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TOXICOLOGICAL EVALUATIONS

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TOXICOLOGICAL EVALUATION

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2-(1-Methyl-propyl)-4,6-dinitrophenol

No. 239

CAS No. 88-85-7

BG Chemie
Berufsgenossenschaft der chemischen Industrie

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2-(1-Methylpropyl)-4,6-dinitrophenol

1 Summary and assessment

In animal studies, 2-(1-methylpropyl)-4,6-dinitrophenol is almost completely absorbed within 72 hours of dermal application, while after oral administration only moderate absorption takes place. Absorbed 2-(1-methylpropyl)-4,6-dinitrophenol is almost completely metabolised. The main metabolites, which are formed through oxidation of the side chain and reduction of a nitro group, are primarily β-methyl-β-(2-hydroxy-3,5-dinitrophenyl)-propionic acid and 6-amino-4-nitro-2-sec-butylphenol and its glucuronide. Within 72 hours, 30 to 60% of an orally administered dose is excreted in the urine, 30 to 40% in the faeces and 2 to 10% in the bile. Only ca. 3% of the orally administered dose is found in the urine of the rat as unchanged 2-(1-methylpropyl)-4,6-dinitrophenol.

On acute oral and dermal exposure, 2-(1-methylpropyl)-4,6-dinitrophenol is highly toxic (LD_{50} rat oral 25 to 60 mg/kg body weight; LD_{50} mouse oral 16 to 40 mg/kg body weight; LD_{50} rat dermal 80 to 200 mg/kg body weight; LD_{50} rabbit dermal 10 to 200 mg/kg body weight). Toxic symptoms include tachypnoea, increased motor activity, elevated body temperature, weight loss, cataract formation, convulsions and lying on the stomach. On acute inhalation exposure, the substance is of very high toxicity, with 4-hour LC_{50} values in rats of 127 to 150 mg/m³ air. In animals, 2-(1-methylpropyl)-4,6-dinitrophenol causes methaemoglobinaemia, icterus due to haemolysis, increased coproporphyrin excretion and oliguria. Damage to the liver and kidney is manifested as progressive necrosis on histopathological examination. The methaemoglobinaemia is probably attributable to the metabolite 6-amino-4-nitro-2-sec-butylphenol.

2-(1-Methylpropyl)-4,6-dinitrophenol is not irritating to the skin of the rabbit but is severely irritating to the eye.

On administration of 2-(1-methylpropyl)-4,6-dinitrophenol to rats in the diet for 13 weeks at concentrations of 0, 12.5, 40, 120 and 360 ppm (equivalent to daily intakes of 0, 1.09, 3.49, 11.95 and 56.80 mg/kg body weight in males and 0, 1.12, 3.76, 11.97 and 52.80 mg/kg body weight in females), a clear deterioration in general health and increased mortality occurred at the top dose. Signs of haemoconcentration, with increasing values for erythro-

cytes, haemoglobin and the haematocrit, were seen dose-dependently in the males from 40 ppm. At 120 ppm, the relative weights of the heart (both sexes) and of the kidneys and spleen (males) were increased, without corresponding histopathological changes. The no-effect level was determined to be 12.5 ppm, equivalent to 1.09 and 1.12 mg/kg body weight/day in males and females, respectively. When 2-(1-methylpropyl)-4,6-dinitrophenol was given to rats in their diet at doses of 2.5, 5.0 and 10 mg/kg body weight/day for 27 weeks, decreased body weight gain and increased liver weights (without corresponding histopathological effects) were seen at the highest dose, with increased urea nitrogen levels in the blood from 5 mg/kg body weight/day. A dose of 25 mg/kg body weight/day led to increased mortality. On dietary administration of 0, 10, 40 or 160 ppm 2-(1-methylpropyl)-4,6-dinitrophenol to rats for 12 months (equivalent to daily intakes of the substance of 0, 0.62, 2.46 and 11.89 mg/kg body weight in males and 0, 0.81, 3.28 and 13.76 mg/kg body weight in females), slight to marked reductions in body weight gain were seen, with a tendency to haemoconcentration in the males from 40 ppm, which was statistically significant at 160 ppm. The weights of the heart, lungs and kidneys were increased in the males at 160 ppm while thyroid gland weights were increased in the females at all doses. Histopathological examination revealed glycogen depletion in the liver and a slight increase in siderin storage in the liver and kidneys at 40 ppm and above. The no-effect level was given as 10 ppm, equivalent to 0.62 and 0.81 mg/kg body weight/day in males and females, respectively.

There have been no indications of mutagenic activity in the Salmonella/microsome test or in the HPRT test in Chinese hamster V79 cells either with or without metabolic activation, but 2-(1-methylpropyl)-4,6-dinitrophenol is weakly mutagenic in the mouse lymphoma test. No clastogenic activity is detectable either in vitro (chromosome aberration test in human lymphocytes) or in vivo (micronucleus test in the mouse). A DNA-damaging effect has been seen in vitro in the mitotic recombination test in *Saccharomyces cerevisiae*, and in the Rec assay in *Bacillus subtilis* and *Salmonella typhimurium*, but not in the UDS test in rat hepatocytes and human lymphocytes. 2-(1-Methylpropyl)-4,6-dinitrophenol causes no increase in sex-linked recessive lethal mutations in *Drosophila melanogaster*.

In chronic studies in which mice were fed 2-(1-methylpropyl)-4,6-dinitrophenol at 1, 3 or 10 mg/kg body weight/day for 100 weeks, no increase in

the tumour incidence was evident in the treated mice compared with the controls. In a preliminary study in mice involving oral administration for 18 months or the administration of a single dose by subcutaneous injection followed by an 18-month observation period, the tumour incidences were within the range of those seen in the controls.

In male rats, 2-(1-methylpropyl)-4,6-dinitrophenol impairs fertility when given in the diet, an effect arising from inhibition of sperm motility, a decrease in the sperm count and degeneration of the spermatogenic epithelium. The no-effect level for effects on male fertility has been reported to be 3.8 mg/kg body weight/day on oral administration to rats for 11 weeks. In mice and rats, the administration of 2-(1-methylpropyl)-4,6-dinitrophenol at maternally toxic doses causes embryotoxicity and visceral and skeletal malformations. Malformations are observed in rabbits on oral and dermal exposure, even at doses that are not maternally toxic. Mortality, fertility, mating behaviour, the pregnancy index and macroscopic and microscopic findings do not differ from those in control animals in multi-generation feeding studies in rats.

In the available case studies in man, which have sometimes had fatal outcomes, the symptoms reported have included diarrhoea, pyrexia, dyspnoea, hyperthermia, convulsions, jaundice and disturbances in liver function. 2-(1-Methylpropyl)-4,6-dinitrophenol has been detected in the body tissues.

2-(1-Methylpropyl)-4,6-dinitrophenol has been legally classified by the European Union in the TRGS 905 and placed in category R_D2 of substances toxic to reproduction (i. e. “substances which should be regarded as if they cause developmental toxicity to humans”) and in category R_F3 of substances toxic to reproduction (i. e. “substances which cause concern for human fertility”).

2 Name of substance

2.1	Usual name	2-(1-Methylpropyl)-4,6-dinitrophenol
2.2	IUPAC name	2-(1-Methylpropyl)-4,6- dinitrophenol
2.3	CAS No.	88-85-7
2.4	EINECS No.	201-861-7

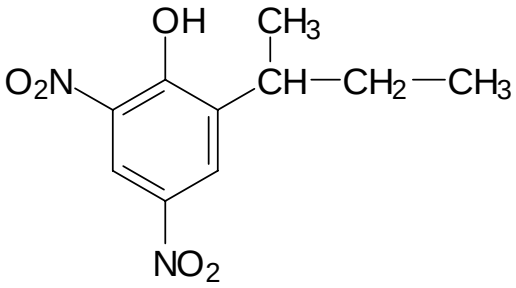
3 Synonyms, common and trade names

Aatox
Basanite
Blaartox
BNP 20
BNP 30
Butaphene
2-sec-Butyl-4,6-dinitrophenol
2-(sec.-Butyl)-4,6-dinitrophenol
6-sec-Butyl-2,4-dinitrophenol
Caldon
Chemox General
Chemox P.E.
DBNF
Desicoil
Dibutox
Dinitro
Dinitro-3
Dinitrobutylphenol
2,4-Dinitro-6-sec-butylphenol
2,4-Dinitro-6-(1-methylpropyl)phenol
4,6-Dinitro-2-(1-methyl-n-propyl)phenol
4,6-Dinitro-2-sec-butylphenol
4,6-Dinitro-o-sec-butylphenol
Dinoseb
DN 289
DNBP
DNOSBP
DNSBP
Dow General Weed Killer
Dow Selective Weed Killer
Dytop
Elgetol
Elgetol 318
ENT 1,122
Gebutox
Hel-Fire

Hivertox
Ivosit
Kiloseb
Ladob
Laseb
Liro DNBP
6-(1-Methylpropyl)-2,4-dinitrophenol
Nitropone C
Phenol, 2-(1-methylpropyl)-4,6-dinitro- (9CI)
Phenol, 2-sec-butyl-4,6-dinitro- (6CI, 9CI)
Phenotan
Premerge
Premerge 3
RCRA Waste Number P020
Sinox General
Sparic
Spurge
Subitex
Unicrop DNBP
Vertac Dinitro Weed Killer
Vertac General Weed Killer
Vertac Selective Weed Killer
WSX 8365

4 Structural and molecular formulae

4.1 Structural formula



4.2 Molecular formula $C_{10}H_{12}N_2O_5$

5 Physical and chemical properties

5.1	Molecular mass, g/mol	240.24	
5.2	Melting point, °C	36–40	(Hoechst, 1992)
		38–42	(Anonymous, 1984; Windholz et al., 1983)
5.3	Boiling point, °C	220 (at 1013 hPa; decomposition)	(Hoechst, 1992)
5.4	Vapour pressure, hPa	0.0000504 (at 20 °C)	
		0.0103 (at 60 °C)	(Hoechst, 1992)
5.5	Density, g/cm³	1.29 (at 30 °C)	(Verschueren, 1983)
5.6	Solubility in water	0.050 g/l (at 20 °C)	(Anonymous, 1984)
		0.097 g/l (at 20 °C)	(Hoechst, 1992)
		0.0734 g/l (at 25 °C)	(Spencer et al., 1948)
5.7	Solubility in organic solvents	480 g/1000 g ethanol	(Anonymous, 1984)
		235 g/1000 g ethanol	(Spencer et al., 1948)
		800 g/1000 g mineral oil	(Anonymous, 1984)
		Infinitely soluble in toluene and ether	(Anonymous, 1984)
5.8	Solubility in fat	n-Octanol-water partition coefficient, log P_{ow}: 3.7	(Hoechst, 1992)
5.9	pH value	7 (at 0.097 g/l water; 20 °C)	(Hoechst, 1992)
5.10	Conversion factor	1 ml/m³ (ppm) ≙ 9.83 mg/m³	
		1 mg/m³ ≙ 0.10 ml/m³ (ppm) (at 1013 hPa and 25 °C)	

6 Uses

Active ingredient in pesticides; polymerisation inhibitor for styrene Hoechst, 1990).

Under the German Pflanzenschutz-Anwendungsverordnung (Use of Pesticides Order), there is a complete ban on the use of 2-(1-methylpropyl)-4,6-

dinitrophenol (Dinoseb), its acetate and salts (Das Deutsche Bundesrecht, 1995).

7 Experimental results

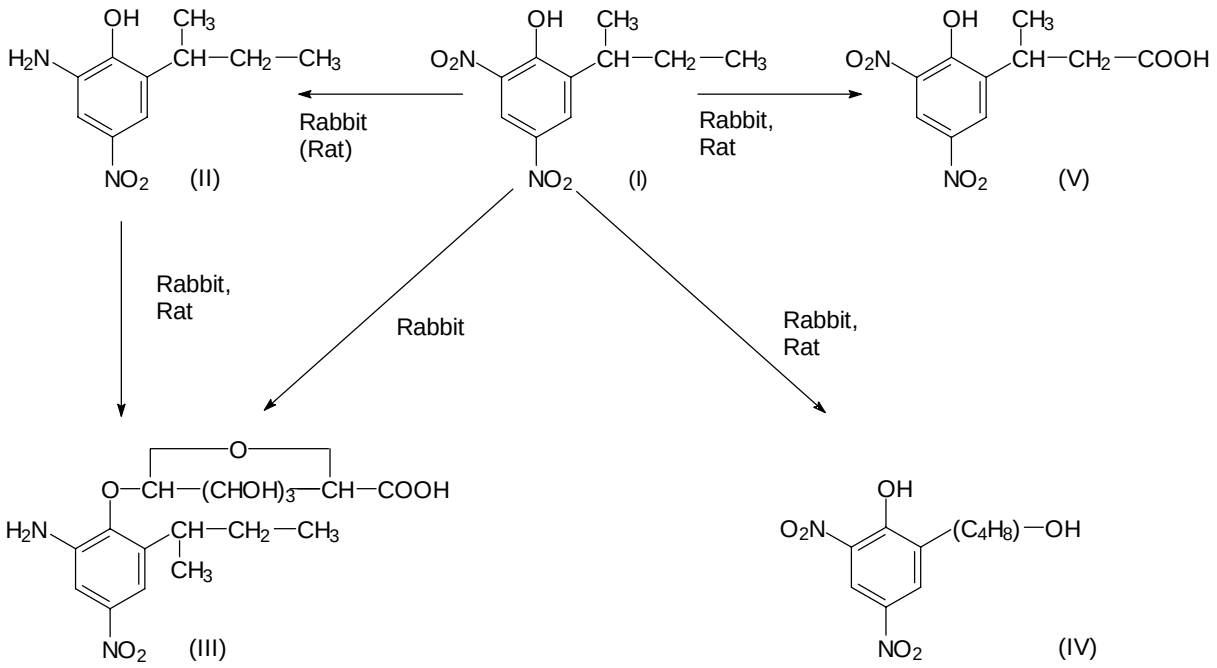
7.1 Toxicokinetics and metabolism

In vivo

After oral administration of single doses of 5.8, 8.9 and 11.5 mg 2-(1-methylpropyl)-4,6-dinitrophenol/kg body weight to the rat in the diet (no data on duration of administration), a total of 13.6 to 16.0% of the administered dose was excreted in the urine within 48 hours; only 1.9 to 2.4% of the administered dose could be detected as the unchanged test substance. Metabolites detected included β-methyl-β-(2-hydroxy-3,5-dinitrophenyl)-propionic acid (5.8 to 6.2% of the administered dose) and traces of 6-amino-4-nitro-2-sec-butylphenol, as well as an unidentified non-uniform chemical fraction (probably a metabolite that had undergone side chain hydroxylation; 5.7 to 7.9% of the administered dose). The O-glucuronide of 6-amino-4-nitro-2-sec-butylphenol could not be detected as a metabolite in the urine (Ernst and Bär, 1964).

