

## DIMETHIPIN

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### Explanation

Dimethipin was first evaluated by the 1985 Joint Meeting (Annex 1, reference 44), when a temporary ADI of 0–0.003 mg/kg bw was established on the basis of a NOAEL of 100 ppm, equivalent to 2.5 mg/kg bw per day, in a 90-day study in dogs treated in the diet. Dimethipin was evaluated again by the 1988 Joint Meeting (Annex 1, reference 53), which reviewed additional data and established an ADI of 0–0.02 mg/kg bw on the basis of a NOAEL of 2 mg/kg bw per day for increases in the absolute and relative (to body weight) weights of the liver in female rats fed dimethipin in the diet in a 2-year study. Further data have been provided. The compound was re-evaluated within the periodic review programme of the Codex Committee on Pesticide Residues.

### Evaluation for Acceptable Daily Intake

#### 1. Biochemical aspects

##### (a) *Absorption, distribution, and excretion*

##### *Rats*

Three male and two female CD rats received a single oral dose of approximately 3.8 mg/kg bw of [2,3-<sup>14</sup>C-dithiin ring]dimethipin (radiochemical purity, 96%) in distilled water. About 89% of the administered radiolabel was excreted in urine and faeces within 48 h, although one of the females eliminated only about 42% of the dose by these routes over the same period. The reason for the low recovery of label in this animal was unclear, as the tissue and blood concentrations were similar to those in the other treated animals. In general, faecal elimination slightly exceeded urinary excretion. Less than 0.1% of the administered label was detected in expired air. At sacrifice 96 h after treatment, the mean concentration of total residue in the tissues analysed (excluding blood) amounted to about 1% of the administered dose. The concentrations of residue were highest in the lung, heart, liver, and kidney and lowest in the gastrointestinal tract, brain, muscle, and fat. The concentration in blood represented 2–7.7% of the administered dose. No significant sex difference was apparent in the rate, route of elimination, or concentrations of residue in tissues (Caplan & Merricks, 1978; JMPR, 1985).

Groups of four to five male and female CD Sprague-Dawley rats received a single oral dose of 1.2 mg/kg bw of labelled dimethipin; a single intravenous dose of 2 mg/kg bw (targeted at

1.2 mg/kg bw) of labelled dimethipin; a single oral dose of 50 mg/kg bw of labelled dimethipin; or 1000 ppm of unlabelled dimethipin in the diet continuously for 14 days followed immediately on day 15 by a single oral dose of 50 mg/kg bw radiolabelled dimethipin. Urine, faeces, blood, and tissue samples were analysed at numerous intervals after treatment. Significant absorption and rapid excretion were found (Billings, 1987; JMPR, 1988).

(b) *Biotransformation*

The metabolic pathways of dimethipin in animals are shown in Figure 1.

*Rats*

In the study of Caplan & Merricks (1978) described above, analysis of pooled urinary and faecal samples from the treated rats, except for the female rat with an unusually low excretory rate, showed that about 5% of the label was in the urine or faeces as the unchanged parent compound. The other radiolabelled components were highly polar (Smilo, et al., 1978; JMPR 1985).

In the study of Billings et al. (1987) described above, urine samples collected from rats treated in the diet were analysed by high-performance liquid chromatography (HPLC). During the first 24-h collection period, the amounts of the reduced product, *N*-acetylcysteine, and polar fractions increased while those of cysteinylglycine conjugates decreased. There were no sex differences. These results supported the proposed metabolic pathway involving glutathione conjugation. The glutathione concentrations in liver and blood from three male and three female rats treated in the diet were no different from control values (McManus, 1987a; JMPR, 1988).

Three young male Charles River CD rats weighing approximately 225 g each received a single oral dose of 800 mg/kg bw of <sup>14</sup>C-dimethipin. Urine and faeces were collected separately 24, 48, and 72 h after dosing. The samples collected at 24 h were used for identification of metabolites. Only trace amounts of dimethipin were detected in the urine by HPLC, but seven metabolites were detected. The three identified polar products, which accounted for 77% of the urinary radioactivity, were a cysteinylglycine conjugate (54%), an *N*-acetylcysteine conjugate (12%), and a reduced metabolite, 2,3-dimethyl-5,6-dihydro-1,4-dithiane-1,1,4,4-tetraoxide (11%). The proposed metabolic pathway involves conjugation with glutathione followed by degradation to cysteinylglycine and cysteine conjugates and formation of mercapturic acid conjugates (McManus, 1987b; JMPR 1988).

