues 2,4-DNHPC (meptyldinocap) and 2,6-DNHPC (purity 95%) singly or in combination at 25 mg/kg bw per day. Dosing was by gavage in corn oil vehicle on days 7–16 of gestation. On GD 18, dams were killed and fetuses examined for cleft palate and external lesions. The only treatment that produced adverse effects was dinocap, which reduced fetal body weight by about 20% and produced cleft palate in 84% of the fetuses.

In the second phase, groups of 7–12 pregnant mice received dinocap (purity 84%; lot No. 3-83122) or the analogues 2,4-DNHPC (meptyldinocap) or 2,6-DNHPC (purity 95%) singly at 25 mg/kg bw per day. Dosing was by gavage in corn oil vehicle on days 7–16 of gestation; dams were allowed to litter normally. On day 45 postpartum, 20 pups per group were investigated for their swimming ability, head tilt (tortocollis) and otolith score. Results for corn oil and individual analogue groups were similar, with no adverse findings. The dinocap group showed reduced pup weights, reduced pup survival, increased incidences of tortocollis and inability to swim and a marked reduction in otolith score.

This study indicates that the developmental effects of dinocap in mice are not due to the two methylheptyl analogues tested and therefore not relevant to meptyldinocap, which is more than 90% (w/w) 2,6-DNHPC (Rogers et al., 1987).

In a further investigation of dinocap analogues, groups of 10 mated CD-1 mice received one of four different dinocap technical analogues on GD 6–17. The compounds were suspended in 0.5% methylcellulose and administered by gavage at dose levels of 0 or 10 mg/kg bw per day for the

Table 16. Findings (mean $\pm$ standard deviation) in mice exposed to dinocap analogues during gestation

	Control	4-EH	4-PP	6-EH	6-PP
	Dose (mg/kg bw per day)				
	0	5	5	10	10
Litters with viable fetuses	8	8	6	10	9
No. of implantations/dam	13.0 $\pm$ 3.9	13.6 $\pm$ 3.2	14.8 $\pm$ 1.8	13.7 $\pm$ 3.5	14.9 $\pm$ 1.7
Mean % postimplantation loss	7.05 $\pm$ 5.64	0.96* $\pm$ 2.72	38.77* $\pm$ 47.64	7.61 $\pm$ 17.09	7.23 $\pm$ 4.98
Viable fetuses/litter	12.0 $\pm$ 3.5	13.5 $\pm$ 3.3	9.0* $\pm$ 7.1	12.8 $\pm$ 4.3	13.8 $\pm$ 1.4
Mean fetal weight (g)	1.42 $\pm$ 0.11	1.37 $\pm$ 0.07	0.76* $\pm$ 0.22	1.38 $\pm$ 0.16	1.34 $\pm$ 0.08
Fetuses with cleft palate	0	0	83*	0	0
Fetuses with ablepharia	1	0	4	0	0
Fetuses with hindlimb club foot	0	0	5*	0	0
Mean otolith score	11.8 $\pm$ 0.2	11.6 $\pm$ 0.3	1.8* $\pm$ 2.3	11.8 $\pm$ 0.3	11.7 $\pm$ 0.3

From Carney & Tornesi (2005b)

* $P < 0.05$

2,4-dinitro-6-(1-ethylhexyl)phenyl crotonate (6-EH) and 2,4-dinitro-6-(1-propylpentyl)phenyl crotonate (6-PP) analogues and 5 mg/kg bw per day for the 2,6-dinitro-4-(1-ethylhexyl)phenyl crotonate (4-EH) and 2,6-dinitro-4-(1-propylpentyl)phenyl crotonate (4-PP) analogues. Maternal investigations included clinical observations, body weight and body weight gain. On GD 18, all surviving mice were killed and examined for gross pathological alterations. Gravid uterine weights and numbers of uterine implantations, resorptions and live/dead fetuses were recorded. All fetuses were weighed and examined for external alterations, including cleft palate, and otoconia. Adverse effects were seen only with the 4-PP analogue; severe maternal toxicity, requiring euthanasia in four dams, was evident from GD 12, with a range of fetal effects typical of dinocap (Table 16).

This study indicates that the developmental effects produced in mice by dinocap are associated with the 4-PP analogue (Carney & Tornesi, 2005b).

Rats

In a GLP-compliant study, groups of CD rats (26 mated females per group) received meptyldinocap (purity 97.4%; lot No. 2004-03140-64) by gavage in 0.5% methylcellulose on GDs 6–20. Dose levels were 0, 50, 150 or 500 mg/kg bw per day. The study complied with OECD guideline 414. Dams were subjected to routine in-life observations. On GD 21, they were sacrificed and examined grossly, and their uterine contents were examined. All fetuses were examined for external alterations; approximately half the fetuses were examined for visceral and craniofacial alterations, with the remainder examined for skeletal and otoconial alterations.

Bright yellow urine staining was evident from the beginning of dosing. Marked maternal toxicity (e.g. mortality, poor condition and body weight loss) was evident at 500 mg/kg bw per day, and this group was terminated on or before GD 11. At the next highest dose level of 150 mg/kg bw per day, there were reductions from GD 6 to GD 9 in feed consumption (approximately 18 g per rat) and maternal body weight gain (approximately 14 g per rat) at the start of the dosing period, with a body weight deficit persisting until termination. There was no evidence of maternal toxicity at 50 mg/kg bw per day; minor effects (< 10%) on feed consumption on GDs 6–9 and liver weights were considered not to be adverse. There was no fetotoxicity at 150 mg/kg bw per day (Table 17).