A similar metabolic profile was found in rabbits following the administration of single oral doses of 15.8 and 20.0 mg 2-(1-methylpropyl)-4,6-dinitrophenol/kg body weight in the diet (duration of administration unspecified). Independent of the dose, a total of 32.8 to 48.1% of the administered dose was excreted in the urine within 48 hours. The amount of unchanged test substance excreted in the urine during this period was 2.4 to 2.7% of the administered dose. Metabolites detected in the urine included β-methyl-β-(2-hydroxy-3,5-dinitrophenyl)-propionic acid (10.0 to 13.5% of the administered dose), 6-amino-4-nitro-2-sec-butylphenol (in trace amounts) and 6-amino-4-nitro-2-sec-butylphenol-O-glucuronide (5.2 to 13.7% of the administered dose) as well as an unidentified non-uniform chemical fraction (probably a metabolite with a hydroxylated side chain; 14.9 to 18.5% of the administered dose; Ernst and Bär, 1964).

Figure 1. Metabolism of 2-(1-methylpropyl)-4,6-dinitrophenol after oral administration (after Ernst and Bär, 1964)



Substance I:	2-(1-Methylpropyl)-4,6-dinitrophenol
Substance II:	6-Amino-4-nitro-2-sec-butylphenol
Substance III:	6-Amino-4-nitro-2-sec-butylphenol-O-glucuronide
Substance IV:	Unidentified (probably a metabolite of 2-(1-methylpropyl)-4,6-dinitrophenol with a hydroxylated side chain)
Substance V:	β-Methyl-β-(2-hydroxy-3,5-dinitrophenyl)-propionic acid

In a further study male mice (20 g) and rats (180 g) were given 8 to 10 µmol [^{14}C]2-(1-methylpropyl)-4,6-dinitrophenol/kg body weight (equivalent to 1.9 to 2.4 mg/kg body weight) as a single dose by gavage. In the mouse ca. 25 to 35% of the administered radioactivity was excreted in the urine within 24 hours and ca. 35 to 40% was excreted in the faeces within 72 hours. In the rat ca. 35 to 40% of the administered radioactivity was excreted in the urine within 24 hours and ca. 60 to 65% within 72 hours, while in the faeces, ca. 20 to 25% and ca. 25 to 30% was excreted within 24 and 72 hours, respectively (data derived from graphic presentation; Bandal and Casida, 1972).

In 3 Swiss Webster mice that were each given 32 mg [^{14}C]2-(1-methylpropyl)-4,6-dinitrophenol/kg body weight as a single dose by gavage, 26.3%

of the administered radioactivity was excreted in the urine and 30.4% in the faeces within 64 hours, while 1.4% was excreted in the bile within 8 hours (Gibson and Rao, 1973).

In a study of the distribution of 2-(1-methylpropyl)-4,6-dinitrophenol in the body, rats were given a single oral dose of 50 mg /kg body weight. Within 4 hours of dosing, the unchanged test substance could be detected in the muscles (10 to 20 µg/g tissue), spleen (10 to 30 µg/g tissue) and lungs (up to 80 µg/g tissue). Between 5 and 50 µg/g tissue was found in the heart, liver and kidneys, while no 2-(1-methylpropyl)-4,6-dinitrophenol was detectable in the central nervous system. The maximum concentration (250 µg/ml) was detected in the blood after 24 hours, while after 96 hours a level of 30 µg/ml was still present and after 120 hours, 2-(1-methylpropyl)-4,6-dinitrophenol was no longer detectable (Burkatzkaya, 1962).

After the administration of a single dose of 40 mg technical grade 2-(1-methylpropyl)-4,6-dinitrophenol/kg body weight to guinea pigs (n=8) as a suspension in 10% gum arabic by gavage, 86 µg of the substance was detected/ml blood when death occurred after 1.7 to 3.0 hours (no further details; Bough et al., 1965).

In sheep (n=7), the maximum level of 2-(1-methylpropyl)-4,6-dinitrophenol in the plasma ca. 90 minutes after the administration of a single oral dose of 45 or 49 mg/kg body weight via a tube into the rumen, was between 23 and 60 µg/ml plasma, decreasing significantly within 3 to 6 hours (no further details; Fröslie, 1974).

In a metabolism study, a Holstein dairy cow was given 2-(1-methylpropyl)-4,6-dinitrophenol at a concentration of 5 ppm in the diet for 3 days. This was equivalent to a dose of ca. 0.25 mg/kg body weight/day at a total administered dose of 340.5 mg/animal and an assumed body weight of 450 kg. About 3.5% (11.8 mg) of the total dose was excreted in the urine as the unchanged substance within 6 days. No metabolites were detectable. No residues of 2-(1-methylpropyl)-4,6-dinitrophenol were detectable in the milk (detection limit ca. 0.25 µg/ml; St. John et al., 1965).

2-(1-Methylpropyl)-4,6-dinitrophenol was given to 3 Holstein cows in the diet in successively increasing concentrations of 1 ppm for 14 days, 3 ppm for a further 17 days, 10 ppm for a further 15 days, 30 ppm for a further 14 days and 100 ppm for the remaining 21 days (total study duration 81 days).

This was equivalent to respective doses of ca. 0.02, 0.06, 0.2, 0.6 and 2 mg 2-(1-methylpropyl)-4,6-dinitrophenol/kg body weight/day. A further 3 cows served as controls. The milk was analysed daily and the cream was analysed after 6 weeks of the study. No 2-(1-methylpropyl)-4,6-dinitrophenol residues were detectable in the milk or cream by gas chromatography (detection limit 0.01 and 0.05 µg/ml, respectively). There were no effects on milk production, body weight gain, appearance or behaviour (McKellar, 1971).

After a single application of [^{14}C]-2-(1-methylpropyl)-4,6-dinitrophenol in acetone to the clipped dorsal skin of 82-day-old female F344 rats (application site 5.6 cm², equivalent to ca. 2.3% of the surface area of the body) at a dose of 285 nmol/cm² (equivalent to 68.5 µg/cm²), groups of 3 animals were killed at different times and the proportion of the applied radioactivity that had been absorbed was determined in the animals and their excreta. Within 1, 6, 24, 48 and 72 hours, on average 22.3, 43.1, 85.5, 85.1 and 84.3% of the administered radioactivity had been absorbed (no further details). When the same experiment was carried out in young rats (age 33 days; application site 2.8 cm², equivalent to ca. 2.3% of the surface area of the body), absorption through the skin was significantly lower that that seen in the adult animals; within 1, 6, 24, 48 and 72 hours, 11.1, 38.8, 63.0, 55.3 and 55.7% of the administered radioactivity was absorbed (Shah et al., 1990; Hall et al., 1992).

The effect of dose on absorption was investigated in a further study carried out in accordance with the protocol described above following a single dermal application. [^{14}C]-2-(1-Methylpropyl)-4,6-dinitrophenol in acetone was applied to young rats at doses of 250, 535 and 2680 nmol/cm² (equivalent to 60, 129 and 644 µg/cm²) and to adult rats at 210, 535 and 2680 nmol/cm² (equivalent to 52, 129 and 644 µg/cm²). The amount of radioactivity in the skin, liver, kidneys and carcass was determined 72 hours after dosing, as was that in the urine and faeces. The amount absorbed was higher in the adults (86.4 to 93.2%) than in the young animals (77.7 to 82.9%). In both the young and adult animals, the highest concentrations were detected in the blood and the kidneys. The tissue content and concentration increased with increasing dose except in the kidneys, where the level and concentration fell with increasing dose. The amount and concentration in the urine was unaffected by the dose administered (Hall et al., 1992).

Single doses of 10, 20 and 40 mg/kg body weight technical grade 2-(1-methylpropyl)-4,6-dinitrophenol (purity unspecified; 10% solutions in acetone) were applied to the clipped dorsal skin (application site 50 cm²) in groups of 4 rabbits. At the lowest dose all of the animals survived the 24-hour observation period. In this group, the concentration in the blood was 33 µg 2-(1-methylpropyl)-4,6-dinitrophenol/ml 8 hours after application and 9 µg/ml after 24 hours. In the intermediate and high dose groups, the animals died within 2.5 to 5.5 hours of application. The concentrations of 2-(1-methylpropyl)-4,6-dinitrophenol in the blood at the time of death ranged from 45 to 53 µg/ml (no further details; Bough et al., 1965).

Albumin-2-(1-methylpropyl)-4,6-dinitrophenol complexes could be detected in the serum of rats after oral, dermal or inhalation exposure to 2-(1-methylpropyl)-4,6-dinitrophenol. Between 60 and 94% of an administered oral dose was found in the stomach and 2% in the blood in this species. Only metabolites were detectable in the liver, kidneys, spleen and brain (no further details, particularly about the doses administered, time points at which determinations were made and metabolites; Henneberg, 1964, 1965).

Within 36 hours of oral and intraperitoneal administration, 6 and 11%, respectively, of an administered dose of 15 mg 2-(1-methylpropyl)-4,6-dinitrophenol/kg body weight was excreted in the urine in the form of metabolites in the rat (no further details; Rahn, 1961).

In 3 Swiss-Webster mice which had each been given a single intraperitoneal injection of 17.7 mg [^{14}C]2-(1-methylpropyl)-4,6-dinitrophenol/kg body weight, 28.2% of the administered radioactivity was excreted in the urine and 40.8% in the faeces within 64 hours, with 9.6% excreted in the bile within 8 hours (Gibson and Rao, 1973).

After intraperitoneal injection of 15.8 mg [^{14}C]2-(1-methylpropyl)-4,6-dinitrophenol/kg body weight (equivalent to 25 µCi/kg body weight), the half lives for elimination from the plasma and the liver in female Swiss-Webster mice were 10.8 and 10.0 hours, respectively (Preache and Gibson, 1975a).

In a study of the toxicokinetics and metabolism of 2-(1-methylpropyl)-4,6-dinitrophenol, groups of 5 pregnant Swiss-Webster mice were given single oral (gavage) or intraperitoneal doses of 32 and 17.7 mg [^{14}C]2-(1-methylpropyl)-4,6-dinitrophenol/kg body weight, respectively, on day 11 of pregnancy. The radioactivity of the doses was ca. 25 µCi/kg body weight in both

cases. 2-(1-Methylpropyl)-4,6-dinitrophenol was detectable 3 hours after oral and intraperitoneal administration in the liver, kidney and blood of the dams and in the embryo at concentrations of 14.2, 13.8, 29.7 and 1.8 µg/g tissue (oral) and 15.6, 15.4, 46.6 and 2.9 µg/g tissue (intraperitoneal). The concentration in the embryo was at most only ca. 2.5% of the concentration in the plasma of the respective mother. Metabolites (not identified) were found in the liver and kidneys of the dams and in the embryo, but not in the blood of the dams. Maximum concentrations in the embryo were attained 8 minutes after intraperitoneal injection and 12 hours after oral administration. The rate of absorption of 2-(1-methylpropyl)-4,6-dinitrophenol was 40 times higher after intraperitoneal injection than after oral administration (absorption constant K_a (intraperitoneal) 299 h^{-1}, K_a (oral)7 h^{-1}). The half lives for elimination from the plasma in the dams were 7.7 and 34.7 hours after intraperitoneal and oral administration, respectively (Gibson and Rao, 1973).

In vitro

When 2-(1-methylpropyl)-4,6-dinitrophenol (concentration unspecified) was incubated for 48 hours with the caecal contents of rats or an *Escherichia coli* strain (W3110) from a volunteer, the metabolites 6-amino-4-nitro-2-sec-butylphenol (ca. 63 and 1.7%, respectively, of the concentration added) and 6-acetylamino-4-nitro-2-sec-butylphenol (ca. 7 and 0.9%, respectively, of the concentration added) were detected qualitatively (thin layer chromatography) and quantitatively (densitometry; Fujita et al., 1982).

In a further study, the incubation of 2-(1-methylpropyl)-4,6-dinitrophenol with rat caecal contents at ca. 180 mg/l at a temperature of 37 °C led to almost complete reduction of 2-(1-methylpropyl)-4,6-dinitrophenol to 6-amino-4-nitro-2-sec-butylphenol and then to the diaminophenol, 4,6-diamino-2-sec-butylphenol within ca. 2 hours (Ingebrigtsen and Fröslie, 1980).

The incubation of fresh ruminal fluid from a cow with 300 mg 2-(1-methylpropyl)-4,6-dinitrophenol/l for 4 hours at 39 °C under anaerobic conditions also led to almost complete reduction of 2-(1-methylpropyl)-4,6-dinitrophenol to 6-amino-4-nitro-2-sec-butylphenol and the diaminophenol, 4,6-diamino-2-sec-butylphenol within 30 minutes. Following the appearanceof the metabolite 6-amino-4-nitro-2-sec-butylphenol within the first 30 minutes there was, with its disappearance, a constant increase in the concentration of 4,6-diamino-2-sec-butylphenol (Fröslie and Karlog, 1970).

The dermal absorption of [^{14}C]2-(1-methylpropyl)-4,6-dinitrophenol in acetone was studied in vitro at a dose of 285 nmol/cm² (equivalent to 68.6 µg/cm²) in static and flow systems with isolated dorsal skin from adult (age 82 days) and young (33 days) female F344 rats. In the static system, 1.5, 17.0, 52.3, 66.2 and 70.8% of the administered radioactivity was absorbed through the skin of the adult animals within 1, 6, 24, 48 and 72 hours, while in young skin, 3.0, 20.7, 59.1, 70.9 and 74.9%, respectively, were absorbed. In the flow system, the maximum amount of the administered radioactivity absorbed within 72 hours was 20.6% through skin from adult animals and 47.3% through that from young animals (Shah et al., 1990; Hall et al., 1992).

7.2 Acute and subacute toxicity

Acute toxicity

LD_{50} and LC_{50} values determined after the administration of single doses of 2-(1-methylpropyl)-4,6-dinitrophenol are shown in Table 1. These show that 2-(1-methylpropyl)-4,6-dinitrophenol is toxic on acute oral or dermal exposure and highly toxic after a 4-hour inhalation exposure.