*Goats*

A goat with a cannulated bile duct received <sup>14</sup>C-dimethipin (radiochemical purity, 98%) at a dietary concentration of 500 ppm for three days. Samples of urine, liver, bile, and kidney were collected for characterization and identification of metabolites. No details of the experimental conditions were provided, and although two animals appear to have been treated, data were presented on only one. Only 18% of the labelled residues in liver and 15% of those in kidney were identified: (butan-3-one-2-yl)-2-hydroxyethyl sulfone (due to ring cleavage) and 2,3,5,6,-tetrahydro-5-hydroxy-5,6-dimethyl-1,4-dithiine 1,1,4,4,-tetraoxide being the predominant residues. Metabolites 3 and 4 were invariably identified together with unchanged parent compound in urine, bile, liver, and kidney. Metabolite 4 accounted for 2–8% of the radiolabel recovered in each case. Some alcohol metabolites of dimethipin were identified only in urine and bile. Most of the radiolabelled components in urine, bile, liver, and kidney were highly polar and were present as glucuronide, cysteine, and acetylcysteine conjugates. Studies *in vitro* confirmed that these are the likely major metabolic steps. It was not reported whether total radioactivity was measured or whether residues were identified in muscle, fat, or milk (McManus, 1984; FAO/WHO, 1985).

Goats received a normal ration supplemented with <sup>14</sup>C-dimethipin at 1 or 10 mg/kg of feed until a plateau was reached in the milk after 17 days. After 18 days of dosing, about 97% of the total administered radiolabel was excreted in the urine and faeces, but none were detected in muscle and the concentrations were low in milk, liver, and kidney. The concentrations added to the feed were higher than the so-called 'worst case' situation in which every component of the diet contains residues at the tolerable maximum residue level (TMRL) proposed by the JMPR in 1985. Thus,



if animals are exposed to a normal feeding regime, the concentrations in meat, milk, and edible offal will not exceed the limit of determination (JMPR, 1987).

In a study conducted in compliance with GLP standards and with a signed and dated quality assurance statement, two lactating goats received  $^{14}\text{C}$ -dimethipin (radiochemical purity, > 98%) at a concentration of 0.15 or 50 mg/kg bw per day for 5 days before being killed about 22 h after the last dose. The doses were equivalent to dietary concentrations of 3.1 ppm (10 times actual exposure) and 1000 ppm (3200 times actual exposure), respectively. Urine and faeces were collected once daily, and milk was collected twice daily. Overall, 97% of the total radiolabel administered was recovered from the animal given the low dose and 96% from that given the high dose. More than 95% of the total administered dose was identified in the excreta, and 0.1–0.2% was eliminated in the milk. The average daily concentrations in milk were 0.005 ppm at the low dose and 1 ppm at the high dose. Fat, muscle, and whole blood contained the lowest concentrations of radiolabel at both the low dose (0.001 ppm in fat, 0.002 ppm in muscle, and 0.006 ppm in whole blood) and the high dose (0.32 ppm in fat, 0.64 ppm in muscle, and 2.7 ppm in whole blood), whereas the liver and kidney contained the highest concentrations, with 0.27 and 0.14 ppm at the low dose and 79 and 28 ppm at the high dose, respectively. The sensitivity of the method was < 0.0004 ppm for the low dose and < 0.011 ppm for the high dose (Byrd, 1992).

Enzymatic digestion followed by acid hydrolysis of liver tissue from this study suggested that the radiolabelled residues of dimethipin were covalently bound to tissue proteins. After acid hydrolysis, acetyldithiane was identified as the single radiolabelled peak in liver. Kidney samples from the animals at the low dose contained ethane disulfonic acid as the only metabolite, whereas renal tissue from the animal at the high dose also contained ethane disulfonic acid; acid hydrolysis of the bound material from this kidney showed that acetyl dithiane was also present. Milk from both animals contained dimethipin cysteine conjugate as the major metabolite. Acid hydrolysis of muscle from the goat at the high dose, which contained 0.64 ppm of radiolabelled material, yielded reduced dimethipin as the major metabolite. The results of this study suggests that dimethipin is metabolized primarily via Michael addition to glutathione or its derivatives or to protein (Gay & Lau, 1996).