The NOAEL for maternal toxicity is 50 mg/kg bw per day, based on increased (> 20%) absolute liver weights and decreased body weight gain at 150 mg/kg bw per day. The NOAEL for devel-

Table 17. Findings in rats exposed to meptyldinocap during gestation^a

	Dose (mg/kg bw per day)		
	0	50	150
Body weight gain, GDs 6–9 (g)	20 ± 4.5	16 ± 5.1	5.7* ± 6.6
Body weight, GD 18 (g)	361 ± 21	357 ± 17	346* ± 21
Feed consumption, GDs 6–9 (g/day)	23 ± 1.8	21* ± 1.8	17* ± 2.4
Absolute liver weight (g)	14.4 ± 1.9	15.7* ± 1.4	17.7* ± 1.5
Number bred	26	26	26
Pregnant (%)	25/26 (96.2)	25/26 (96.2)	26/26 (100)
Number of litters totally resorbed	0	0	0
Number of litters with viable fetuses	25	25	26
Number of corpora lutea/dam	13.8 ± 1.9	13.8 ± 0.9	14.0 ± 2.3
Number of implantations/dam	13.1 ± 2.1	13.5 ± 1.0	13.2 ± 2.0
Number of resorptions/litter	0.1 ± 0.3	0.2 ± 0.5	0.2 ± 0.6
Viable fetuses/litter	13.0 ± 2.2	13.2 ± 1.1	13.0 ± 2.0
Fetal weight, sexes combined (g)	5.8 ± 0.30	5.9 ± 0.24	5.8 ± 0.25
Gravid uterine weight (g)	102 ± 14.8	108 ± 8.5	105 ± 13.7
Fetuses with external malformations	0/325	0/331	0/338
Fetuses with craniofacial malformations	0/168	0/172	0/176
Fetuses with visceral malformations	0/168	4/172	0/176
Fetuses with skeletal malformations	2/157	1/159	0/162
Mean otoconia scores	12.0 ± 0.1	11.8 ± 0.4	11.9 ± 0.32

From Carney & Tornesi (2005c)

* $P < 0.05$

^a Findings are ± standard deviation.

opmental toxicity is 150 mg/kg bw per day, the highest dose tested for the full duration of the study (Carney & Tornesi, 2005c).

Rabbits

In a GLP-compliant study, groups of New Zealand White rabbits (26 mated females per group) received meptyldinocap (purity 97.4%; lot No. 2004-03140-64) by gavage in 0.5% methylcellulose on GDs 7–27. Dose levels were 0, 3, 12 or 48 mg/kg bw per day. The study complied with OECD guideline 414. Dams were subjected to routine in-life observations. On GD 28, they were sacrificed and examined grossly, and their uterine contents were examined. All fetuses were examined for external, visceral and skeletal alterations; approximately half the fetuses were examined for craniofacial alterations or for otoconia.

Yellow urine staining was evident in most top-dose animals, four mid-dose animals and two low-dose animals. Two top-dose animals died on day 20 after a period of inanition; one of the dams had a low feed consumption even before dosing commenced. Maternal body weight loss was seen in several dams before dosing commenced and in half the dams exposed at 48 mg/kg bw per day early in the dosing period. From GD 7 to GD 10, dams in the top-dose group had a mean deficit of 44 g in body weight gain relative to controls; although this was similar in magnitude to the decrease in feed consumption over the same period, there was no apparent consistency in individual body weight gains or feed consumption. Over the remainder of the study, the body weight gain in the top-dose group was similar to that in controls, and from GD 20 to GD 28 (during which dosing continued), the body

Table 18. Findings in rabbits exposed to meptyldinocap during gestation

	Dose (mg/kg bw per day)			
	0	3	12	48
Body weight gain, GDs 7–10 (g)	42 ± 32	16 ± 42	33 ± 31	–4* ± 56
Body weight gain, GDs 10–13 (g)	50 ± 36	63 ± 41	65 ± 44	43 ± 57
Body weight gain, GDs 20–28 (g)	117	132	114	151
Body weight, GD 28 (g)	3582	3493	3532	3549
Feed consumption, GDs 7–10 (g/day)	545	522	551	513
Pregnant (%)	25/26 (96.2)	25/26 (96.2)	23/26 (88.5)	24/26 (92.3)
Number of litters with viable fetuses	25	25	23	22
Number of corpora lutea/dam	9.4 ± 1.6	9.4 ± 2.7	9.6 ± 1.6	8.9 ± 1.6
Number of implantations/dam	8.7 ± 1.6	8.7 ± 2.5	8.6 ± 1.8	8.1 ± 1.4
Number of resorptions/litter	0.5 ± 0.7	0.4 ± 1.1	0.5 ± 0.8	0.1 ± 0.4
Viable fetuses/litter	8.2 ± 1.3	8.3 ± 2.0	8.1 ± 1.7	8.0 ± 1.4
Fetal weight, sexes combined (g)	37 ± 2.7	36 ± 4.0	37 ± 3.3	35 ± 4.0
Gravid uterine weight (g)	460 ± 70	444 ± 100	445 ± 77	418 ± 52
Fetuses with external malformations	0/206	3/207	0/187	0/175
Fetuses with craniofacial malformations	0/109	2/109	0/99	0/92
Fetuses with visceral malformations	7/206	2/207	1/187	4/175
Fetuses with skeletal malformations	2/192	0/207	1/186	0/175
Mean otoconia scores	11.9 ± 0.2	11.9 ± 0.2	11.7 ± 0.8	11.9 ± 0.2

From Carney & Tornesi (2005d)

* $P < 0.05$

weight gain was greater than that in controls. An increase in kidney weight of approximately 8% was seen in the top-dose group, but this is not considered to be adverse. There were no adverse effects on pregnancy outcome or on the pattern of fetal findings, and ossification was similar in control and treated groups (Table 18).