Beginning of Table 1

Table 1. Acute toxicity of 2-(1-methylpropyl)-4,6-dinitrophenol

Species	Number of animals/ dose	Sex	Route	Observation period	LD_0 (mg/kg body weight)	LD_{50}/LC_{50} (95% confidence intervals; mg/kg body weight)	Reference
Rat	10–20	male female	oral	14 days	5	40	Spencer et al., 1948
Rat	10	male	oral	24 hours	-	25–40	Bough et al., 1965
Rat	20	female	oral	-	-	30	Hoechst, 1957
Rat	-	-	oral	-	-	35	Hoechst, 1958
Rat	-	-	oral	-	-	40–50	Anonymous, 1984
Rat	-	-	oral	-	-	50	Ben-Dyke et al., 1970
Rat	-	-	oral	-	-	58	Dow Chemical, 1978
Rat	-	-	oral	-	-	37–60	Schafer, 1972
Mouse	-	-	oral	-	-	16	Izmerov et al., 1982
Mouse	5	male	oral	24 hours	-	20–40	Bough et al., 1965
Guinea pig	4	female	oral	24 hours	-	20–40	Bough et al., 1965
Guinea pig	-	-	oral	-	-	25	Anonymous, 1984
Guinea pig	1–4	male female	oral	-	20–25	LD_{100} 35	Grolleau, 1976

Table 1. Acute toxicity of 2-(1-methylpropyl)-4,6-dinitrophenol							
Species	Number of animals/ dose	Sex	Route	Observation period	LD_0 (mg/kg body weight)	LD_{50}/LC_{50} (95% confidence intervals; mg/kg body weight)	Reference
Rat	-	-	dermal[4)]	-	-	80–200	Ben-Dyke et al., 1970
Rabbit	4	-	dermal[5)]	-	-	10–20	Bough et al., 1965
Rabbit	-	-	dermal[4)]	-	-	80–200	Anonymous, 1984
Rabbit	-	-	dermal	-	-	80	The British Crop Protection Council, 1991
Rabbit	-	-	dermal	-	-	50–100	Hoechst, 1965
Guinea pig	5	male female	dermal[6)]	-	100	150–400	Spencer et al., 1948
Guinea pig	-	-	dermal[4)]	-	-	100–200	Dow Chemical, 1978
Guinea pig	-	-	dermal[4)]	-	-	500	Anonymous, 1984
Rat	5	male	inhalation (aerosol)	14 days	-	127 mg/m³ (4 hours)	Hoechst, 1983
Rat	5	female	inhalation (aerosol)	14 days	-	150 mg/m³ (4 hours)	Hoechst, 1983
Rat	5	male female	inhalation (aerosol)	14 days	-	138 mg/m³ (4 hours)	Hoechst, 1983
Cat	-	-	inhalation	-	-	LC_{Lo} 45 mg/m³ (3 hours)	Izmerov et al., 1982
Rat	-	-	s.c.	-	-	21.4	Harvey, 1952
Rat	-	female	i.p.	-	-	15	Hoechst, 1958
Mouse	6	male	i.p.	24 hours	-	10	Ilivicky and Casida, 1969
Mouse	≥5	female	i.p.	24 hours	-	14.1 (13.3–14.9)[1)]	Preache and Gibson, 1975b
Mouse	≥5	female	i.p.	24 hours	-	20.2 (18.9–21.6)[2)]	Preache and Gibson, 1975b
Mouse	≥5	female	i.p.	24 hours	-	22.5 (20.8–24.3)[3)]	Preache and Gibson, 1975b

- No data
1) Animals kept at 32 °C during the observation period
2) Animals kept at 25 °C during the observation period
3) Animals kept at 6 °C for 4 hours after exposure, then at room temperature (23 to 24 °C)
4) No data on duration of application or size of area exposed
5) Exposure for 24 hours, application site 50 cm²
6) Exposure for 4 hours, size of area exposed unspecified

End of Table 1

• Oral administration

Tachypnoea, convulsions and prostration were noted in mice, rats and guinea pigs in studies of the acute oral toxicity of 2-(1-methylpropyl)-4,6-dinitrophenol (see Table 1; Bough et al., 1965).

2-(1-Methylpropyl)-4,6-dinitrophenol was very poorly tolerated by 2 cats when administered orally at a dose of 10 mg/kg body weight. Although the animals eliminated the majority of the test substance by vomiting, they still died after the second dose (no further details; Hoechst, 1958).

• Dermal administration

The acute dermal toxicity of 2-(1-methylpropyl)-4,6-dinitrophenol was studied in male albino mice. Groups of 5 mice were treated with a suspension of 2-(1-methylpropyl)-4,6-dinitrophenol in 10% gum arabic, which was applied to ca. 1 cm^2 of their clipped abdominal skin for 24 hours under occlusive cover at doses of 100 and 500 mg/kg body weight. A group of 10 mice served as controls and the study was repeated independently. The mortality rates in the low and high dose groups were 2/10 and 9/10, respectively, while none of the control animals died (no further details; Bough et al., 1965).

The fur of two 7 and 11-year-old male dogs (English setters) was found to be heavily soiled with a yellow fluid after they had been rummaging about for 2 hours in a rural area. Tachypnoea, increased movement and ataxia was seen in the dogs, and one died within 15 minutes on the way to a vet. On reaching the vet, the second dog was disorientated ataxic and weak, with tachypnoea and a weak pupillary reflex. Despite symptomatic treatment of its severe breathing difficulties (atropine i.v., i.m., s.c.), the animal died during a bath to remove the yellow fluid from its coat. No macroscopic findings were evident on dissection of this dog. Cholinesterase activity in the brain was normal. On analysis of the yellow fluid, 2-(1-methylpropyl)-4,6-dinitrophenol was detected by gas chromatography-mass spectroscopy, and 2-(1-methylpropyl)-4,6-dinitrophenol levels of more than 100 µg/g tissue were found in the contaminated skin. Traces of 2-(1-methylpropyl)-4,6-dinitrophenol (<0.1 µg/g) were detected in the stomach contents and liver (no further details; Fikes et al., 1989).

• Inhalation exposure

As shown in Table 1, after male and female Wistar rats (5/group) were exposed to an aerosol of 98% pure 2-(1-methylpropyl)-4,6-dinitrophenol for 4 hours (nose only), and observed for 14 days, LC_{50} values of 127 mg/m^3,

150 mg/m³ and 138 mg/m³ were determined for males, females and both sexes together, respectively. Signs of toxicity included breathing difficulties, disturbances of balance and attenuated reflexes. Post-mortem examinations of the animals that died revealed effects on the lungs (which were dark red or infiltrated with dark red foci) and swollen stomachs, particularly in the rats exposed to the highest tested concentration (577 mg/m³). Of note was the immediate onset of rigor mortis in the dead animals. Overall the toxic effects were non-specific, but indicative of the general toxicity profile of dinitrophenols (increased metabolic rate due to uncoupling of oxidative phosphorylation) because of the rapid rigor mortis. Post-mortem examinations of the rats killed at the end of the study revealed no notable effects (see Table 1; Hoechst, 1983).

- **Studies on methaemoglobin formation**

In 10 male Wistar rats, which were each given a single dose of 60 mg 2-(1-methylpropyl)-4,6-dinitrophenol/kg body weight by gavage, haematological examination revealed a reduction in the average haemoglobin level to 11.63 g/100 ml blood (controls 13.32 g/100 ml) as well as an increase in the average methaemoglobin level to 0.253 g/100 ml blood (controls 0.128 g/100 ml). All of the dosed animals died within 1 to 3.25 hours of administration (no further details; Henneberg, 1971).

2-(1-Methylpropyl)-4,6-dinitrophenol was given to 6 sheep at a dose of 45 mg/kg body weight and one sheep at 49 mg/kg body weight, the single doses being administered via a tube into the rumen (volume delivered ca. 200 ml/animal). A further two sheep, which were given the vehicle alone (33% aqueous acetone) served as controls. Within 24 hours and 3.5 days, respectively, 2 of the 7 sheep died. A further sheep which developed severe haemolysis was killed after 3 days. The 5-day observation period was survived by 4 of the sheep. The following signs of toxicity occurred: dyspnoea, a slight but significant increase in body temperature in some animals within the first hour of dosing and jaundice. Haematological tests revealed methaemoglobin formation in all sheep in the treated group, with maximum levels of ca. 5 to 8 g/100 ml blood 3 hours after administration. The methaemoglobin level was still significantly elevated 3 days after dosing. Within 6 hours of administration, the leukocyte count was increased to ca. 12,000 to 18,000/mm³ blood, but returned to normal within the following

18 hours. Haemolysis occurred in all animals after 2 to 3 days. Biochemical analysis showed a decrease in the glutathione concentration in the erythrocytes by 60 to 80% within 90 minutes of dosing. After 2 days the glutathione concentration rose again, except in 2 of the sheep in which the glutathione concentration fell to the level of detection during the second day. The bilirubin level in the plasma was slightly increased during the first 2 days, increasing in 2 animals to 2.1 and 2.5 mg/100 ml plasma after 2 and 3 days. The activity of aspartate aminotransferase was only slightly increased within 2 days of dosing, increasing in 2 animals to 740 and 880 units (reference level unspecified) after 5 days. The levels of non-protein-bound nitrogen and of creatinine in the plasma increased slightly within 2 days, but then returned to normal. The protein level in the plasma was reduced by ca. 20% within 6 hours of dosing. Investigation of urine parameters (volume, pH, specific gravity, protein, haemoglobin, glucose content and ketone bodies) revealed oliguria within 24 hours of dosing. In some cases protein and haemoglobin could be detected in the urine, whereas glucose and ketone bodies were undetectable. Histopathological examination revealed congestion of the lungs, liver and kidneys, pulmonary oedema, isolated haemorrhages in the heart muscle and kidneys and a brown discoloration of the blood. There were necrotic changes to the kidneys and liver. The sheep that survived the 5-day observation period were jaundiced. The cytoplasm of the liver parenchyma cells was clearly eosinophilic, the proximal and distal kidney tubules showed hyaline droplet degeneration and dilation (Fröslie, 1974).

The introduction of a single dose of 45 mg 2-(1-methylpropyl)-4,6-dinitrophenol/kg body weight by gavage into the rumen led to the deaths of 2 of 4 sheep within 16 to 18 hours and after 2.5 days. Dyspnoea occurred 3 hours after administration, with severe haemolysis after 1 to 2 days. Haematological analyses revealed a maximum methaemoglobin level of 5.6 to 6.8 g/100 ml blood after 3 hours which then decreased, with elimination rates of 0.37 g/100 ml blood/hour over the following 3 hours and 0.07 g/100 ml blood/hour over the next 18 hours. On the second day the average elimination rate for methaemoglobin was still 0.04 g/100 ml blood/hour. The haemoglobin level was reduced at this time because of the onset of haemolysis. NADH-dependent methaemoglobin reductase activity in the blood was inhibited. This was 0.3 to 0.9 units 6 hours after administration

(activity before dosing 3.3 units; reference values not given) and was at the limits of detection after 1 to 2 days (Fröslie, 1976).

Subacute toxicity

After repeated oral administration (by gavage) of 20 mg 2-(1-methylpropyl)-4,6-dinitrophenol/kg body weight/day to male Wistar rats, 30% of the animals had died after 2 days and a further 30% after 3 days. The coproporphyrin excretion in the urine was increased by about 4-fold in the surviving animals on day 4 of the study (6.65 µg/rat/24 hours) compared with the excretion rate prior to dosing (1.55 µg/rat/24 hours; no further details; Henneberg, 1971).

The oral administration (by gavage) of 10 mg 2-(1-methylpropyl)-4,6-dinitrophenol/kg body weight/day for 32 days led to an increase in the average methaemoglobin level to 8.9% (controls 0.9%) in 2 male Wistar rats at the end of the study. The haemoglobin level was within the range of that in the controls. Coproporphyrin excretion in the urine, which was collected over 24-hour periods, was on average ca. 2 µg/rat/24 hours (controls ca. 1.5 µg/rat/24 hours), the maximum increase being apparent on day 28 of the study at which point levels were approximately 2-fold (3.3 µg/rat/24 hours) those in the controls. Urobilinogen excretion in the urine was in the control range (average 0.018 µg/rat/24 hours; controls 0.015 µg/rat/24 hours), but was increased by a maximum of ca. 2.5-fold (0.038 µg/rat/24 hours) compared with controls on day 5 of the study. The differential blood count showed no treatment-related changes. Erythrocyte and leukocyte counts were slightly increased in the treated groups. Histological examination revealed increased fat deposition and reduced glycogen levels in the liver (Henneberg, 1971).

The daily oral administration of a dose of 2 mg 2-(1-methylpropyl)-4,6-dinitrophenol/kg body weight led to accelerated breathing, loss of appetite and weight loss in dogs. Post-mortem examination after the administration of 14 doses within 22 days showed no histologically detectable damage to the organs. The administration of 5 or 20 mg 2-(1-methylpropyl)-4,6-dinitrophenol/kg body weight orally caused vomiting (no further details; Hoechst, 1958).

Groups of 6 male albino mice were treated with 16, 40 or 100 mg 2-(1-methylpropyl)-4,6-dinitrophenol/kg body weight/day which was applied

to the clipped skin of the flanks 5 days/week for 2 weeks. A control group consisting of 9 mice was treated with the vehicle, acetone. All of the mice in the lowest dose group survived the treatment period and their body weight gain was within the control range. The mortality rates in the intermediate and high dose groups were 100% after the first dose (no further details; Bough et al., 1965).

The daily application of a 3% solution of 2-(1-methylpropyl)-4,6-dinitrophenol in ethanol to the shaved abdominal skin of 3 rabbits under occlusive cover was fatal after 1, 3 and 8 applications. The application of a 10% solution of 2-(1-methylpropyl)-4,6-dinitrophenol in butyl carbitol acetate to the shaved abdominal skin or to the ear in 3 rabbits led to their deaths within 24 hours (no further details; Spencer et al., 1948).

Cats (2/group) generally did not survive more than 3 to 5 intraperitoneal injections of 2-(1-methylpropyl)-4,6-dinitrophenol at doses of 0.75, 1.25, 2.5 or 5 mg/kg body weight. Only one of the two cats treated with 0.75 mg/kg survived the planned 14 injections within 20 days. There were no characteristic effects on the organs examined histopathologically at the end of the study in this cat. Methaemoglobin and Heinz bodies were not determined. The activity of alkaline phosphatase in the serum was unchanged (Hoechst, 1958).