### *Chickens*

Laying hens were fed a normal diet fortified with  $^{14}\text{C}$ -dimethipin at concentrations of 1, 6, or 30 mg/kg for 30 days, when half of the birds were slaughtered; the remainder were killed after a withdrawal period of 11 days. By day 30, more than 95% of the total administered radiolabel had been excreted in the faeces. No residues were detectable in muscle or fat at the end of dosing with 1 mg/kg or in fat after administration of 6 mg/kg. The concentrations found in tissues corresponded roughly to the doses. The concentrations found in the tissues of birds after 11 days of withdrawal were considerably lower, and none of the tissues except blood from hens at 1 mg/kg contained measurable residues. The birds fed 6 or 30 mg/kg  $^{14}\text{C}$ -dimethipin had measurable residues in all tissues except fat. In eggs, the residual radiocarbon increased slowly over 10 days and remained fairly constant for the remainder of the dosing period. Except in birds given 30 mg/kg, the concentrations in eggs decreased to below the limit of determination during the 11 days of withdrawal. Extrapolation from these data indicates that residues in birds fed a hypothetical 'worst case' diet in which all or nearly all of the components contain residues at the level of the MRL proposed by the 1985 JMPR would not exceed the limit of determination (JMPR, 1987).

In a study conducted in compliance with GLP, with a signed and dated quality assurance statement, [ $^{14}\text{C}$ -6]dimethipin (radiochemical purity, > 95%) was administered orally to white Leghorn laying hens daily for five consecutive days at a dose of 15.8 mg/kg bw per day (five hens) or 152 mg/kg bw per day (two hens), equivalent to concentrations of 203 ppm (7000 times that calculated to result from use of dimethipin at the permitted level on the cereals in the diet) and 2770 ppm (92 000 times the calculated concentration), respectively. Five untreated birds served as controls. Excreta and eggs were collected once daily during treatment. More than 90% of the total administered radiolabel was found in the excreta. The hens were killed within 24 h of the last dose, and tissues were collected, examined, and weighed. Edible tissues and eggs contained 5.5%

and 5.1% of the total radiolabel at the low and high doses, respectively. The liver and kidney had the highest concentrations, with 9.7 and 65 ppm in liver and 4.5 and 39 ppm in kidney at the low and high doses, respectively. Breast and thigh muscle each contained 10 ppm at the high dose and 0.63 and 0.72 ppm at the low dose, respectively. Egg yolk and egg white contained 6.9 and 6.6 ppm, respectively, at the high dose and 1.1 and 0.68 ppm at the low dose. Fat had the lowest concentrations, with 0.20 ppm at the low dose and 2.4 ppm at the high dose. Multiple metabolites were identified in eggs and tissues, all formed as conjugates of glutathione followed by degradation, resulting in the following compounds: glutathionyl-dimethipin,  $\gamma$ -glutamyl-cysteinyl-dimethipin, cysteinyl-dimethipin, mercaptodimethipin, thioacetyl-dimethipin, thiomethyl-dimethipin, and methyl sulfoxide-dimethipin. The major metabolite was  $\gamma$ -glutamylcysteinyl-dimethipin in most tissues and cysteinyl-dimethipin in liver (Lau & Gay, 1993).

While the metabolism of dimethipin in plants was found to be negligible, because it is applied at or close to the harvesting stage when the biochemical activities of the plant are at a minimum, its metabolism in animals is quite extensive. As a result, the main residues in crops are the parent product, while in animals dimethipin undergoes glutathione conjugation with subsequent degradation. In a second pathway, dimethipin undergoes hydration followed by ring cleavage.

## 2. Toxicological studies

### (a) Acute toxicity

The acute toxicity of dimethipin after administration by the oral, dermal, and inhalation routes and its ocular and dermal irritation and dermal sensitizing capacity are summarized in Table 1.