The NOAEL for maternal toxicity is 12 mg/kg bw per day, based on body weight loss at the beginning of dosing. The NOAEL for developmental effects is 48 mg/kg bw per day, the highest dose tested (Carney & Tornesi, 2005d).

2.6 Special studies

There are no special studies.

3. Observations in humans

Meptyldinocap is a relatively new molecule, and no workplace monitoring data are available.

Comments

Biochemical aspects

No new ADME studies on meptyldinocap in mammals have been conducted. However, in a number of the ADME studies with dinocap, the radiolabel was present on the methylheptyl analogue, which is the primary component of meptyldinocap. The Meeting considered that these ADME studies were applicable to meptyldinocap.

Meptyldinocap is relatively well absorbed, with approximately 60–70% of the radiolabel absorbed in rabbits. Absorption is rapid, with peak plasma radioactivity seen 1–6 hours after oral administration. Radiolabel was widely distributed, with tissue levels generally low and below those in blood. The compound did not tend to concentrate in any particular organ or tissue; highest levels were found in the liver, kidneys and skin. Metabolism was extensive, consisting of hydrolytic cleavage to release the crotonate moiety and subsequent oxidation of the methylheptyl chain. The basic metabolic pathways are similar in rats and mice. Excretion of radiolabel was extensive via urine (39–58% in mice; 31–50% in rats) and faeces and mainly occurred within 48 hours.

Toxicological data

Meptyldinocap is of low acute toxicity when administered orally or dermally ($LD_{50} > 2000$ mg/kg bw) but is of moderate toxicity by inhalation (LC_{50} 1.2 mg/l). Meptyldinocap is a slight irritant to skin and a moderate irritant to the eye; it has been found to produce skin sensitization in a local lymph node assay in mice.

Short-term studies of toxicity with meptyldinocap were performed in mice, rats and dogs. Yellow urine staining was a consistent finding, but this is considered not to be adverse, as it is associated with the excretion of coloured metabolites of meptyldinocap. In a 28-day dietary study of meptyldinocap in mice, there were increases in liver weight (approximately 10–15%) at 750 ppm (equal to 126 mg/kg bw per day), with a NOAEL of 200 ppm (equal to 33 mg/kg bw per day). In a 90-day dietary study of meptyldinocap in rats, altered clinical chemistry parameters and mononuclear cell infiltration of the lacrimal glands were seen at 2000 ppm (equal to 122 mg/kg bw per day), with a NOAEL of 650 ppm (equal to 40 mg/kg bw per day).

In a 90-day dietary study, groups of dogs were exposed to meptyldinocap at 0, 15, 60 or 120 ppm, with a positive control group receiving 60 ppm dinocap. Reduced body weight gain was seen in males after the first week of dosing with 120 ppm (equal to 3.9 mg/kg bw per day), following a gradual introduction to the treated diets. These initial body weight effects showed no consistency between animals or with feed consumption patterns. The body weight gain over 90 days was 41% lower in males receiving 120 ppm than in controls. Ocular changes seen in the dinocap-exposed group were not evident in the meptyldinocap-treated groups. The NOAEL for meptyldinocap was 60 ppm (equal to 1.6 mg/kg bw per day), based on the effects on body weight gain over the duration of the study. In an extension to this 90-day study, a satellite group was exposed to meptyldinocap for 1 year at 120 ppm (equal to 3.5 mg/kg bw per day). The examinations were limited to tibial nerves, eyes and heart. This segment of the study showed that there were no significant eye, heart or nerve lesions evident after exposure to meptyldinocap for 1 year. The reduced body weight gain seen in the 90-day phase was not evident over the extended dosing period. The 1-year study was not designed to permit the identification of a NOAEL.

No evidence of carcinogenicity was seen in long-term studies of toxicity and carcinogenicity with dinocap at the highest doses tested, 150 ppm (equal to 23 mg/kg bw per day) in mice and 2000 ppm (equal to 71 mg/kg bw per day) in rats. The NOAEL for general toxicity in the chronic study in mice with dinocap was 15 ppm (equivalent to 2.8 mg/kg bw per day), based on body weight deficits in females. In the 30-month study of dinocap in rats, there was a significant increase in survival in both sexes at the top dose of 2000 ppm (equal to 71 mg/kg bw per day), which had an impact on the incidences of a number of age-related changes. The NOAEL for general toxicity of dinocap was 200 ppm (equal to 6.4 mg/kg bw per day).

The potential genotoxicity of meptyldinocap has been investigated in an adequate range of tests *in vitro* and *in vivo*. No evidence of mutagenicity or clastogenicity was noted.

The Meeting concluded that meptyldinocap is unlikely to be genotoxic.

The Meeting concluded that dinocap is not carcinogenic and that this conclusion could be extrapolated to meptyldinocap.