7.3 Skin and mucous membrane effects

The repeated application of a 3% solution of 2-(1-methylpropyl)-4,6-dinitrophenol in ethanol to the ear or the shaved abdominal skin of 3 rabbits under occlusive cover did not induce skin irritation. A 10% solution of 2-(1-methylpropyl)-4,6-dinitrophenol in butyl carbitol acetate similarly caused no skin irritation when applied to the ear or shaved abdominal skin of 3 rabbits (no further details; Spencer et al., 1948).

When 50 µg 2-(1-methylpropyl)-4,6-dinitrophenol was instilled into the conjunctival sac of the rabbit eye, the substance was shown to be severely irritating after a 24-hour exposure period (no further details; Marhold, 1986).

7.4 Sensitisation

No information available.

7.5 Subchronic and chronic toxicity

2-(1-Methylpropyl)-4,6-dinitrophenol (98.2% pure) was given to groups of 10 male and 10 female Wistar rats (average initial weights 99 and 103 g, respectively) in the diet for 13 weeks at concentrations of 0 (controls), 12.5, 40, 120 or 360 ppm (OECD guideline No. 408). This was equivalent to daily doses of 0, 1.09, 3.49, 11.95 and 56.80 mg/kg body weight in the males and 0, 1.12, 3.76, 11.97 and 52.80 mg/kg body weight in the females. Yellow discoloration of the fur was evident from 120 ppm. At 360 ppm there was a clear deterioration in general condition and in the first week of the study 5/10 males and 8/10 females died. The rest of the rats in this dose group were killed in a moribund condition after 6 weeks. The high dose led to cessation of body weight gain and emaciation. A marked reduction in feed intake occurred in male rats from 120 ppm and in females at 360 ppm. Drinking water consumption was increased from 120 ppm. Signs of haemoconcentration, with increasing levels of erythrocytes, haemoglobin and haematocrit values were observed dose-dependently in the males from 40 ppm. Increased urea nitrogen levels were found in the 360 ppm group. There was a dose-dependent yellow to yellow-brown discoloration of the urine. At 120 ppm there was a significant decrease in the specific gravity of the urine in the females. Also at 120 ppm there were statistically significant increases in the relative weights of the heart (in both sexes) and the kidneys and spleen (in the males). In the males at the top dose level, the testes were found to be in a juvenile condition, as a secondary effect of the inhibition of body weight gain caused by toxicity. The lymphoreticular tissues of the spleen were hypoplastic in both sexes at this dose. The no effect level based on these results was 12.5 ppm (equivalent to 1.09 mg/kg body weight in males and 1.12 mg/kg body weight in females; Hoechst, 1987a).

In a 27-week feeding study, groups of 20 male rats were given 2-(1-methylpropyl)-4,6-dinitrophenol in the diet at concentrations of 0.005, 0.01 and 0.02%, equivalent to doses of 2.5, 5.0 and 10.0 mg/kg body weight/day at an assumed average feed consumption of 50 g/kg body weight/day. The control group consisted of 30 rats. Mortality and feed consumption were in the range of control values for all dose groups. Body weight gain was slightly reduced in the top dose group (by 3 to 8% compared with controls). Haematological parameters (erythrocyte and leukocyte counts, haemoglobin level and differential blood count) were unaffected. Histopathological examination of the liver, kidneys, heart, testes, lungs, spleen, adrenal glands,

pancreas, stomach and bone marrow revealed no treatment-related effects. The weights of the kidneys, heart and testes were in the range of control values, with only the liver weight in the high dose rats being significantly increased. Blood urea nitrogen levels in the intermediate and high dose groups were raised (209 and 203 µg/ml blood; controls 175 µg/ml blood). A parallel group of 10 male rats, which was given 2-(1-methylpropyl)-4,6-dinitrophenol in the diet at a concentration of 0.05% (equivalent to 25 mg/kg body weight/day at an assumed average feed consumption of 50 g/kg body weight/day), was removed from the study on only day 21 because of high mortality and severe weight loss in the rats that had survived up until this time; 4/10 rats died within 13 days. Examination of the rats that survived until day 21 revealed severe emaciation, an empty gastro-intestinal tract, slight degenerative effects on the liver and kidneys and an increase in the blood urea nitrogen level to 550 µg/ml compared with 175 µg/ml blood in the controls. No histopathological changes were found in the lungs, heart, spleen, adrenal glands, pancreas or testes (Spencer et al., 1948).

Groups of 10 male and 10 female Wistar rats (Hoe:WISkf(SPF71); average initial weights 128 and 117 g, respectively) were given 2-(1-methylpropyl)-4,6-dinitrophenol (96.9% pure) in the diet for 12 months (OECD guideline No. 451) at concentrations of 0 (controls), 10, 40 or 160 ppm (equivalent to a daily intake of the substance of 0, 0.62, 2.46 and 11.89 mg/kg body weight in males and 0, 0.81, 3.28 and 13.76 mg/kg body weight in females). There were no effects on behaviour or general condition. Yellow discoloration of the skin and fur was observed in the top dose group. In the females in the top dose group there was a slight, and in the males a marked and occasionally significant reduction in body weight gain. The relative feed consumption was increased in the females in all dose groups and in the males in the highest group only. At 40 ppm and above there was a dose-dependent tendency towards haemoconcentration in the males (significant at 160 ppm) with increasing values for erythrocytes, haemoglobin and haematocrit. In addition, the thrombocyte count was reduced in the 160 ppm group. Increased urea nitrogen levels were found in males from 40 ppm, decreased serum triglycerides in both sexes at 160 ppm. A yellow to yellow-brown discoloration of the urine occurred dose-dependently. There were significant increases in heart, lung and kidney weights in the males in the 160 ppm group, while thyroid gland weights were increased in females in all dose groups without any corresponding histopathological ef-

fects. Histopathological examination revealed glycogen depletion in the liver and a slight increase in the deposition of siderin in the liver and kidneys in the 40 and 160 ppm groups, but no treatment-related cell or tissue damage. All other organs studied showed no histopathological changes. The no effect level was given as 10 ppm, corresponding to a daily intake of the substance of 0.62 mg/kg body weight in male rats and 0.81 mg/kg body weight in female rats (Hoechst, 1987b, 1988).

7.6 Genotoxicity

7.6.1 In vitro

Gene mutagenic effects

2-(1-Methylpropyl)-4,6-dinitrophenol (97.7% pure) was not mutagenic in the Salmonella/microsome test (direct plate test) in the *Salmonella typhimurium* strains TA 100, TA 1535, TA 1537 and TA 1538 and in *Escherichia coli* WP2 at concentrations ranging from 1 to 1000 µg/plate either with or without metabolic activation (S9-mix from Aroclor 1254-induced mouse liver). The highest concentration tested was bacteriotoxic (EPA, 1977).

2-(1-Methylpropyl)-4,6-dinitrophenol showed no mutagenic activity in a study in 8 strains of *Salmonella typhimurium* (no further details; Andersen et al., 1972).

In a further study in the *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538, there were no indications that 2-(1-methylpropyl)-4,6-dinitrophenol had mutagenic activity in the direct plate test either with or without metabolic activation (no further details; Eisenbeis et al., 1981).

2-(1-Methylpropyl)-4,6-dinitrophenol (96.9% pure) was also tested in a standard plate test at concentrations ranging from 0.8 to 500 µg/plate in the *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 and in *Escherichia coli* WP2uvrA with and without metabolic activation (S9-mix from Aroclor 1254-induced rat liver). The substance was bacteriotoxic from 100 µg/plate. 2-(1-Methylpropyl)-4,6-dinitrophenol was not mutagenic either with or without metabolic activation (Hoechst, 1985).

In *Saccharomyces cerevisiae* strain D3, 97.7% pure 2-(1-methylpropyl)-4,6-dinitrophenol was not mutagenic at concentrations ranging from 1000

to 3000 µg/ml with or without metabolic activation (S9-mix from Aroclor 1254-induced mouse liver) in two independent experiments. The highest concentration tested was bacteriotoxic (EPA, 1977).

2-(1-Methylpropyl)-4,6-dinitrophenol (98.2% pure) was also not mutagenic in *Schizosaccharomyces pombe* P1 at concentrations of 62.5 to 500 µg/ml with and without metabolic activation (S9-mix from Aroclor 1254-induced rat liver; (RBM, 1985).

In the HPRT test in Chinese hamster V79 cells, 2-(1-methylpropyl)-4,6-dinitrophenol (technical grade product, no further details) was negative with and without metabolic activation (S9-mix from phenobarbital and β-naphthoflavone-induced rat liver) at concentrations of 39.1 to 625 µg/ml. Cytotoxicity was observed from 469 µg/ml (LSR-RTC, 1987).

2-(1-Methylpropyl)-4,6-dinitrophenol (technical grade product, 98.2% pure; solvent DMSO) was tested in L5178Y mouse lymphoma cells at concentrations from 4 to 48 µg/ml without metabolic activation and from 1.5 to 90 µg/ml with metabolic activation (S9-mix from Aroclor 1254-induced rat liver). Without metabolic activation there was a slight increase in the total number of mutants and in the mutation rate at the higher concentrations. With metabolic activation there was a marked increase in the mutation rate at toxic concentrations. However, this increase could not be reproduced in a second independent experiment in which the substance was shown to be only weakly positive. Overall therefore, 2-(1-methylpropyl)-4,6-dinitrophenol was evaluated as weakly positive in this test system (Litton Bionetics, 1986a).

Chromosome damaging effects

A cytogenetic test to detect chromosome aberrations in human lymphocytes with and without metabolic activation (S9-mix from Aroclor 1254-induced rat liver) gave no indications that 2-(1-methylpropyl)-4,6-dinitrophenol (technical grade product, 98.2% pure) was mutagenic at the tested concentration range of 0.5 to 15 µg/ml (Litton Bionetics, 1986b).

DNA-damaging effects

2-(1-Methylpropyl)-4,6-dinitrophenol was mutagenic with and without metabolic activation (S9-mix from rat liver) in the modified SOS-chromotest (mi-

crotitration technique) in *Escherichia coli* (cytotoxic dose range covered; no further details; Xu and Schurr, 1990).

In the rec assay in *Bacillus subtilis* H17/Rec$^+$/M45 Rec$^-$ and in *Escherichia coli* W3110 polA$^+$/p3478 polA$^-$, 2-(1-methylpropyl)-4,6-dinitrophenol (97.7% pure) was genotoxic at a concentration of 1000 µg/plate (EPA, 1977).

There were no indications that 2-(1-methylpropyl)-4,6-dinitrophenol was genotoxic in a further rec assay in *Salmonella typhimurium* SL4525 Rec$^+$/SL4700 Rec$^-$ (no further details; Waters et al., 1982).

There were also no indications that 2-(1-methylpropyl)-4,6-dinitrophenol (technical grade product, 98.2% pure) was able to induce DNA damage in a UDS test in primary rat hepatocytes at concentrations of 0.025 to 1.01 µg/ml (Litton Bionetics, 1985).

2-(1-Methylpropyl)-4,6-dinitrophenol (97.7% pure) did not induce DNA damage either with or without metabolic activation (S9-mix from Aroclor 1254-induced mouse liver) in a UDS test in human fibroblasts (WI-38). The substance was tested at concentrations ranging from 0.024 to 24 µg/ml without S9-mix and 2.4 to 240 µg/ml with S9-mix, with 6 and 3 plates, respectively, used at each concentration (EPA, 1977).

Recombination

In *Saccharomyces cerevisiae* (strain heteroallelic at two loci), 2-(1-methylpropyl)-4,6-dinitrophenol increased the induction of mitotic gene conversion (recombination) at concentrations of ca. 200 µg/ml and above. The test was carried out in two independent experiments. Concentrations ranging from ca. 200 to 1700 µg/ml were tested, even the lowest concentration being cytotoxic (Parry, 1973).

In a further gene conversion test in *Saccharomyces cerevisiae* D4 with technical grade 2-(1-methylpropyl)-4,6-dinitrophenol (98.2% pure), concentrations of 50 to 600 µg/ml were tested with metabolic activation (S9-mix from Aroclor 1254-induced rat liver) and concentrations of 1 to 150 µg/ml without metabolic activation. The substance was weakly mutagenic in this study (RBM, 1986).

7.6.2 In vivo

In a micronucleus test (in accordance with OECD guideline No. 474), 5 male and 5 female NMRI mice (strain Hoe:NMRkf (SPF71); weight 24 to 38 g) were given the test substance (technical product, 96.9% pure) orally in a 2% starch slurry at doses of 0 (negative controls), 10, 25 and 40 mg/kg body weight. Positive controls were treated with 50 mg cyclophosphamide/kg body weight. The bone marrow was processed 24, 48 and 72 hours after dosing. There was no increase in the occurrence of micronuclei compared with the negative controls. The ratio of polychromatic to normochromatic erythrocytes was unaffected. Thus the substance was not mutagenic in this study (Hoechst, 1986).

2-(1-Methylpropyl)-4,6-dinitrophenol did not cause any increase in sex-linked recessive lethal mutations in a test in *Drosophila melanogaster* at concentrations ranging from 0.5 to 4.0 µg/ml. when administered in the nutrient solution for 48 hours. Only very low concentrations could be employed because of the highly toxic effect of 2-(1-methylpropyl)-4,6-dinitrophenol and the investigator therefore considered the test to be inadequate (Valencia, 1981).

7.7 Carcinogenicity

In a chronic 100-week feeding study, groups of 70 male and 70 female CD1 mice (40 days old at the beginning of the study) were given 2-(1-methylpropyl)-4,6-dinitrophenol (98% pure) in the diet at concentrations corresponding to daily intakes of 1, 3 and 10 mg/kg body weight. The control group consisted of 70 male and 70 female animals, which were given normal feed. Certain organs were examined histologically in 10 mice/sex and dose after 6 and 12 months. The remaining mice were killed after 100 weeks. Body weight gain in the female mice in the intermediate and high dose groups was significantly below that of the control animals from the beginning of the study onwards (ca. 90 and 87% of control values, respectively, at the end of the study). No differences were found in feed consumption in either sex in any group. Mortality was not increased. There were no findings on analysis of blood and urine samples during the study. The plasma of the mice in the highest dose group appeared jaundiced at the later collection times. The total bilirubin concentration was therefore determined after 52 weeks in a few control animals and in the mice in the highest dose group.