In rats (strain unspecified), the LD<sub>50</sub> for dimethipin (purity, > 97.5%) administered orally was 1200 mg/kg bw for animals of each sex (Varner & Matthews, 1977; JMPR, 1985). In another study, the LD<sub>50</sub> for dimethipin (purity, 98.0%) was 550 mg/kg bw in male and 460 mg/kg bw in female Sprague Dawley CD rats (Blaszczak, 1992a). Surviving animals lost weight during week 1, but gained weight during the remainder of the observation period. The signs of toxicity included nasal discharge, hypoactivity, and rales. At necropsy, discoloured lungs, red distended stomachs, and red fluid in the intestines were noted.

The LC<sub>50</sub> in Sprague-Dawley CD rats exposed by inhalation by nose only for 4 h was 1.5 mg/L for males and 0.88 mg/L for females (Hoffman, 1992). The clinical signs of toxicity included respiratory distress. Body weights were substantially decreased (41% in males and 18% in females) during the first week, but weight was gained after that time. At necropsy, discoloured lungs were observed.

The LD<sub>50</sub> in rabbits was > 5000 mg/kg bw, as no deaths were seen (Blaszczak, 1992b). It severely irritated the eyes (Griffiths & Koschier, 1980), but the material tested was a recrystallized form of dimethipin, which may not be representative of the technical-grade material. Technical-grade dimethipin was not irritating to rabbit skin (Blaszczak, 1992c). It was a weak dermal sensitizer in Hartley guinea-pigs (Madison, 1983).

**Table 1. Acute toxicity of dimethipin**

Species	Purity (%)	Sex	Route	LD <sub>50</sub> or LC <sub>50</sub> (mg/kg bw or mg/L)	Reference
Mouse	> 97.5	M	Oral	440	Shapiro (1977a)
		F		600	
Rat	> 97.5	M&F	Oral	1 200	Varner & Matthews (1977)
Rat	98	M	Oral	460	Blaszczak (1992a)
		F		550	
Rat	NR	M	Intraperitoneal	240	Shapiro (1977b)
		F		240	
Rat	NR	M&F	Inhalation (1 h)	> 20	Babish (1977)
Rat	98.9	M	Inhalation (4 h)	1.5	Hoffman (1992)
		F		0.88	
Rabbit	NR	M&F	Dermal (24 h)	> 12 000	Reagen & Becci (1982)
Rabbit	98	M&F	Dermal (24 h)	> 5 000	Blaszczak (1992b)

NR, not reported

WHO.(1999) has classified dimethipin as 'slightly hazardous'.

(b) *Short-term studies of toxicity*

*Rats*

Groups of six male and six female Charles River CD Sprague-Dawley rats, eight weeks of age, received technical-grade dimethipin (purity, 98%) moistened with distilled water dermally for 6 h/day at doses of 0, 10, 100, or 1000 mg/kg bw per day for 21 days. The study was conducted under GLP requirements, and a signed and dated quality assurance statement was available. There were no treatment-related alterations in mortality rate, body weight, food consumption, clinical signs, clinical pathology, or gross and histopathological appearance. Traces of hyperkeratosis were present in the skin of treated males at all doses and in females at the intermediate and high doses. The weights of the liver relative to body weights were statistically significantly increased in males (20%) and females (15%) at 1000 mg/kg bw per day, but the absolute liver weights of these animals were statistically nonsignificantly increased, by 18% in males and 12% in females. In the absence of clinical pathological or histopathological effects, the NOAEL for systemic toxicity was 1000 mg/kg bw per day, the highest dose tested (Goldenthal, 1991).

Groups of 15 male and 15 female Charles River rats, 28 days old, were fed diets containing technical-grade dimethipin (purity, > 99.2%) at concentrations of 0, 100, 300, or 1000 ppm for 95 days. There was no treatment-related change in mortality rate or abnormal behaviour. Food consumption was slightly depressed in females at the highest dose throughout the study, but body weights were unaffected. Haematology, blood chemistry, and urinalysis performed in 10 males and 10 females from the control and high-dose groups after 45 and 85 days of treatment indicated no significant compound-related effects. At termination, the ratios of organ:body weight of liver and kidney were increased in females at the highest dose. No significant differences in gross pathological changes were observed between control and treated groups. Histopathological evaluation of a variety of tissues, including liver and kidney, from 10 males and 10 females from the control and high-dose groups showed no lesions attributable to treatment. The NOAEL was 300 ppm, equivalent to 15 mg/kg bw per day (Marias et al., 1976; JMPR, 1985).