No effects on fertility, reproductive parameters, sperm or reproductive tissues were seen in a two-generation dietary study with dinocap at doses up to 400 ppm (equal to 27 mg/kg bw per day), the highest dose tested in the second generation. In the first generation, at 1000 ppm (equal to 65 mg/kg bw per day), reduced pup survival led to a reduction in the dose level to 400 ppm for the second generation, which was without effect on pups. The NOAEL for pup development and parental toxicity was 200 ppm (equal to 13 mg/kg bw per day).

In a developmental toxicity study in mice investigating effects on the palate and inner ear, meptyldinocap did not produce any such effects on fetuses at the highest dose of 500 mg/kg bw per day, whereas a dinocap dose of 25 mg/kg bw per day produced cleft palate in nearly all fetuses and had marked effects on otoconia formation. Additional studies showed that the teratogenicity of dinocap in mice was associated with the 4-propylpentyl analogue and not the methylheptyl analogue present in meptyldinocap. In a developmental toxicity study in rats, marked maternal toxicity and marked reductions (approximately 50%) in feed consumption were seen with a meptyldinocap dose of 500 mg/kg bw per day, such that this dose level had to be terminated by GD 11. At the next highest dose level of 150 mg/kg bw per day, there were reductions from GD 6 to GD 9 in feed consumption (approximately 18 g per rat) and maternal body weight gain (approximately 14 g per rat) at the start of the dosing period. The body weight deficit and increased absolute liver weights (23%) were evident at the end of the study, but there were no indications of fetotoxicity. The NOAEL for maternal toxicity was 50 mg/kg bw per day, with the NOAEL for fetotoxicity being 150 mg/kg bw per day. In a rabbit developmental toxicity study with meptyldinocap, maternal body weight loss was seen in several dams before dosing commenced and in half the dams exposed at 48 mg/kg bw per day early in the dosing period. From GD 7 to GD 10, dams in the top-dose group had a mean deficit of 44 g in body weight gain relative to controls; although this was similar in magnitude to the decrease in feed consumption over the same period, there was no apparent consistency in individual body weight gains or feed consumption. Over the remainder of the study, the body weight gain in the top-dose group was similar to that in controls, and from GD 20 to GD 28 (during which dosing continued), the body weight gain was greater than that in controls. There were no effects on the fetuses at any dose level. The maternal NOAEL was 12 mg/kg bw per day, and the NOAEL for fetotoxicity was 48 mg/kg bw per day.

The Meeting concluded that meptyldinocap did not induce developmental toxicity and that it was not teratogenic.

There are no specific neurotoxicity studies on meptyldinocap, but there were no indications of neurotoxicity in routine studies, including a 90-day study in rats that included an FOB.

No information on medical surveillance or poisoning incidents was available.

The Meeting concluded that the existing database on dinocap and meptyldinocap was adequate to characterize the potential hazards of meptyldinocap to fetuses, infants and children.

Toxicological evaluation

The Meeting established an ADI of 0–0.02 mg/kg bw on the basis of the NOAEL of 1.6 mg/kg bw per day in the 90-day dietary study in dogs, based on reduced body weight gain in males at 3.9 mg/kg bw per day. A safety factor of 100 was applied.

The Meeting concluded that an ARfD was unnecessary, as there were no effects that could be attributed to a single exposure. Meptyldinocap did not produce neurotoxicity, fetotoxicity or reproductive effects and has an oral LD₅₀ of greater than 2000 mg/kg bw. The Meeting reviewed in depth the reduced body weight gains and feed consumption seen in the early stages of the 90-day study

in dogs and the developmental toxicity studies in rats and rabbits. In the rat developmental toxicity study, the body weight deficits were considered secondary to reduced feed consumption, which was probably associated with palatability issues. The Meeting concluded that the body weight and feed consumption patterns seen in the early stages of the dog and rabbit studies were not consistent between individual animals. The findings in these three studies did not provide an appropriate basis for establishing an ARfD for meptyldinocap.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Twenty-eight-day study of toxicity with meptyldinocap ^a	Toxicity	200 ppm, equal to 33 mg/kg bw per day	750 ppm, equal to 126 mg/kg bw per day
	Seventy-eight-week study of toxicity and carcinogenicity with dinocap ^a	Toxicity	15 ppm, equal to 2.8 mg/kg bw per day	100 ppm, equal to 18 mg/kg bw per day
		Carcinogenicity	150 ppm, equal to 23 mg/kg bw per day ^b	—
Rat	Ninety-day study of toxicity with meptyldinocap ^a	Toxicity	650 ppm, equal to 40 mg/kg bw per day	2000 ppm, equal to 122 mg/kg bw per day
	Thirty-month study of toxicity and carcinogenicity with dinocap ^a	Toxicity	200 ppm, equal to 6.4 mg/kg bw per day	2000 ppm, equal to 71 mg/kg bw per day
		Carcinogenicity	2000 ppm, equal to 71 mg/kg bw per day ^b	—
	Multigeneration study of reproductive toxicity with dinocap ^a	Reproductive toxicity	400 ppm, equal to 27 mg/kg bw per day ^b	—
		Parental toxicity	200 ppm, equal to 13 mg/kg bw per day	400 ppm, equal to 27 mg/kg bw per day
		Offspring toxicity	200 ppm, equal to 13 mg/kg bw per day	400 ppm, equal to 27 mg/kg bw per day
	Developmental toxicity study with meptyldinocap ^c	Maternal toxicity	50 mg/kg bw per day	150 mg/kg bw per day
		Embryo and fetal toxicity	150 mg/kg bw per day ^b	—
Rabbit	Developmental toxicity study with meptyldinocap ^c	Maternal toxicity	12 mg/kg bw per day	48 mg/kg bw per day
		Embryo and fetal toxicity	48 mg/kg bw per day ^b	—
Dog	Ninety-day study of toxicity with meptyldinocap ^a	Toxicity	60 ppm, equal to 1.6 mg/kg bw per day	120 ppm, equal to 3.9 mg/kg bw per day

^a Dietary administration.