There were no differences, and the investigators therefore suggested that the unusual plasma colour was attributable to high blood levels of 2-(1-methylpropyl)-4,6-dinitrophenol and/or its metabolites. In week 78 of the study, ophthalmological studies revealed an increased incidence of cataracts in the intermediate and high dose groups. High incidences of cataracts were observed in both the control and the test animals up until the end of the study, although the cataracts in the high dose group were significantly more marked. The investigators therefore suggested that chronic administration of 2-(1-methylpropyl)-4,6-dinitrophenol accelerated age-related changes. The yellow discoloration of the fur in the mice in the high dose group and in some animals in the intermediate dose group resulted from direct contact of the test substance in the diet with the fur. The absolute and relative liver weights were increased at the end of the study at the high dose. With respect to non-neoplastic changes, histopathological examination revealed no differences between the treated animals and the controls. A non-significant increase in the incidence of hepatocellular adenomas and carcinomas was found at the end of the study in males in all dose groups (see Table 2). In the females, the absence of hepatocellular adenomas/carcinomas in the control group led to a statistically increased incidence in the treated group (see Table 2). The investigators did not consider these increased incidences to be of biological significance, among other reasons because of the low number of animals affected, the lack of dose-dependency and the absence of other hepatocellular changes (Hazleton, 1981c). The effects on the liver were subsequently re-examined by an independent pathologist. It was concluded that the lesions considered to be adenomas by the investigators were in fact hyperplastic nodules and not neoplasias, and that 2-(1-methylpropyl)-4,6-dinitrophenol thus showed no carcinogenic activity in CD1 mice in this study (BIBRA, 1985).

Table 2. Tumour incidences in CD1-mice at the end of a 100-week feeding study

		2-(1-Methylpropyl)-4,6-dinitrophenol			
		0 mg/kg body weight	1 mg/kg body weight	3 mg/kg body weight	10 mg/kg body weight
Hepatocellular adenomas	Male	6/11*	10/18	9/18	7/14
	Female	0/21	3/24	5/23	5/26
Hepatocellular carcinomas	Male	0/11	0/18	2/18	2/14
	Female	0/21	1/24	0/23	0/26
Hepatocellular adenomas/carcinomas	Male	6/11 (55%)	10/18 (56%)	11/18 (61%)	9/14 (64%)
	Female	0/21 (0%)	4/24 (17%)	5/23 (22%)	4/26 (19%)
* Number of mice with tumours/number of mice examined					

In a further carcinogenicity study, groups of 18 male and 18 female B6C3F1 and B6AKF1 mice were given 2-(1-methylpropyl)-4,6-dinitrophenol in 0.5% gelatine once daily by gavage from day 7 to day 28 of life. The test substance was 95 to 98% pure. At the beginning of the study, the dose was equivalent to 2.15 mg/kg body weight/day, but this was not increased in line with body weight gain. From day 28 of life onwards, the mice were given 2-(1-methylpropyl)-4,6-dinitrophenol at a concentration of 7 ppm in the diet (equivalent to 2.2 mg/kg body weight/day, based on assumed average feed consumption of 5 g/mouse and an average body weight of 16 g on day 28). The dose corresponded to the maximum tolerated dose determined in a 19-day preliminary dietary study in 6 animals. The duration of administration was 18 months. A control group consisted of 18 male and 18 female mice of each strain. Those mice that died during the study and those killed at the end were examined macroscopically for tumours and lesions. Blood smears were examined from mice with enlargement of the spleen or liver and those with lymphadenopathy. At the end of the study, the liver, spleen, kidneys, adrenal glands, stomach, gut and genital organs were examined histologically; in the mice that died prematurely, histological examinations were carried out on a case-by-case basis (no further details). There were no effects on body weight on examination after 1, 6, 12 and 20 months. No data on feed consumption were presented. The investigators evaluated 2-(1-methylpropyl)-4,6-dinitrophenol as not being carcinogenic to the mouse on oral administration, as tumour types and incidences were within the range of control figures. The tumour incidences are shown in Table 3 (Innes et al., 1969; Bionetics, 1968).

Table 3. Tumour incidences in B6C3F1 and B6AKF1 mice after oral administration of 2-(1-methylpropyl)-4,6-dinitrophenol for 18 months

	Mice with tumour/mice examined (%)			
	B6C3F1		B6AKF1	
	male	female	male	female
Control group(gelatine vehicle)	0/16 (0%)	0/16 (0%)	3/18 (17%)	2/17 (12%)
Untreated control group (background)	22/63 (35%)	8/71 (11%)	16/72 (22%)	5/65 (8%)
Treated group*	7/18 (39%)	1/18 (6%)	6/17 (35%)	2/17 (12%)
* See text for dose of 2-(1-methylpropyl)-4,6-dinitrophenol				

Groups of 18 male and 18 female B6C3F1 and B6AKF1 mice were given single subcutaneous injections of 21.5 mg 2-(1-methylpropyl)-4,6-dinitrophenol/kg body weight in the neck on day 28 of life. The test sub-

stance was 95 to 98% pure. A control group of 24 mice/sex and strain was treated with the vehicle (corn oil). The observation period was 18 months. Mice that died prematurely and those killed at the end of the study were examined macroscopically for tumours and lesions. Blood smears were examined from mice with enlargement of the spleen or liver and those with lymphadenopathy. At the end of the study, the liver, spleen, kidney and adrenal gland, stomach, gut and genital organs were examined histologically; in the mice that died prematurely, histological examinations were carried out on a case-by-case basis (no further details). The investigators evaluated 2-(1-methylpropyl)-4,6-dinitrophenol as being not carcinogenic to the mouse after a single subcutaneous injection, as tumour types and incidences were within the range of control figures. The tumour incidences are shown in Table 4 (Bionetics, 1968).

Table 4. Tumour incidences in B6C3F1 and B6AKF1 mice after a single subcutaneous injection of 2-(1-methylpropyl)-4,6-dinitrophenol and an 18-month observation period

	Mice with tumour/mice examined (%)			
	B6C3F1		B6AKF1	
	male	female	male	female
Control group (gelatine vehicle)	7/21 (33%)	1/21 (5%)	2/19 (11%)	1/20 (5%)
Untreated control group (background)	14/80 (18%)	7/92 (8%)	4/100 (4%)	12/95 (13%)
Treated group*	7/18 (39%)	0/18 (0%)	1/18 (6%)	0/18 (0%)
* See text for dose of 2-(1-methylpropyl)-4,6-dinitrophenol				

7.8 Reproductive toxicity

Fertility studies

Four male Charles-River mice (C57BL/6XC3H, F1) were given 20 mg 2-(1-methylpropyl)-4,6-dinitrophenol/kg body weight/day on 5 consecutive days by gavage. A control group, consisting of 4 male mice, was given the carrier (corn oil). The observation period was 30 days. Testis weights were within the control range. On examination of the sperm (number and morphology) there were no significant differences from the controls (no further details; Osterloh et al., 1983).

When male Charles-River mice (C57BL/6XC3H, F1) were given intraperitoneal injections of 2.0, 4.3, 9.3, 20 and 43 mg 2-(1-methylpropyl)-4,6-

dinitrophenol/kg body weight/day on 5 consecutive days there were no significant findings on examination of the sperm (number and morphology) of the animals in the 2.0 to 20 mg/kg dose groups, and testis weights were within the control range. In the high dose group (43 mg/kg) the mortality was 100%, and an evaluation could not therefore be undertaken in this dose group. The sperm were examined and testis weights determined on day 35 of the study. The test substance was 98% pure. In each dose and control group there were 4 mice, with 8 in the top dose group. The mice in the control group were treated with the vehicle (corn oil; Osterloh et al., 1983).

Groups of 10 male Sprague-Dawley rats (97 days old) were given 2-(1-methylpropyl)-4,6-dinitrophenol at concentrations of 125, 150 and 175 ppm in the diet for 25 days. This was equivalent to doses of ca 6.3, 7.5 and 8.8 mg 2-(1-methylpropyl)-4,6-dinitrophenol/kg body weight/day at an assumed feed consumption of 50 g/kg body weight/day. The control group consisted of 10 rats. Examination of the sperm from the caudae epididymides revealed a significant reduction in sperm motility (by 40 to 45%) in the low and intermediate dose groups. In the high dose group, sperm motility was almost completely inhibited. Determination of the motility index in the high dose group was only possible to a limited degree, as increased numbers of anomalous sperm and cell clumping made evaluation of the sperm impossible in many samples (Linder et al., 1986).

In a study to assess the effect of (97.3% pure) 2-(1-methylpropyl)-4,6-dinitrophenol on the male sex organs, male Sherman rats (99 to 115 days old) were given 75, 150, 225 and 300 ppm 2-(1-methylpropyl)-4,6-dinitrophenol in the diet for 11 weeks. This was equivalent to average doses of 3.8, 9.1, 15.6 and 22.2 mg 2-(1-methylpropyl)-4,6-dinitrophenol/kg body weight/day, determined from the average feed consumption. Each dose group consisted of 20 rats, with 36 in the control and high dose groups. Macroscopic and histopathological examination of the epididymides, testes, seminal vesicles, prostate, lungs and bronchi, the determination of organ weights (sex organs) and examination of the sperm were carried out in half of the surviving animals at the end of treatment (after 11 weeks of the study) and in the other half at the end of a 16-week observation period. These animals were mated with untreated females at the beginning and end of the observation period, and the fertility index, number of implantations/dam, foetal mortality and foetal weights were determined. In 4 rats in each case from the 300 ppm and control groups, these studies

were also undertaken after 10, 20, 30 and 50 days of treatment. The mortality rates were ca. 28% (10 of 36 rats) in the 300 ppm group, ca. 5% (1 of 20 rats) in the 225 ppm group and ca. 3% (1 of 36 rats) in the control group. None of the rats in the 75 and 150 ppm groups died during the treatment or observation periods. Signs of toxicity seen at concentrations of 225 ppm and above in the diet included dyspnoea, noises on breathing and salivation. Body weight gain and feed consumption in the 75 ppm group were similar between the treated rats and the controls. The body weight of the rats in the 150 ppm group stayed relatively constant during treatment and was within the control range at the end of the observation period. In the rats in the 225 and 300 ppm groups the body weights were reduced on average by 19 and 38% at the end of treatment, while at the end of the observation period, those of the 225 ppm group were slightly under control values and those of the 300 ppm group were ca. 20% below control values. Feed consumption in the 3 highest dose groups was increased compared with the controls from week 4 of the study. The relative testis and epididymis weights of the rats in the 225 and 300 ppm groups were significantly reduced compared with control values both at the end of treatment and at the end of the observation period. The relative weights of the seminal vesicles in the rats in the 150 and 225 ppm groups were significantly increased at the end of treatment, while in the rats in the 300 ppm group they were significantly reduced; they were within the range of control values for all animals at the end of the observation period. The relative prostate weight in the rats in the 300 ppm group was significantly reduced at the end of the observation period. Macroscopic examination revealed testes, epididymides, prostates and seminal vesicles that were markedly reduced in size in the rats in the 225 and 300 ppm groups at the end of the 11-week treatment period. In the rats in the 300 ppm group this effect (atrophied testes) was already apparent after 20, 30 and 50 days. There were no histopathological effects on the lungs or bronchi. Histopathological changes to the testes (including abnormally large spermatids and morphologically abnormal sperm) and epididymides (including morphologically abnormal spermatozoa and increased desquamation in spermatogenic cells) occurred in the rats in the 300 ppm group after 20 and 30 days of treatment. After 50 days of treatment necrotic spermatogenic cells and cell debris in the seminiferous tubules were evident, and at the end of treatment after 11 weeks, seminiferous cells were completely absent and only Sertoli cells were present; no spermatozoa were detectable in the epididymides.

Similar findings occurred in the rats in the 225 ppm group at the end of treatment. In the rats in the 150 ppm group, only slight histopathological effects were found in the testes and/or epididymides at the end of treatment (occasional multinucleate spermatogenic cells and morphologically abnormal spermatozoa). These histopathological effects were still evident at the end of the 16-week observation period. In the 300 ppm group, atrophy of the accessory reproductive organs (particularly the seminal vesicles) was observed at the end of treatment, although this effect was no longer evident at the end of the observation period. In the spermatogram, the sperm count (epididymis, vas deferens) in the 225 and 300 ppm groups was significantly reduced (oligozoospermia) both at the end of treatment and at the end of the observation period. There were no histopathological effects in the 75 ppm group. The mating behaviour of rats in all dose groups remained unaffected. However, in the 225 ppm group only 1 of 10 and 2 of 10 male rats were fertile at the end of treatment and the end of the observation period, respectively, and in the 300 ppm group, none of the males was fertile. The number of live litters/dam and the fertility index were slightly reduced in the 75 and 150 ppm groups both at the end of treatment (number of live litters/dam 12/20 and 13/19, controls 16/20; fertility index 60 and 68%, controls 80%) and at the end of the observation period (number of live litters/dam 8/10 and 7/10, controls 9/10; fertility index 80 and 80%, controls 90%). The average number of implantations/dam, average foetal mortality and foetal weights were within the range of control values. At the end of treatment and the end of the observation period in the 225 ppm group, there were reductions in the fertility index (10 and 20%, respectively, controls, 80 and 90%, respectively) and the average number of implantations/dam (9.5 and 9.0%, controls 11.7 and 11.4%); however, foetal mortality (0 and 10.0%, controls 13.1 and 23.2%) was decreased and foetal weight remained unaffected. The no effect level was 75 ppm (equivalent to 3.8 mg/kg body weight/day; Linder et al., 1982).

Male and female Sherman rats (35 to 38 days old) were given technical grade 2-(1-methylpropyl)-4,6-dinitrophenol (80% pure; no data on contaminants) at nominal concentrations of 0 (controls), 50, 100, 150, 200, 300, 400 and 500 ppm in the diet over a period of 153 days in total (60 days prior to mating, during mating and during rearing of the offspring). This was equivalent to doses of ca. 2.5, 5.0, 7.5, 10.0, 15.0, 20.0 and 25.0 mg 2-(1-methylpropyl)-4,6-dinitrophenol/kg body weight/day at an assumed

average feed consumption of 50 g/kg body weight/day. Each group consisted of 14 rats of each sex. In the 300 ppm group 14% of the rats died and in the 400 and 500 ppm groups all rats died. Because of the high mortality, these groups were taken out of the study on day 21. In the 4 dose groups remaining in the study, body weight gain was reduced, the absolute organ weights (liver, spleen, heart, lungs and brain) were decreased and relative organ weights were increased (no further details). Biochemical analysis revealed significant increases in the activities of alkaline phosphatase and alanine aminotransferase, a significant increase in potassium and urea nitrogen levels and significant inhibition of the activities of lactate dehydrogenase and cholinesterase in the blood (no further details). The concentrations of 2-(1-methylpropyl)-4,6-dinitrophenol in the various body compartments and excreta were dependent on dose, the following series being determined: blood > faeces > urine > fat > brain > liver. Aminopyrine-N-demethylase activity in the liver was increased. Discrimination learning was unaffected, while locomotor activity of the rats in the highest remaining dose group (200 ppm) was increased. Diffuse tubular atrophy of the testes, particularly in the top dose group (200 ppm) was a significant histopathological finding. Fertility, lactation, body weight gain and viability of offspring were decreased and the number of live-borne offspring was reduced (abstract; no further details; Hall et al., 1978).