Groups of 10 male and 10 female Charles River CD Sprague-Dawley rats aged four week were fed diets containing technical-grade dimethipin (purity, 98.5%) at concentrations of 0, 40, 1750, or 3500 ppm (equal to 2.5, 110, and 220 mg/kg bw per day in males and 3.1, 130, and 260 mg/kg bw per day in females) for 13 weeks. The study was conducted under GLP requirements, and a signed quality assurance statement was available. The animals were observed for deaths and clinical signs of toxicity twice daily, and body weight and food consumption were measured weekly. Ophthalmoscopic examinations were made before treatment and at the end of the study. Clinical data were collected on all surviving animals at 13 weeks. The weights of the brain, kidneys, liver, and testis were recorded for all animals, and all tissues were examined microscopically.

One male at the low dose was killed *in extremis*, but this animal showed no significant gross or microscopic changes, and the cause of death was not identified. No clinical signs of toxicity or ophthalmoscopic findings were reported. Males at the high dose had significantly decreased food consumption (11%), body weight (6%), and body-weight gain (8%) in comparison with controls; and females at the intermediate and high doses had significantly decreased food consumption (12% at the intermediate and 16% at the high dose), body weight (10% and 11%), and body-weight gain (20% and 28%). Males at the high dose had slightly decreased erythrocyte volume fractions (8%) and haemoglobin values (7%). Significantly increased cholesterol concentrations were found in females at the intermediate (37%) and high doses (41%), and those at the high dose had increased serum activity of aspartate aminotransferase (30%). There were no gross findings at necropsy that were considered to be related to treatment. The weight of the liver relative to body weight was significantly increased in males at the high dose (18%) and was correlated with microscopic hepatocellular hypertrophy in 5 of 10 animals. Females at the intermediate and high doses showed increased relative weights of the brain, kidney, and liver, in the absence of microscopic changes, and these changes were considered to be related to the decreased body weights of those animals. The NOAEL was 40 ppm, equal to 2.5 mg/kg bw per day (Goldenthal, 1993).

### Dogs

Groups of four male and four female pure-bred beagles, about six months old, were given diets containing technical-grade dimethipin (purity, > 99.2%) at concentrations of 0, 100, 300, or 1000 ppm for 90 days. No deaths occurred, and no treatment-related effects were seen on behaviour, body weight, or food consumption or in blood chemistry or haematology conducted after 42 and 85 days of treatment. Urinary analysis at the same two intervals indicated an increase (not dose-related) in the incidence of moderate-to-large amounts of 'crystals' in the urinary sediments of all treated females after 85 days. No other urinary parameters were affected. At terminal sacrifice, the organ weights and gross appearance were not affected by treatment. Microscopic examination of a large number of tissues, including the testis, from each animal revealed oesophageal lesions characterized by focal mucosal vesicles containing a few acute inflammatory cells in one of eight animals at 300 ppm and three of eight animals at 1000 ppm, but in none of the concurrent controls or those at the lowest dose. The NOAEL was 100 ppm, equivalent to 2.5 mg/kg bw per day (Burtner et al., 1976).

Groups of six male and six female pure-bred beagles, about 7.5 months old and caged individually, were given diets containing technical-grade dimethipin (purity, 99.7%) at concentrations of 0, 300, 1000, or 3000 ppm for one year. One male and three females at the highest dose died or were sacrificed *in extremis* between weeks 13 and 52. 'Thinness', a major clinical sign, was seen frequently in most animals at 3000 ppm and infrequently in one animal at 1000 ppm. Other signs, including dehydration and paleness of the gums, were also noted infrequently at 3000 ppm. Weight loss or growth depression and decreased food consumption were seen in animals of each sex at 3000 ppm throughout most of the study. Bitches at 300 or 1000 ppm showed a marginal (10%) but not consistently dose-dependent reduction in growth between weeks 12 and 48. Animals of the highest dose had abnormalities in the T-wave on electrocardiograms at the end of the study, and ophthalmoscopic examination showed increased incidences of conjunctival discharge, inflammation and corneal irregularities and roughening at week 27 but not at week 52. 'Severe to slight thinness and irregular or erratic heartbeat' were seen almost exclusively in animals at 3000 ppm throughout the study.