^b Highest dose tested.

^c Gavage administration.

Estimate of acceptable daily intake for humans

0–0.02 mg/kg bw

Estimate of acute reference dose

Unnecessary

Information that would be useful for the continued evaluation of the compound

Results from epidemiological, occupational health and other such observational studies of human exposure

Critical end-points for setting guidance values for exposure to meptyldinocap*Absorption, distribution, excretion and metabolism in mammals*

Rate and extent of oral absorption	Rapid; moderately well absorbed (60–70%)
Distribution	Widely distributed
Potential for accumulation	None
Rate and extent of excretion	Relatively rapid
Metabolism in animals	Extensively metabolized, initially hydrolysis to remove the crotonate side-chain and then via oxidation of the methylheptyl chain
Toxicologically significant compounds (animals, plants and the environment)	Meptyldinocap

Acute toxicity

Rat, LD ₅₀ , oral	> 2000 mg/kg bw
Rat, LD ₅₀ , dermal	> 5000 mg/kg bw
Rat, LC ₅₀ , inhalation	1.2 mg/l (4 h, nose only)
Rabbit, dermal irritation	Slight
Rabbit, ocular irritation	Moderate
Mouse, dermal sensitization	Sensitizer (local lymph node assay)

Short-term studies of toxicity

Target/critical effect	Body weight gain (males)
Lowest relevant oral NOAEL	Dog: 1.6 mg/kg bw per day (meptyldinocap)
Lowest relevant dermal NOAEL	No data
Lowest relevant inhalation NOAEC	No data

Long-term studies of toxicity and carcinogenicity

Target/critical effect	Body weight
Lowest relevant NOAEL	Mouse: 2.8 mg/kg bw per day (dinocap)
Carcinogenicity	Not carcinogenic in rats or mice

Genotoxicity

Not genotoxic in vitro or in vivo

Reproductive toxicity

Reproduction target/critical effect	Pup survival
Lowest relevant reproductive NOAEL	Rat: 13 mg/kg bw per day (dinocap)
Developmental target/critical effect	None
Lowest relevant developmental NOAEL	Rabbit: 48 mg/kg bw per day (meptyldinocap)

Neurotoxicity/delayed neurotoxicity

No indications in routine studies

Other toxicological studies

No data

Medical data

No data

Summary

	Value	Study	Safety factor
ADI	0–0.02 mg/kg bw	Ninety-day study of toxicity in dogs	100
ARfD	Unnecessary		

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Appendix 1: Summaries of studies performed with dinocap and relevant to the evaluation of meptyldinocap

Long-term studies of toxicity and carcinogenicity

Mice

In a non-GLP chronic toxicity/carcinogenicity study, groups of CD-1 rats (60 of each sex per group) received dinocap technical (purity 96%; lot No. 2893) incorporated into the diet at levels of 0, 15, 100 or 200/150 ppm for 78 weeks. Mortality, clinical signs, body weight, behaviour, and feed and water consumption were recorded routinely. Ophthalmoscopy examinations were performed on all animals at weeks 52 and 78. Haematology samples were obtained from all animals at weeks 52 and 78. Gross examinations were conducted for all animals sacrificed or dying during the course of the study, main organs were weighed and an extensive range of organs from all control and top-dose animals was examined histopathologically. Lung, liver, kidneys, gross lesions and suspected tumours from all groups were examined histopathologically.

Homogeneity and stability of the test diets were confirmed analytically; although there was a degree of variability in the level of exposure over the duration of the study, this is considered insignificant. Mean intakes were 0, 2.3, 15 and 23 mg/kg bw per day in males and 0, 2.8, 18 and 27 mg/kg bw per day in females. As a result of deaths in females at the 200 ppm dose level during week 1, the top dose level was reduced to 150 ppm; subsequently, survival was similar in all groups (> 55% in all groups at termination). There were no clinical signs associated with treatment other than urine stains in top-dose males. Body weights and feed consumption were reduced throughout the study in top-dose animals of both sexes, with mean body weight gain also reduced significantly in mid-dose females (Table 19). Slight deficits in feed consumption and body weight in 15 ppm males were not considered to be adverse due to the small magnitude (< 10%) and absence of an effect on body weight in 100 ppm males. There were no treatment-related ophthalmoscopy or haematological findings. Testicular atrophy (bilateral) was increased at 150 ppm; absolute seminal vesicle weights were also decreased at 150 ppm, but this appeared to be secondary to the body weight deficits. A slight reduction in absolute testes weight at 100 ppm is not considered to be adverse, as there are no related pathological changes and the value is within normal variation (Table 19). There were no increases in tumour incidences in treated animals.

The NOAEL for general toxicity is 15 ppm (equal to 2.8 mg/kg bw per day for females), based on body weight deficits at 100 ppm (equal to 18 mg/kg bw per day). The NOAEL for carcinogenicity is 150 ppm (equal to 23 mg/kg bw per day), the highest dose tested (Moore, 1991).