Teratogenicity studies in rats

- **Oral administration**

Groups of 25 Wistar rats were given 2-(1-methylpropyl)-4,6-dinitrophenol (96.1% pure) in corn oil by gavage from days 6 to 15 of pregnancy at doses of 0 (controls), 1, 3 or 10 mg/kg body weight (OECD guideline No. 414). Laparotomy was performed on day 21 p.c. No overt signs of toxicity were observed in the dams, although body weight gain and feed consumption were impaired in the highest dose group. None of the rats died and there were no findings in the dams at post-mortem. There were no differences in the number of implantations or in post-implantation losses. In the highest dose group foetal weights were reduced and increased incidences of incompletely ossified skulls, phalangeal nuclei, heal bones and cervical vertebrae were observed. These findings were evaluated as being effects of maternal toxicity. No malformations were found. The no observed effect level for dams and foetuses was thus 3 mg/kg body weight (RCC, 1986a).

Pregnant CD rats (15 to 17/group) were given 2.5, 5, 10 or 15 mg 2-(1-methylpropyl)-4,6-dinitrophenol/kg body weight/day from days 6 to 15 of pregnancy by gavage. A control group was given the vehicle (corn oil). Laparotomy was carried out on day 21 of pregnancy. Body weight gain in the dams was significantly reduced in the two higher dose groups (10 and 15 mg/kg body weight/day) during the treatment period, but was then (from day 15 to 21 of pregnancy) within the range of control values. In the top dose group, 4 of the 15 dams died within the first 5 days of treatment. For all treatment groups, the numbers of corpora lutea and implantations/dam, the numbers of resorptions and live foetuses/litter and the percentages of dams with resorptions and post-implantation losses were within the range of control values. Foetal weights were significantly reduced in the top dose group. In the 5 mg/kg dose group one foetus had a cerebral haemorrhage. A foetus in the top dose group had several skeletal malformations (including scoliosis), but no skeletal malformations occurred in any other dose group or in the controls. The occurrence of skeletal variations (particularly supplementary ribs) was dose-dependently increased, significantly in the two higher dose groups, and the ossification of metacarpal bones and sternebrae was significantly delayed in the highest dose group (Giavini et al., 1986a, b). In summary there was a maternal no effect level in this study of 5 mg/kg body weight/day and a foetal no effect level of 2.5 mg/kg body weight/day.

Pregnant CD rats were given 2-(1-methylpropyl)-4,6-dinitrophenol from days 6 to 13 of pregnancy by gavage at doses of 7.5 or 10 mg/kg body weight twice daily in corn oil, with a further group receiving a dose of 15 mg/kg body weight once daily in saline. A control group consisting of 10 rats was given the vehicle (corn oil). Each dose group consisted of 10 to 16 rats. A laparotomy was performed on day 21 of pregnancy. Mortality rates in the dose groups ranged from 19 to 40% (3/13, 4/10 and 3/16 dams in the 7.5, 10 and 15 mg/kg dose groups, respectively). Body weight gain in the dams was significantly reduced during the treatment period in all dose groups, but thereafter (from day 15 to day 21 of pregnancy) was within the range of control values in the 7.5 and 10 mg/kg dose groups. Only in the 15 mg/kg dose group (vehicle NaCl) was body weight gain significantly increased during this time. The numbers of corpora lutea and implantations/dam, the number of live foetuses/litter and the percentage post-implantation loss were within the range of control values in all dose groups. Foetal weights were significantly reduced in all dose groups. The occur-

rence of skeletal variations (including supplementary ribs and asymmetric sternebrae) was significantly increased in the 10 and 15 mg/kg dose groups; ossification of the sternebrae and tail vertebrae was significantly delayed in the 15 mg/kg dose group (vehicle NaCl). The malformation rate in all dose groups was within the range of control values (Giavini et al., 1986a). All doses administered (15 to 20 mg/kg body weight/day) caused severe maternal toxicity and embryotoxicity, but no teratogenicity.

In a further experiment, in which two groups of 15 and 11 CD-rats were given 2-(1-methylpropyl)-4,6-dinitrophenol in the diet at 200 ppm daily from days 6 to 15 of pregnancy (equivalent to 17.9 and 18.7 mg 2-(1-methylpropyl)-4,6-dinitrophenol/kg body weight/day, determined from the average daily feed consumption), body weight gain was significantly reduced in the dams during the treatment period in both dose groups, but was then significantly increased from days 15 to 21 of pregnancy (when laparotomy was performed). Foetal weights were significantly reduced compared with controls, the numbers of corpora lutea and implantations/dam, the numbers of resorptions and live foetuses/litter and the percentages of dams with resorptions and post-implantation losses were within the range of control values. In the treated groups there were significant increases in the numbers of foetuses with visceral malformations (unilateral and bilateral microphthalmia in 9 to 17% of foetuses; controls 0%) and skeletal variations (supplementary ribs in 37 to 90% of foetuses; controls 7 to 9%; Giavini et al., 1986a, b). 2-(1-Methylpropyl)-4,6-dinitrophenol administered in the feed (at 17.9 and 18.7 mg/kg body weight/day) therefore caused maternal toxicity, embryotoxicity and teratogenic effects.

Pregnant Sprague-Dawley rats (6/group) were given 2-(1-methylpropyl)-4,6-dinitrophenol (95% pure) in the diet at concentrations of 0 (controls), 50, 100, 150, 200, 250, 300 and 350 ppm from days 6 to 15 of pregnancy. These concentrations corresponded to daily ingested doses of 3.26, 6.90, 9.23, 10.86, 9.38, 9.49 and 8.60 mg 2-(1-methylpropyl)-4,6-dinitrophenol/kg body weight/day, as concentrations above 200 ppm in the diet reduced the actual intake of the substance and gave rise to signs of toxicity (ataxia, lethargy). In some of the rats in the control and dose groups, a laparotomy was carried out on day 12 of pregnancy, while the remaining animals concluded their pregnancies (no information on numbers of animals in either case). Body weight gain was significantly reduced compared with controls at 150 ppm and above. Embryo mortality was significantly in-

creased on day 12 of pregnancy, concentration dependently from 200 ppm in the diet, and was 100% in the 350 ppm group. The percentage of live foetuses/litter at birth was concentration dependently significantly reduced from 150 ppm. The birth weights of the foetuses were significantly decreased in the 200 ppm group, with higher concentrations not being evaluated. Shortened tails were found in 8 of 62 foetuses in the 200 ppm group. Biochemical analysis of the placenta (which was undertaken in a second experiment on day 16 of pregnancy in 6 rats/concentration and control group) revealed a concentration-dependent significant reduction in glycogen and protein levels compared with the controls from a concentration of 200 ppm. The protein content of the ovaries was significantly reduced at 200 ppm and above. The accumulation of a yellow fluid was observed in the fatty tissue surrounding the ovaries and uterus (Spencer, 1981; Spencer and Sing, 1982). Thus the maternal and foetal no effect level in this study was 100 ppm, equivalent to 6.9 mg/kg body weight/day.

In a supplementary study by the same investigators on the effect of 2-(1-methylpropyl)-4,6-dinitrophenol on the placenta, Sprague-Dawley rats were given 2-(1-methylpropyl)-4,6-dinitrophenol (95% pure) at doses of 1.6, 3.6, 4.9, 8.9, 12.8, 13.8 and 24.3 mg/kg body weight/day in the diet from days 6 to 15 of a false pregnancy (induced by mechanical stimulation of the cervical region of the uterus). On day 10 of the false pregnancy, the absolute and relative uterus weights were dose-dependently reduced from 8.9 mg/kg, significantly at the highest dose level; the glycogen and protein levels in the uterus tissue were significantly reduced dose-dependently from 1.6 mg/kg and the ovarian protein level was reduced from 13.8 mg/kg. Histopathological examination revealed a yellow discoloration of the uterus and its mesenteric adipose tissue in the rats at the top two dose levels. Marked signs of toxicity (ataxia and lethargy) occurred in the rats at the top dose level during the study, while the rats in all other dose groups showed less marked symptoms (Spencer, 1981; Spencer and Sing, 1982).

- **Intraperitoneal injection**

Pregnant Sprague-Dawley rats were given 6.3, 8.0, 9.0, 11.2, 12.5 and 15.8 mg 2-(1-methylpropyl)-4,6-dinitrophenol/kg body weight/day from days 10 to 12 of pregnancy by intraperitoneal injection, with 4 to 16 pregnant rats used per dose group. A control group consisted of 12 pregnant rats. In some of the rats in the control and dose groups, a laparotomy was carried

out on day 21 of pregnancy, while the remaining animals concluded their pregnancies and the offspring were observed until at most day 42 of life (no information on numbers of animals in any case). In the three highest dose groups (11.2, 12.5 and 15.8 mg/kg) all of the pregnant rats died within a week of the end of treatment. The mortality rate in the 9.0 mg/kg dose group was ca. 20% (no data on maternally toxic effects). The number of live foetuses and the percentage of resorbed and dead foetuses in the remaining three dose groups (6.3, 8.0 and 9.0 mg/kg) were within the control range. There was a dose-dependent significant reduction in foetal weights in both high dose groups (8.0 and 9.0 mg/kg) and in foetal length at 9.0 mg/kg. The relative weights of the liver, kidneys, heart and lungs of the foetuses remained unaffected. The liver cells of foetuses from dams given 8.0 and 9.0 mg/kg were vacuolised and in ca. 40% of these foetuses dilation of the kidney pelvis, renal tubules and/or ureter was evident, mesenteric tissue was predominant in the kidney and the transitional epithelium of the ureter was vacuolised. Body weight in 1- and 7-day-old offspring from dams in the highest dose group remaining in the study (9.0 mg/kg) was significantly reduced, while in 42-day-old offspring it was in the control range. The relative liver and kidney weights in 1-, 7- and 42-day-old offspring from dams in the 9.0 mg/kg dose group were unaffected. Urine parameters (sodium and potassium levels, osmolality and output) were in the control range in the 42-day-old offspring of dams in the 9.0 mg/kg dose group. Histopathological examination of 1-day-old pups from dams in the 9.0 mg/kg dose group revealed similar effects on the kidneys and ureter to those seen in the foetuses. In addition, vacuolisation and necrosis of the liver was observed in 42-day-old offspring, and 3 of the 28 offspring of dams in the 9.0 mg/kg dose group showed dilated kidney pelves. Tests of kidney function in vitro in 1- and 42-day-old offspring from dams in the 9.0 mg/kg dose group gave no indications of functional disturbances, the para-aminohippuric acid (PAH) level and the N-methylnicotinamide content of the kidney cortex being within the range of control values. Tests of kidney function in vivo (PAH clearance and inulin clearance) also gave no indications of functional disturbances in 42-day-old offspring of dams in the 9.0 mg/kg dose group (McCormack et al., 1980).

In order to study the relevance of morphological and functional effects on the kidney and urinary tract found by McCormack et al. (1980; see above), extensive follow-on studies were carried out. Pregnant Sprague-Dawley

rats (11 to 13/group) were given 2-(1-methylpropyl)-4,6-dinitrophenol (97.3% pure) in preliminary studies at doses of 4.5, 6.0, 7.5, 9.0, 12.0 and 18.0 mg/kg body weight/day by intraperitoneal injection on days 10 to 12 or 11 to 13 of pregnancy. A control group consisting of 46 pregnant rats was given the vehicle (NaCl) by intraperitoneal injection on days 10 to 13 of pregnancy. A laparotomy was performed on day 21 of pregnancy. Between 3 and 5 litters/dose were evaluated (13 litters in the controls). Dose-dependent increases in mortality occurred from 9.0 mg/kg and all of the pregnant rats in the top dose group died. The number of implantations/dam, the number of live foetuses/litter, the percentages of dead and resorbed foetuses per litter and the developmental stage of the kidney papillae of the foetuses were similar to the controls. The percentage of foetuses with severely dilated kidney pelves was significantly increased in the 6.0 and 12.0 mg/kg dose groups (days 10 to 12 of pregnancy). Average foetal weights were significantly reduced in the 7.5, 9.0 and 12.0 mg/kg dose groups (days 10 to 12 of pregnancy) and in the 7.5 and 9.0 mg/kg dose groups (days 11 to 13 of pregnancy; Daston et al., 1988).

To study the reversibility of the morphological and functional effects on the urinary tract caused by 2-(1-methylpropyl)-4,6-dinitrophenol in maternally toxic doses, the substance was given to pregnant Sprague-Dawley rats by intraperitoneal injection at doses of 8 and 10.5 mg/kg body weight/day. Maternal mortality was 4% (1/27 rats) and 47% (9/19 rats) in the 8.0 and 10.5 mg/kg dose groups, respectively. Body weights and absolute and relative kidney weights in the F_1 generation were within the control range on day 2 and day 15 of life. On day 30 of life, the body weights of the F_1 generation in the low and high dose groups were significantly increased and decreased, respectively, compared with the controls. The relative kidney weights of the F_1 generation were significantly increased on day 30 of life in the high dose group. There were only a few cases of dilation of the kidney pelves in the F_1 generation on day 31 of life (2 and 5% in the 8.0 and 10.5 mg/kg dose groups, respectively, controls 5%). Serum analysis (levels of sodium, potassium, urea-nitrogen, creatinine and total protein, creatinine clearance and urea nitrogen clearance) was only undertaken in the F_1 generation in the low dose group on day 30 of life and revealed no treatment-related effects. Urine analysis (osmolality, volume, pH level and excretion of chloride, sodium and potassium) revealed a dose-dependent significant reduction in urine volume in the F_1 generation in both dose

groups on day 2 of life, whereas on day 15 of life the urine volume did not differ from that in the controls. Osmolality was in the range of control values on days 2 and 15 of life. The pH of the urine in the F_1 generation in the high dose group was significantly reduced on day 2 of life, while on day 30 of life there were no treatment-related effects on urine parameters. There was a significant increase in chloride excretion in the urine after 24 hours of water deprivation in the F_1 generation of the high dose group on day 30 of life. On day 6 of life the ability to concentrate urine (test parameters: urine volume, osmolality) 1 and 2 hours after subcutaneous administration of desmopressin (a vasopressin analogue) was significantly inhibited in the F_1 generation of both dose groups. The ability to concentration urine was also inhibited on day 14 of life in the F_1 generation of the high dose group, although on day 30 there were no treatment-related effects on the ability to concentrate urine in the F_1 generation of either dose group. A functional test of the proximal kidney tubules, carried out on days 3, 17 and 30 of life in the F_1 generation of the low and high dose groups, revealed a significant decrease in the para-aminohippuric acid (PAH) level in the kidney cortex of the F_1-generation of the low dose group on day 30 of life. On days 3 and 30 of life, the PAH-content of the kidney cortex was significantly increased in the F_1 generation of the high dose group. In their discussion the investigators concluded that the morphological and functional changes observed at maternally toxic doses in the kidneys and urinary tract were reversible by day 42 of post-natal development (Daston et al., 1988).