Monthly haematological and blood chemical determinations revealed deviations from control values in many parameters mainly in animals of each sex at 3000 ppm, including decreased erythrocyte volume fraction, increased platelet count, and depressed values of total protein, albumin, globulin, calcium, blood urea nitrogen, and creatinine at most sampling intervals. Animals at 1000 ppm had decreased values of blood urea nitrogen (both sexes) and creatinine (dogs) at many sampling intervals. When compared with concurrent controls, treated dogs showed a slight but consistent, generally dose-related decrease in erythrocyte counts and haemoglobin levels. These findings were considered unlikely to be related to treatment, because the values were within the normal ranges for control beagles in the published literature (Bushby, 1970) and those recorded for control beagle dogs maintained in the testing laboratory. Urinalysis, including microscopy of urinary sediments, conducted twice monthly showed no significant changes related to treatment.

At termination, the gross pathological appearance in the treated groups was not significantly different from that in the controls. The ratio of organ:body weight for the kidneys was increased at both 1000 and 3000 ppm (both sexes), for liver in dogs at 3000 ppm and in bitches at  $\geq 1000$  ppm, for brain at 3000 ppm (both sexes), and for testis at 3000 ppm. Histopathological evaluation of a large number of tissues from each animal showed testicular degeneration in 0/6, 2/6, 1/6, and 3/6 dogs at 0, 300, 1000, and 3000 ppm, respectively. Severe and diffuse testicular degeneration was seen in one affected dog at 300 ppm and one at 3000 ppm, while generally mild focal degeneration of the testis was seen in the other affected animals. Although the incidence and severity of testicular degeneration did not show a dose-response relationship, the complete absence of this lesion in concurrent controls and in seven similar 1-year studies conducted in the same laboratory and comprising over 20 control dogs (McGee, 1983) indicated that the possibility that the testicular lesion is related to treatment cannot be ruled out. Nevertheless, the testicular lesions were considered not to be a direct effect of dimethipin on the testes, but rather the result of the prolonged poor nutritional status of the dogs or an incidental finding, as they were similar to incidental findings in other studies in dogs in the same laboratory. Additionally, no testicular lesions were

seen in dogs in a 90-day study. Other microscopic findings likely to be attributable to treatment included hypocellularity of the bone marrow, lesions in the gastrointestinal tract (gastritis, oedema, and ulceration) and heart (haemorrhage), and thymic atrophy at the high dose, and an increased incidence of nephritis, centrilobular degeneration in the liver, lymphadenitis, and splenic hyperplasia at both 1000 and 3000 ppm. The NOAEL was 300 ppm, equivalent to 7.5 mg/kg bw per day, on the basis of the increased relative liver weight, increased alanine aminotransferase and alkaline phosphatase activities; and hepatocellular degeneration in bitches at 1000 ppm (Benson, 1981; JMPR, 1985, 1999).

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

*Mice*

Groups of 50 male and 50 female CD-1 mice, about 50 days old, housed five per sex per cage, were fed diets containing technical-grade dimethipin (purity, 97–98%) at 0, 80, 400, or 2000 ppm for 78 weeks. The animals fed dimethipin were kept for about 35 weeks in the same room as animals receiving a highly photodegradable compound identified only by a code name. It was stated, but unsubstantiated by data, that dietary analysis showed that dimethipin was stable in the diet for 7 days and that the mixture of dimethipin and basal diet was satisfactorily homogeneous. All animals killed *in extremis* or that died during the study and those killed at the end were examined grossly, and a wide range of tissues, including the brain, were examined microscopically.

The mortality rate was not affected by treatment: 58–78% of males and 76–86% of females in all groups were still alive at the conclusion of the study. A slight (< 10%), non-dose-related decrease in body-weight gain was seen in males at doses  $\geq$  400 ppm during the first 13 weeks. Food consumption was not affected in a consistent dose-related pattern. There were no significant differences between control and treated groups in the incidence of clinical signs or palpable nodules or tissue masses. Haematological examination of five males and five females per group at three intervals during the study revealed a significant increase in erythrocyte volume fraction in males at 2000 ppm at week 13 and in erythrocyte volume fraction, haemoglobin, and erythrocyte values at week 78. At termination, the erythrocyte volume fraction was elevated in females at both 400 and 2000 ppm. All treated females showed a statistically significant, albeit not strictly dose-dependent, increase in erythrocyte counts at week 78. Blood chemical and urinary parameters were not evaluated. No significant gross pathological alterations or changes in organ weights were seen. No detailed histopathological data with morphological descriptions of lesions in individual animals were available, but tabulated 'individual histopathology findings' indicated that no compound-induced non-neoplastic changes were found.