Rats

In a GLP-compliant 1-year toxicity study, groups of CrI:CD®BR rats (20 of each sex per group) received dinocap (purity 97%; batch No. 632 6C21) in the diet at 0, 20, 200 or 2000 ppm for 52 weeks. Rats were observed routinely for mortality, clinical signs, body weight, feed consumption and water consumption. After 13, 26 and 52 weeks of treatment, samples from 10 rats of each sex per group were collected for haematology, clinical chemistry and urinalysis examinations. Ophthalmological examinations were performed on all animals prior to initiation and prior to termination of treatment. The weights of seven organs were recorded after autopsy from 10 animals of each sex per group. An extensive range of organs from all animals was preserved in buffered 10% formalin for histopathological examination. Histopathological examinations were performed on tissues of all animals in the control and 2000 ppm groups and for all animals found dead or killed as moribund during the course of the study. Homogeneity and stability of the test diets were confirmed analytically. Achieved intakes were 0, 1.1, 11 and 121 mg/kg bw per day in males and 0, 1.4, 14 and 144 mg/kg bw per day in females.

Table 19. Findings in mice (n = 60) receiving dinocap in the diet for 78 weeks

	Males				Females			
	Dietary concentration (ppm)							
	0	15	100	150	0	15	100	150
Body weight, week 1 (g)	30.9	30.3	29.7*	28.8*	23.8	23.5	23.7	20.5*
Body weight, week 78 (g)	39.2	37.4*	37.9	37.0*	32	31.5	30.3	28.9*
Body weight gain, weeks 0–78 (g) (mean ± SD)	9.5 ± 2.9	8.5 ± 2.5	8.6 ± 2.5	7.7* ± 3.4	9.3 ± 3.9	9.2 ± 3.4	7.2* ± 2.4	6.2* ± 2.1
Mean feed consumption, week 1 (g/week)	38.3	37.2	35.0*	31.4*	36.0	35.1	31.3*	26.2*
Mean total feed consumption, weeks 0–78 (g)	1224	1165*	1158*	1121*	1143	1132	1111	1059*
Testicular atrophy (bilateral)	11	4	11	25*	—	—	—	—
Testes (small)	3	1	6	12*	—	—	—	—
Testes weight, absolute (g)	0.21 ± 0.05	0.23 ± 0.04	0.19* ± 0.04	0.16* ± 0.06	—	—	—	—
Seminal vesicle weight, absolute (g)	0.56 ± 0.19	0.55 ± 0.20	0.54 ± 0.29	0.45* ± 0.12	—	—	—	—
Testes weight, relative to body weight (%)	0.61 ± 0.14	0.68* ± 0.12	0.56 ± 0.13	0.48* ± 0.18	—	—	—	—
Seminal vesicle weight, relative to body weight (%)	0.40 ± 0.05	0.42 ± 0.06	0.39 ± 0.05	0.40 ± 0.06	—	—	—	—

From Moore (1991)

* $P < 0.05$

Five animals died or were sacrificed during the study, but, as none of these were in the top-dose groups, they are considered to be unrelated to treatment. The only clinical sign was bright yellow-stained urine in both sexes at 200 and 2000 ppm and yellow-stained anogenital area in both sexes at 2000 ppm. Body weight deficits consistent with reduced feed consumption were evident throughout the study at 2000 ppm. There were no notable changes in haematological values. Statistically significant reductions in levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), cholesterol and triglycerides were seen at 2000 ppm at some sampling times, but values were within normal variation at week 52. Blood urea nitrogen was increased in top-dose females at all sampling times. Statistically significant increases in relative organ weights (liver, brain and kidney of both sexes; testis of males; adrenals, lungs, spleen and ovaries of females) were observed in animals in the 2000 ppm group; absolute weights were similar to control values. Histopathological findings were limited to hypertrophy of the liver and thyroid and altered colloid in the thyroid at 2000 ppm (Table 20).

Table 20. Findings in rats receiving dinocap in the diet for 1 year

	Males				Females			
	Dietary concentration (ppm)							
	0	20	200	2000	0	20	200	2000
Body weight, week 1 (g)	243	243	240	213*	178	175	177	160*
Body weight, week 49 (g)	672	665	666	540*	380	375	373	279*
Mean feed consumption, week 1 (g/week)	160	163	157	120*	199	193	193	182
Mean feed consumption, week 49 (g/week)	124	122	121	86*	146	149	144	127*
AST activity, week 52 (U/l) (mean ± SD)	168 ± 41	163 ± 52	201± 199	133 ± 42	209 ± 176	139 ± 51	136 ± 39	125 ± 23
ALT activity, week 52 (U/l) (mean ± SD)	70 ± 54	67 ± 47	98 ± 150	44 ± 41	110 ± 124	62 ± 45	52 ± 27	41* ± 14
Triglycerides, week 52 (mg/dl) (mean ± SD)	123 ± 61	104 ± 41	99 ± 71	70 ± 27	76 ± 35	109 ± 35	101 ± 45	51 ± 31
Cholesterol, week 52 (mg/dl) (mean ± SD)	109 ± 46	83 ± 20	76* ± 22	62* ± 14	95 ± 21	99 ±27	103 ± 27	96 ± 18
Blood urea nitrogen, week 52 (mg/dl) (mean ± SD)	13.8 ± 1.7	13.6 ± 1.9	14.5 ± 3.6	13.9 ± 3.2	14.2 ± 1.7	14.2 ± 1.9	13.9 ± 2.3	17.8* ± 3.8
Liver panlobular hypertrophy	0/20	0/20	1/20	14/20	0/20	0/20	0/20	0/20
Thyroid follicular cell hypertrophy	7/20	5/20	8/20	13/20	7/20	10/20	9/20	15/20
Thyroid altered colloid	7/20	6/20	7/20	13/20	2/20	3/20	3/20	7/20

From Ferguson, Morrison & Bernacki (1999)

SD, standard deviation; U, units; * $P < 0.05$

The NOAEL is 200 ppm (equal to 11 mg/kg bw per day), based on reduced body weight gain and feed consumption, liver and thyroid changes and increased blood urea nitrogen at 2000 ppm (equal to 121 mg/kg bw per day) (Ferguson, Morrison & Bernacki, 1999).