Pregnant Wistar rats (5 and 6 animals) were given 2-(1-methylpropyl)-4,6-dinitrophenol at a dose of 10 mg/kg body weight as a single intraperitoneal injection on days 9 and 10 of pregnancy, and then ca. 10 embryos/dam were then removed 24 and 4 hours, respectively, after administration. Their survival rate was studied in an incubator at 37 °C. The mortality of the embryos after 24 and 42 hours was similar to that in the controls. The percentage of embryos with closed neural tubes and successful axial rotation was, however, significantly lower compared with the controls after 24 hours. There were no effects on the number of somites or on the growth of anterior limb buds (Beaudoin and Fisher, 1981).

Teratogenicity studies in mice

In a screening test in which pregnant CD1-mice were given 15 mg 2-(1-methylpropyl)-4,6-dinitrophenol/kg body weight by gavage daily from

days 8 to 12 of pregnancy, body weight gain in the dams and the survival rate and body weight gain in the pups until 3 days after birth were in the control range. The dose was equivalent to the minimal maternally toxic dose determined in a preliminary study, in which one of the 23 mice in the dose group died (controls 0/24; Chernoff and Kavlock, 1982, 1983).

CD-1 mice were given 26 or 33 mg (97% pure) 2-(1-methylpropyl)-4,6-dinitrophenol/kg body weight as a single dose by gavage on day 8 of pregnancy. The treated groups consisted of 20 and 40 mice, respectively, the control group 15 mice. A laparotomy was carried out on day 18 of pregnancy. The high dose was in the maternally toxic range, mortality being 5%. None of the animals in the low dose or control groups died. In the high dose group, body weight gain in the dams and the number of litters with live foetuses were reduced by ca. 20 and 27%, respectively, compared with controls, although the values were still within the distribution range of the controls. Resorption of an entire litter was found in only one dam in the high dose group (no data on further effects indicative of maternal toxicity). In the foetuses, pre-natal mortality, body weight and the numbers of sternal and caudal ossification centres and enlarged cerebral ventricles were within the range of control values. Malformations (encephalocele, exencephaly, various skull defects and umbilical hernia) seen in 2 of 181 (1.1%) and 4 of 223 (1.8%) of the foetuses examined in the low and high dose groups, respectively, were not considered to be treatment-related. Malformations occurred in 12 of 1682 foetuses examined (0.7%) in the control group. The occurrence of supernumerary lumbar ribs was significantly increased in the foetuses in both dose groups compared with the controls, by 34% in each case (Kavlock et al., 1985). Thus, while the no effect level for maternal toxicity was 26 mg/kg body weight, this dose was associated with embryotoxic effects.

Pregnant Swiss Webster mice (4 to 14/dose) were given 2-(1-methylpropyl)-4,6-dinitrophenol during the entire (days 8 to 16 of pregnancy), early (days 11 to 12) or late (14 to 16) period of organogenesis, by the oral, intraperitoneal and subcutaneous routes. Control mice (5 to 8 animals) were treated in the same way with the vehicle, water. Laparotomy took place on day 19 of pregnancy. The following parameters were determined: numbers of live and dead foetuses, foetal weights, crown-rump length of the foetuses, external, visceral and skeletal malformations, resorptions and implantations. 2-(1-Methylpropyl)-4,6-dinitrophenol had no effects on the development of the embryo or the foetus when administered at 5 mg/kg

body weight/day by intraperitoneal injection, 10 mg/kg body weight/day by subcutaneous injection or 20 mg/kg body weight/day orally on days 8 to 16 of pregnancy. Doses of 17.7 mg 2-(1-methylpropyl)-4,6-dinitrophenol/kg body weight/day by intraperitoneal injection on days 10 to 12 or 14 to 16 of pregnancy gave rise to maternal toxicity (hyperthermia) and embryotoxicity (a significant increase in resorptions and a significant decrease in foetal length); teratogenic effects (such as significantly increased occurrences of anal atresia, acaudia, amelia, oligodactyly, absence of bones in the extremities and hydronephrosis) were evident on intraperitoneal injection of 17.7 mg/kg body weight/day on days 10 to 12 of pregnancy. 2-(1-Methylpropyl)-4,6-dinitrophenol caused maternal and embryotoxicity but not teratogenicity when administered by subcutaneous injection on days 14 to 16 of pregnancy at a dose of 17.7 mg/kg body weight/day. The oral administration of 2-(1-methylpropyl)-4,6-dinitrophenol during early or late organogenesis at doses of 20 and 32 mg/kg body weight/day was maternally toxic, but not embryotoxic or teratogenic (Gibson, 1973).

Two groups of pregnant Swiss Webster mice (20 to 36 animals) were given 2-(1-methylpropyl)-4,6-dinitrophenol at doses of 14.1 and 15.8 mg/kg body weight/day on days 10, 11 and 12 of pregnancy by intraperitoneal injection. Some of the mice in each dose group had their feed (but not their drinking water) withdrawn from day 9 of pregnancy for 24 or 48 hours. Corresponding controls (7 to 14 mice) were treated with the vehicle, dilute sodium hydroxide neutralised with hydrochloric acid, by intraperitoneal injection. A laparotomy was carried out on day 19 of pregnancy. No dams died in either the control or treated groups (no further details). The proportion of resorbed and dead foetuses/litter was similar to the corresponding control values for both dose groups with and without feed withdrawal. There were no effects on foetal weight in either dose group without feed withdrawal, while with feed withdrawal, there was a significant reduction in foetal weight compared to the corresponding control group. In the low dose group there were no indications of an increased incidence of external or visceral malformations with or without feed withdrawal. In the high dose group with and without feed withdrawal, the incidence of external malformations (including amelia or micromelia, ectrodactyly or brachydactylia, club foot, acaudia and microcaudia) was significantly increased, while the incidence of visceral malformations (hydronephrosis, ectopic kidneys, internal hydrocephalus) was only increased significantly in the high dose group with 24-hour feed

withdrawal. The frequency of skeletal variations and malformations (absent or unossified toe bones, large toes or sternebrae; absence or shortening of tibia, fibula, femur, ischium or pubis; fused ribs or vertebrae, incomplete vertebrae, split vertebrae or sternebrae) was significantly increased in both the low and high dose groups with and without feed withdrawal, with the percentage of foetuses with skeletal variations and malformations in the group with feed withdrawal being higher compared with the incidence in the same dose group without. The investigators did not differentiate between skeletal variations and malformations (Preache and Gibson, 1974, 1975a). The embryotoxic and teratogenic effects of 2-(1-methylpropyl)-4,6-dinitrophenol were thus increased by temporary withdrawal of feed, but were not qualitatively changed.

A single intraperitoneal injection of 17.7 mg 2-(1-methylpropyl)-4,6-dinitrophenol/kg body weight on day 11 of pregnancy or 18.8 mg /kg body weight on day 12 of pregnancy caused no maternal mortality in pregnant Swiss-Webster mice. A laparotomy was carried out on day 19 of pregnancy. Each dose and control group consisted of 6 to 7 pregnant mice. The number of resorptions/litter was significantly increased and the foetal weights were significantly reduced in the 18.8 mg/kg dose group. The frequency of occurrence of external malformations in the foetuses in both dose groups was in the range of that in the controls, while the occurrence of visceral malformations was significantly increased (no further details; Preache and Gibson, 1975a).

Pregnant Swiss-Webster mice (3 to 6/group) were given intraperitoneal injections of 2-(1-methylpropyl)-4,6-dinitrophenol at doses of 0 (controls), 6.3, 7.5, 10.0, 11.7, 15.8 or 17.7 mg/kg body weight on days 10, 11 and 12 of pregnancy. After dosing, the mice were kept either for 2 hours at 6 °C, 24 hours at 32 °C or at room temperature (23 to 25 °C). Laparotomy was performed on day 19 of pregnancy and the foetuses were examined. When the mice were kept at 32 °C the maternal mortality was increased at 7.5 mg/kg body weight, and was 100% at 10.0 mg/kg body weight and above. In the 7.5 mg/kg group the body weights of the foetuses were below those in the controls and there was an increased incidence of visceral malformations (particularly hydronephrosis and hydrocephalus). In addition, ossification was delayed. In contrast, a short period at 6 °C did not change the number of malformations compared with being housed at room temperature (Preache and Gibson, 1975b).

Teratogenicity studies in rabbits

Groups of 15 and 16 Chinchilla rabbits (Kfm: CHIN, hybrids; initial weights 2.36 to 2.78 kg) were given 98.1% pure 2-(1-methylpropyl)-4,6-dinitrophenol in corn oil by gavage from days 6 to 18 of pregnancy at doses of 0 (controls), 1, 3 or 10 mg/kg body weight/day (OECD guideline No. 414). Laparotomy was carried out on day 28 p.c. No clinical signs of toxicity or deaths occurred during the study. There were no signs of maternal toxicity or foetotoxicity or teratogenic effects in the two lower dose groups. There were also no signs of maternal toxicity in the 10 mg/kg group, but malformations were evident in a total of 40 of 122 foetuses (32.8%) in 11 of 16 litters. In 26 foetuses (21.3%), multiple malformations were found, 11 (9%) had only malformations of the cephalic viscera and 4 had only skeletal malformations. The main effects were dyscrania linked to hydrocephalus internus and micro- and anophthalmia. Based on these results the no observed effect level for dams and foetuses was given as 3 mg/kg body weight (RCC, 1986b).

Groups of 16 to 17 pregnant New Zealand rabbits were treated with 2-(1-methylpropyl)-4,6-dinitrophenol (purity unspecified) which was applied to the clipped skin of their backs at doses of 1, 3, 9 or 18 mg/kg body weight/day from days 7 to 19 of pregnancy. The duration of exposure was 6 hours/day, after which the application site was washed (with 50% alcohol) and dried. Each animal had 7 application sites which were used in rotation so that no site was used more than twice. Ingestion of the test substance was prevented by fitting the animals with ruff collars. The highest dose (18 mg/kg body weight/day) was dropped from the study because of high maternal toxicity (no further details). Laparotomy was carried out the day before birth was expected. Feed intake was reduced dose-dependently during treatment (from days 7 to 16 of pregnancy), thereafter being similar to or slightly under that in the controls (no further details). Body temperature was dose-dependently increased from 3 mg/kg body weight/day. Mortality rates were ca. 19 and 71% in pregnant rats in the intermediate (3 mg/kg) and high (9 mg/kg) dose groups, respectively (controls and low dose groups ca. 13%). The number of implantations/dam and the average foetal weights were in the control range for all dose groups. The number of litters with live foetuses was reduced in the high dose group (3 compared with 10 in the controls) and the average percentage of dead and resorbed foetuses/litter was increased (51.4%; controls 11.2%). External malforma-

tions (anophthalmia in 1%, hydrocephalus in 2%) and abnormal tail vertebrae (in 1%) occurred in live foetuses in the intermediate dose group, while in live foetuses in the top dose group there were also cleft palates (in 10%), microencephaly (in 10%), microphthalmia (in 5%) and asymmetric thoracic vertebrae (in 20%; controls: microphthalmia in 2%, asymmetric thoracic vertebrae in 2%). The no effect level for maternal toxicity, foetotoxicity and teratogenic effects was 1 mg/kg body weight/day (Johnson et al., 1988).

Groups of 18 Chinchilla rabbits (Kfm:CHIN, hybrids; initial weights 2.52 to 3.43 kg) were treated with 97.2% pure 2-(1-methylpropyl)-4,6-dinitrophenol in sesame oil, which was applied to the clipped skin on their backs at doses of 0 (controls), 3, 10 or 30 mg/kg body weight from days 6 to 18 of pregnancy for 6 hours/day under occlusive cover (OECD guideline No. 44). Laparotomy was performed on day 28 p.c. Neither systemic nor local treatment-related effects were observed in the dams. Values for body weight gain, feed consumption, numbers of implantations and live and dead foetuses, foetal weights and sex ratios were within the range of control values in all dose groups. There were no treatment-related effects at post-mortem. Malformations were present in 24 of 127 foetuses (18.9%) in the high dose group in 7 of 17 litters, with 15 foetuses (11.8%) showing various malformations. In 9 foetuses (7.1%), malformations of the cephalic viscera, hydrocephalus internus and encephalocele were observed, often linked with holes in the parietal bones (9.4%) and/or micro- or anophthalmia (6.2%). Cleft palates were detected in two foetuses and encephalocele in one. Based on these results, the no observed effect level for foetuses in this study was 10 mg/kg body weight/day (RCC, 1987).