In a non-GLP lifetime toxicity/carcinogenicity study, groups of Wistar rats (80 of each sex per group) received dinocap (purity 77.5%; batch not specified) incorporated into the diet at a level of 0, 20, 200 or 2000 ppm for 30 months (130 weeks). Eight animals of each sex per dose level were sacrificed after 13, 26 and 52 weeks. Mortality, clinical signs, body weight, behaviour, feed consumption and water consumption were recorded routinely. Urinalysis, clinical chemistry and haematology were examined in samples obtained prior to the sacrifice of the satellite groups. Gross examinations were conducted for all animals sacrificed or dying during the course of the study, and weights of 11 major organs were recorded. In addition to the major organs, a range of other organs/tissues (approximately 15) and grossly observable lesions were examined histopathologically. Statistical analyses were not performed on a number of parameters.

Table 21. Findings in rats receiving dinocap in the diet for 30 months

	Males				Females			
	Dietary concentration (ppm)							
	0	20	200	2000	0	20	200	2000
Body weight, week 1 (g)	190	187	190	167*	138	140	136	120*
Body weight, week 110 (g)	697	712	692	566	557	554	533	354*
Mean feed consumption, week 1 (g/day) ^a	19.2	19.5	19.1	13.7	14.3	15.6	14.4	8.6
Mean feed consumption, week 110 (g/day) ^a	18.9	17.3	18.2	15.0	13.9	15.4	14.4	11.7
Mean feed efficiency (g weight gain/100 g feed) ^a	2.9	3.0	3.1	2.5	3.2	3.0	3.4	2.4
Liver fatty degeneration	22/80	20/80	13/80	3*/80	17/80	19/80	13/80	5/80
Liver necrosis	15/80	12/80	13/80	5*/80	12/80	6/80	8/80	3*/80
Liver hyperplasia	21/80	26/80	22/80	36*/80	22/80	15/80	22/80	25/80
Kidney tubular atrophy/cast	20/80	29/80	20/80	34*/80	17/80	27/80	21/80	16/80
Kidney chronic nephrosis	22/80	13/80	18/80	3*/80	5/80	3/80	4/80	1/80

From Maita et al. (1981)

* $P < 0.05$

^a Standard deviation not calculated, statistics not performed.

Mean dinocap intakes were 0, 0.65, 6.4 and 71 mg/kg bw per day in males and 0, 0.8, 8.1 and 90 mg/kg bw per day in females. Significantly reduced mortality was observed in both sexes at the high dose, survival at 30 months in controls and 2000 ppm groups, respectively, being 4 of 56 versus 25 of 56 males and 6 of 56 versus 24 of 56 females. Group mean body weight gains and feed consumption were reduced in both sexes at the high dose level from initiation of the study to termination (Table 21). The only clinical sign of note was yellowing of the urine and fur at the high dose level. Both males and females at 2000 ppm showed decreased haematocrit, haemoglobin and red blood cell counts intermittently during the first year of the study. There were no notable or consistent effects on clinical chemistry results. Absolute kidney weights were increased significantly in top-dose males at week 52; relative weights of many organs were increased at 2000 ppm, consistent with the lower body weights. Decreased fat deposition was noted in the 2000 ppm dose groups. The pattern of neoplastic findings was similar in test and control groups, with the exception of mammary gland tumours, which were significantly less prevalent in top-dose females. The pattern of non-neoplastic lesions in some organs was altered in the top-dose groups, probably related to the markedly increased survival (Table 21); these changes are not considered to be adverse. An increase in total number of top-dose animals with calf muscle or sciatic nerve degeneration and cataracts was associated primarily with surviving animals and is considered secondary to the increased survival (Table 22).

The NOAEL for general toxicity is 200 ppm (equal to 6.4 mg/kg bw per day), based on body weight deficits at 2000 ppm (equal to 71 mg/kg bw per day). The NOAEL for carcinogenicity is 2000 ppm (equal to 71 mg/kg bw per day), the highest dose tested (Maita et al., 1981).

Dogs

In a pre-GLP study, groups of Beagle dogs (four of each sex per group) received dinocap (purity 78%; lot No. 2-0375) in the diet at 0, 15, 60 or 240 ppm for up to 2 years. Because of high toxicity seen at 240 ppm, the top dose was varied downwards during the study, and this group was terminated at week 62. Mortality, clinical signs, body weight, behaviour, feed consumption and water consumption were recorded routinely. Samples for urinalysis, clinical chemistry and haematology were taken pretest and on five occasions during the study. Ophthalmoscopy was performed pretest, at

Table 22. Incidence of cataracts and degeneration of muscle and nerves in rats receiving dinocap in the diet for 30 months