Multi-generation studies

In a 3-generation study, groups of 25 male and 25 female Sprague-Dawley rats in three generations (F_0, F_1, F_2) were given 98% pure 2-(1-methylpropyl)-4,6-dinitrophenol in the diet for 29 weeks (total study duration 87 weeks) at concentrations equivalent to daily doses of 0 (controls), 1, 3 or 10 mg/kg body weight. The parent generations were each fed the appropriate diet for 13 weeks, and then mated twice while undergoing further treatment (2 times 2 weeks of mating, 3 weeks gestation and 3 weeks lactation). The pups from the first mating were observed until weaning and then killed, those of the second mating were reared. A proportion of the dams from

each group and generation were killed 21 days after the second mating and the foetuses examined for teratogenic effects. Post-natal development was studied in the litters of 5 dams each from the F_0 and F_1 generations. From each of these litters 2 males and 2 females were observed untreated for 3 months for the study of the later stages of development. Of the offspring of the remaining females from each generation, 25 males and 25 females/group were used as parents for the next generation. The parents of the F_0, F_1 and F_2 generations were killed after the second litters were weaned, and a histopathological examination was carried out in 10 males and 10 females from the F_2 generation. Groups of 5 male and 5 female rats from the second litters of the F_2 generation were also histopathologically examined after weaning. There was a significant reduction in body weight gain at the 10 mg/kg body weight/day dose in all generations in male and female rats. Yellow discoloration of the fur was observed in the high dose group in all generations, an effect which was attributed to direct contact with the test substance in the feed. 2-(1-Methylpropyl)-4,6-dinitrophenol did not affect mortality, macroscopic effects, fertility, fecundity or the reproductive indices studied. The number of offspring in the F_0 and F_1 generations of the 10 mg/kg body group was slightly below that in the controls. Body weight gain of the pups was below that of the controls in the high dose group, an effect of which there were also suggestions in both lower dose groups. Development, survival rates, sex ratio, clinical symptoms and macroscopic effects did not differ between the offspring of treated and control rats. The numbers of corpora lutea and foetuses/dam were reduced in the 10 mg/kg group in all three generations, with average foetal weights also being reduced in the F_2 generation, the foetuses showing delayed skeletal ossification. Histological examination of the parents and their young revealed no treatment-related effects (Hazleton, 1981a).

Subsequent to the 3-generation study described above, two further generations (F_3 and F_4) were investigated. Groups of 25 male and 25 female rats were given 98% pure 2-(1-methylpropyl)-4,6-dinitrophenol for 22 weeks in the diet (total study duration 44 weeks) at concentrations that also corresponded to daily doses of 0 (controls), 1, 3 or 10 mg/kg body weight. The F_3 and F_4 generations received the diets for 14 weeks prior to mating and during mating, gestation and lactation (8 weeks). Histopathological examinations were made in 10 male and 10 female parent rats of the F_3 and F_4 generations and 5 male and 5 female offspring of the F_4 generation. Ex-

cept for yellow discoloration of the fur in all rats in the high dose group there were no clinical signs of toxicity or deaths. Body weight gain was significantly reduced at the high dose in both sexes in both generations. There were no effects on fertility, fecundity, mating behaviour, pregnancy indices or macroscopic and microscopic effects. The number of offspring in the 10 mg/kg group was significantly below that in the controls in the F_3 generation and slightly but not significantly under that in the controls in the F_4 generation (Hazleton, 1981b).

7.9 Effects on the immune system

No information available.

7.10 Neurotoxicity

In rats (n=5) which were given 2-(1-methylpropyl)-4,6-dinitrophenol as a single dose of 6.66 mg/kg body weight by intraperitoneal injection, the acetylcholine content of the cerebral cortex and the brain stem was significantly increased 2 hours after dosing, while acetylcholine synthesis was significantly inhibited compared with controls (n=26; vehicle olive oil, intraperitoneal; Gorny and Miszczak, 1981).

The locomotor activity of individuals and groups of male Swiss mice (20 to 25 g) was studied over a period of 4 hours immediately after the administration of single doses of 2-(1-methylpropyl)-4,6-dinitrophenol of 20, 200 and 2000 mg/kg body weight (dermally) and 0.4, 4.0 and 40 mg/kg body weight (orally). For each dose and control group 30 mice were used to assess group activity, while to assess individual activity after dermal administration 10 mice/dose and control group were employed. The mice in the control groups were treated with the vehicle, xylene, by the dermal or oral routes. Locomotor activity was significantly increased dose-dependently from 200 mg/kg in grouped subjects after dermal application and from 4 mg/kg after oral administration. Locomotor activity was similarly dose-dependently increased in individual subjects after dermal exposure, significantly in the high dose group compared with the controls (Mitchell et al., 1989).

7.11 Other effects

The effects of 2-(1-methylpropyl)-4,6-dinitrophenol are based on uncoupling of cell respiration and phosphorylation (respiratory-chain phosphorylation). However, because biological oxidation is not inhibited in the process, heat is produced in place of energy-rich phosphate compounds. In this way an excess of adenosine diphosphate and inorganic phosphate arises, cell respiration is increased to a maximum and glycolysis takes place under aerobic conditions. This results in an increase in the basal metabolic rate with an increase in temperature and weight loss (no further details; DFG, 1990).

When single intraperitoneal injections of 15, 24 or 36 mg 2-(1-methylpropyl)-4,6-dinitrophenol/kg body weight were given to pairs of male mice, the administration of 24 mg/kg body weight and above caused dose-dependent partial or complete uncoupling of oxidative phosphorylation in the mitochondria of liver and brain cells after 20 minutes. At these doses, severe signs of toxicity occurred that were dose-dependent (no further details; Ilivicky and Casida, 1969).

The minimum effective concentrations leading to uncoupling of respiratory-chain phosphorylation in the mitochondria of isolated cells from mouse liver or brain in vitro were 1.0 and 0.5 µmol/l, respectively (equivalent to 240.24 and 120.12 µg/l at 25 °C; Ilivicky and Casida, 1969).

Freshly isolated hepatocytes from male Wistar rats were incubated with 2-(1-methylpropyl)-4,6-dinitrophenol at concentrations of 1, 5 or 10 µM for 3 hours at 37 °C. 1 µM was cytotoxic to 30% of the cells and 10 µM to 65%. At 10 µM there was a slight fall in the intracellular glutathione concentration (from 21 to 16.5 nmol/10^6 cells) and a fall in the intracellular adenosine triphosphate level (from 11.7 to 6.9 nmol/10^6 cells). A 2-hour incubation with 10 µM reduced the NADH level from 2.0 to 0.5 nmol/10^6 cells. This concentration also induced an increase in thiobarbituric acid reactive substances (lipid peroxidation) from 6.4 nmol/10^6 cells to 9.2 nmol/10^6 cells after incubation for 3 hours. There was a significant decrease in protein-bound thiol groups by ca. 20% after an incubation period of 3 hours at concentrations of 5 and 10 µM (Palmeira et al., 1994a, 1995a).

2-(1-Methylpropyl)-4,6-dinitrophenol (concentrations used 50 to 500 nM) inhibited the activity of succinate dehydrogenase by 10% and that of cyto-

chrome c reductase by ca. 20% in the mitochondria from the livers of male Wistar rats. The activity of cytochrome c oxidase was unaffected. The substance caused an increase in oxygen consumption in the mitochondria, stimulated ATPase activity, induced increased permeability of the mitochondrial membrane to H^+ and suppressed membrane potential in a concentration-dependent manner (Palmeira et al., 1994b)

The incubation of hepatocytes from male Wistar rats with 5 µM 2-(1-methylpropyl)-4,6-dinitrophenol resulted in time-dependent loss of viability by 20%. The exposure of hepatocytes to the substance had no effect on the intracellular Ca^{2+} level, but there was a marked concentration-dependent decrease in the calcium concentration in response to vasopressin after a 60-minute incubation. 2-(1-Methylpropyl)-4,6-dinitrophenol also inhibited the stimulation of inositol phosphate formation in response to vasopressin (Palmeira et al., 1995b).

The repeated subcutaneous administration of 24 mg 2-(1-methylpropyl)-4,6-dinitrophenol/kg body weight to rats (n=20) every third day for a month led to a decrease in succinate dehydrogenase activity and an increase in the activity of cytochrome oxidase in the brain (Pallade and Goldstein, 1963).

In male Swiss mice (20 to 25 g), single doses of 2-(1-methylpropyl)-4,6-dinitrophenol of 20, 200 or 2000 mg/kg body weight applied dermally or 0.4, 4.0 or 40 mg/kg body weight given orally led to a dose-dependent significant taste aversion to 0.3% saccharin solution from 20 mg/kg (dermally) and 4.0 mg/kg (orally) 24 hours after dosing. For both routes, 8 mice were used per dose and control group. The mice in the control group were treated with the vehicle, xylene, which was administered either dermally or orally. After oral administration, mortality in the top dose group was 10% (Mitchell et al., 1989).

In male WAG/MBL rats, a single intraperitoneal injection of 0.03 mM 2-(1-methylpropyl)-4,6-dinitrophenol/kg body weight (equivalent to 7.2 mg/kg body weight) caused a significant decrease in the thyroxine (T_4) level in the plasma by 40%, while the triiodothyronine (T_3) level remained unchanged (Van den Berg et al., 1991).

In vitro, 100 µM 2-(1-methylpropyl)-4,6-dinitrophenol (equivalent to 24 mg/l) led to a 71 to 100% displacement of the thyroid hormone thyroxine (T_4) from the binding sites of the carrier transthyretin (Van den Berg et al., 1991).

On incubation of bovine erythrocytes with 2-(1-methylpropyl)-4,6-dinitrophenol or its metabolite 6-amino-4-nitro-2-sec-butylphenol at 39 °C, for 6 and 4 hours, respectively, only the metabolite induced methaemoglobin formation (Fröslie, 1971).

The effects of 2-(1-methylpropyl)-4,6-dinitrophenol on isolated guinea pig hearts was investigated with the method of Langendorff. The substance was shaken with Ringer's solution (which was used to perfuse the heart), at a ratio of 1:100 at room temperature, and a filtrate was obtained. Further dilutions of the filtrate were added to the hearts (10^{-6}, 10^{-5}, 10^{-4} etc.) in 0.1 ml aliquots. 1:1000 and higher dilutions led to an increase in coronary perfusion. After an initial acceleration, the heart rate decreased until it stopped almost completely, and then slowly recovered (Hoechst, 1958).

Pairs of rabbits were given oral doses of 20 mg 2-(1-methylpropyl)-4,6-dinitrophenol, and their body temperature was then recorded for 8 hours. A maximum increase of 0.2 °C in body temperature was seen in only one animal, and this was evaluated as an extremely slight increase in oxidative processes in the body (Hoechst, 1958).

In mice, 20 mg 2-(1-methylpropyl)-4,6-dinitrophenol given orally increased oxygen consumption by 23% (Hoechst, 1958).

8 Experience in humans

Patch tests were carried out with 20 different pesticides in 305 workers (age 31 to 50 years) who were employed in production, packaging and storage of pesticides in three chemical factories; 7 subjects (2.3%) gave positive reactions to 2-(1-methylpropyl)-4,6-dinitrophenol (1% solution in naphtha; no further details; Chodorowska et al., 1993).

Frequent vomiting and tiredness occurred in an agricultural worker 5 days after he had been employed in treating fields with the herbicide Nitrador 40, consisting of 40% dinitro-ortho-cresol and Dinorsol PL (14% 2-(1-methylpropyl)-4,6-dinitrophenol, no further details). On the sixth day severe spasms occurred with thirst and tachycardia. The worker died in hospital on the seventh day. An autopsy revealed no histopathological effects. 2-(1-Methylpropyl)-4,6-dinitrophenol could not be detected by thin layer chromatography in the skin or subcutaneous fatty tissue (hand, foot), the liver, stomach

and gut contents, kidneys or lungs; an unidentified metabolite was found in the urine. 2-(1-Methylpropyl)-4,6-dinitrophenol and dinitro-ortho-cresol were detected on the work clothes (overall, cap), the non-specific breathing mask and the insides of the rubber gloves that the worker had worn during herbicide application (Heyndrickx et al., 1964).

In a further case study, an agricultural worker showed non-specific signs of toxicity (headache, malaise, lassitude, sweating) on the same day as fields were sprayed with a contact herbicide which contained 2-(1-methylpropyl)-4,6-dinitrophenol (concentration unspecified). His fingers were stained yellow because of direct skin contact with the herbicide during an operation to repair the spraying nozzle of the agricultural machinery in the field without hand protection. Pyrexia was evident (38.7 °C) on the day after the spraying operation. Effects occurring over the next 5 days included anorexia, bouts of excessive sweating alternating with shivering fits, pains in the abdomen and chest, excessive thirst (9 l water in one night), restlessness, insomnia, weight loss (10 kg in a week), jaundice of the skin and sclera, loose stools, dyspnoea, haemoptysis and personality changes. On the sixth day the man was admitted to hospital. At this point he showed the following clinical symptoms: pyrexia (39.8 °C), dyspnoea, spasmodic coughing, dullness at the base of one lung, crepitations, and photophobia with neck stiffness, and a positive Kernig's sign. The urine was discolored yellow, but no dinitro-compounds could be detected in the blood. Liver function was impaired and lung function tests revealed reductions in forced expiratory volume in one second and in vital capacity. X-rays showed patchy shadows at the base of the lungs. The clinical symptoms resolved after a week in hospital, with only the liver function remaining impaired. About 2 weeks later the patient still complained of lethargy, night sweats and absent-mindedness. After a further 10 to 12 weeks he was completely free of symptoms. However, the blood urea level was still elevated 6 months after intoxication (Smith, 1981).

A 2.5-year-old child became unconscious immediately after the accidental ingestion of a fluid containing 2-(1-methylpropyl)-4,6-dinitrophenol (no further details). On admission to hospital, generalised spasms and hyperthermia occurred and the child subsequently died of heart failure. Macroscopic and histopathological examination revealed yellow staining around the mouth, yellow discoloration of the hands and meninges and the mucous membranes of the airways, oesophagus and stomach, and degenera-

tive damage to the liver cells at the margins of the liver lobes. 2-(1-Methylpropyl)-4,6-dinitrophenol was detected in the brain, liver and stomach contents at concentrations of 0.02, 20 and 1700 µg/g (no further details; Vycudilik et al., 1986).

A 50-year-old man died after intentionally consuming small amounts of the pesticide Gebutox (24% 2-(1-methylpropyl)-4,6-dinitrophenol, 40% methanol, 16% triethanolamine, 20% water) over several months. After death occurred the official post-mortem revealed localised yellow discoloration around the nostrils and on the hands, yellow discoloration of the conjunctiva, yellow-brown stomach contents with an aromatic smell, numerous haemorrhages of the gastric mucosa, swelling of the lungs and brain and liquid blood in the corpse. Substantial amounts of 2-(1-methylpropyl)-4,6-dinitrophenol were detectable in the body (in the blood, liver and stomach contents; no further details; Grabner et al, 1967).

9 Classifications and threshold limit values

2-(1-Methylpropyl)-4,6-dinitrophenol has been legally classified by the European Union in the TRGS 905 and placed in category R_D2 of substances toxic to reproduction (i. e. “substances which should be regarded as if they cause developmental toxicity to humans”) and in category R_F3 of substances toxic to reproduction (i. e. “substances which cause concern for human fertility”; TRGS 905, 2000).

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