Parameter	Males								Females							
	Dietary concentration (ppm)															
	0		20		200		2000		0		20		200		2000	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
No. of animals examined	28 / 4	52	26 / 2	52	28 / 4	52	49 / 25	31	30 / 6	50	30 / 6	50	28 / 4	52	48 / 24	32
Calf muscle degeneration / atrophy	4	38	3	34	4	31	24	19	6	16	5	11	4	21	22	12
Sciatic nerve degeneration / atrophy	4	28	4	15	4	18	25	16	6	12	6	8	2	13	24	11
Incidence of cataract	—	5	2	5	1	3	6	4	—	3	1	2	—	3	3	—

From Maita et al. (1981)

a. Killed on schedule / surviving to 30 months.

b. Dead or killed in extremis.

week 52 and then every 13 weeks. Main organs from all animals were weighed, and a wide range of tissues was examined histopathologically. The only finding of note at the intermediate dose level was bilateral retinal atrophy with greyish discoloration of the tapetum lucidum, seen in seven animals at week 52 and subsequently; this was seen in three males during histopathological examination.

The NOAEL is 15 ppm (equivalent to 0.4 mg/kg bw per day), based on retinal atrophy seen at 60 ppm (equivalent to 1.5 mg/kg bw per day) (Weatherholtz et al., 1979).

Multigeneration studies

Rats

In a GLP-compliant study, groups of CD:CrI®BR rats (26 of each sex per group) received dinocap (purity 96%; batch No. 2893) in the diet at 0, 40, 200 and 1000/400 ppm for 70 days before mating, during mating and during gestation until terminal sacrifice. At weaning of the F₁ pups, the highest dose was reduced to 400 ppm because of a high rate of mortality. Pairing was one male to one female. On day 4 postpartum, litter size was reduced to eight pups, ideally four of each sex. Each litter was weaned on lactation day 21. Observations included clinical signs and mortality and recording of body weight, feed consumption and reproductive performance. In addition, gait, righting reflex and behaviour were evaluated during the growth phase of the F₀ and F₁ parental animals. All adult animals of the F₀ and F₁ generations were subjected to a gross necropsy, and their reproductive organs, liver and thyroid were weighed. Pups culled on day 4 postpartum and postweaning offspring not selected for continuation of the study were examined externally and internally for macroscopic abnormalities. Histological examinations were performed on all gross lesions and the reproductive organs, liver, thyroid (including parathyroid) and eyes from the control and high dose group parental animals.

In addition to the guideline requirements, a supplemental investigation of effects of dinocap on male reproductive capabilities was conducted on selected F₁ males at 0, 40 and 200 ppm (12 rats per dose group). Clinical observations, body weights and feed consumption were evaluated weekly until termination after 16 weeks. At necropsy, terminal body weight, left testicular, epididymal and caudal weights, sperm motility and sperm count per gram caudal tissue were recorded.

Table 23. Intakes of dinocap in the rat reproduction study

Dietary concentration (ppm)	Dose (mg/kg bw per day)					
	Males (premating)		Females (premating)		Females (gestation)	
	F ₀	F ₁	F ₀	F ₁	F ₀	F ₁
40	2.6	3.1	3.2	3.7	2.8	2.8
200	12.9	14.9	15.7	18.0	13.9	13.7
1000/400	65.1	32.4	77.3	38.7	69.5	27.4

Homogeneity and stability were confirmed analytically. Achieved intakes are outlined in Table 23. There were no deaths or overt clinical signs of systemic toxicity related to treatment. Yellow-stained fur and orange-discoloured urine were seen in most animals of the two highest dose groups (200 and 1000/400 ppm). This is not considered adverse, as it is most likely related to the presence of dinocap or its metabolites in the urine. Body weight gain and feed consumption were reduced at 1000 ppm from the start of treatment, by 10–20% and 10%, respectively. Body weight gain during gestation was not adversely affected by dinocap treatment. At 400 ppm, body weight gain was similar to that of controls. There were no treatment-related effects on mating performance, fertility, litter size, gestation length and index, parturition or viability index (number of pups surviving to day 4) at all doses of both generations. At the top dose (1000 ppm), the body weight gain of the F₁ offspring during the lactation phase was decreased compared with controls, and more pups appeared to be weak/thin and dehydrated. Pup survival during lactation was not affected by treatment; however, after weaning, the mortality rate of the high-dose pups increased. A total of 28 pups died within 7 days after weaning (mortality rate 26%). No adverse effects on parameters investigated (litter size, body weight, clinical observations, viability) were noted in F₂ pups at any dose level. In parental animals of the F₀ and F₁ generations receiving 1000/400 ppm, the relative liver and testes weights were increased (< 10%), with no evidence of a histological correlate. No other effects on organ weights were observed. In addition, necropsy (including histomorphological evaluation) of parental animals and gross pathology of F₁ and F₂ pups did not reveal any treatment-related effects at dose levels up to 400/1000 ppm dinocap. In F₁ pups that had been culled after weaning, the cause of mortality could not be determined, but the presence of orange or red discoloration of the contents of the gastrointestinal tract and bladder was noted, indicating the presence of dinocap or its metabolites. In the investigation of male reproductive tissues, no adverse effects were seen on reproductive organs or sperm counts/motility.

Dinocap produced no adverse effects on reproduction at the highest dose tested (1000 ppm, equal to 65 mg/kg bw per day in the first mating; 400 ppm, equal to 27 mg/kg bw per day in the second mating). The NOAEL for pup development is 200 ppm (13 mg/kg bw per day, based on reduced body weight and survival at 1000 ppm). The NOAEL for parental toxicity is 200 ppm (13 mg/kg bw per day), based on reduced body weight gains (Morseth, 1990).