The only notable neoplastic finding was an increased incidence of pulmonary (alveolar and bronchiolar) tumours in males at 2000 ppm. The incidence of lung adenocarcinomas, but not of adenomas alone, was significantly increased in males at 2000 ppm when compared with concurrent or historical controls from five studies ( $p < 0.05$ , Fisher exact test), but not when compared with the maximum incidence of lung adenocarcinomas in historical controls. The time to appearance of adenocarcinomas and the multiplicity of tumours were not modified by treatment. Additionally, the pulmonary tumours were not associated with an increase in hyperplastic pulmonary changes. The combined incidence of lung adenocarcinoma and adenoma was significantly increased in comparison with the incidence in historical controls from five studies but not when compared with the incidence in concurrent controls or the maximum incidence in historical controls. There was no dose-related increase in the incidence of benign or malignant lung tumours in females. The incidence, location, and type of tumours other than lung tumours were comparable to those in controls. About 30% of the male and 40% of the female concurrent controls were found to have tumours. Lymphoma (in males), lung tumours (in both sexes), and hepatocellular carcinoma (in males) were the most frequently observed spontaneous tumours. The fact that the animals were about 50 days old at initiation of the study may have compromised the sensitivity of the test. The NOAEL was 80 ppm, equal to 12 mg/kg bw per day, on the basis of criteria other than tumours. The data on lung tumours are unclear (Serota et al., 1981a; JMPR, 1985). As lung adenomas and adenocarcinomas occur commonly in this strain of mice, this finding was considered to be of no toxicological relevance (JMPR, 1988).

### *Rats*

Groups of 50 male and 50 female Sprague-Dawley CD rats, about 40 days old and caged individually, were fed diets containing technical-grade dimethipin (purity, 97–98%) at concentrations of 0, 40, 200, or 1000 ppm for 104 weeks. The control group was used for both this study and another study on a chemical identified only by a code, and it was not reported whether treated animals in the two studies were kept in the same room. All animals that were killed in moribund condition or died during the study and all survivors killed at the end of the study during weeks 105 and 106 were necropsied, and a variety of tissues including the brain and any 'unusual' lesions were examined histopathologically. Sections of the spinal cord and 'head' of 10 male and 10 female survivors per group were also evaluated microscopically. Five males and five females per group were studied for haematological and blood chemical parameters at five intervals during the study and similar numbers at the same intervals for urinary indices.

The survival of animals at the highest dose appeared to be better than that in other groups. By 104 weeks, the survival rates were 44% for males at 200 ppm and 50–72% for males and females in all groups, including controls. No clinical signs related to treatment were seen, and there were no dose- or compound-related effects on food consumption or on the incidence of palpable nodules, tissue masses, or wart-like lesions. A slight but consistent depression of growth ( $\leq 5\%$  in males and  $\leq 10\%$  in females) was seen at 1000 ppm between weeks 43 and 95 in males and between weeks 51 and 87 in females. Females in all treated groups had increased total protein at week 13 and decreased platelet counts at week 104, the only time at which this parameter was measured. Other deviations from control values were observed in certain haematological and blood chemical parameters, but essentially only in animals at the highest dose. No significant differences were seen between control and treated groups in urinary parameters. The gross pathological changes seen in treated animals were not significantly different from those in controls. A non-dose-related increase in the ratio of liver:body weight was seen in males in all treated groups, and a dose-related increase was seen in females at 200 and 1000 ppm. The absolute weight of the adrenals and that relative to body weight were decreased in females in all treated groups. Histopathological examination showed focally dilated bile ducts containing basophilic homogeneous material in one male and one female control, two males and three females at 40 ppm, five males and nine females at 200 ppm, and 33 males and 18 females at 1000 ppm. This finding was presumed to be related to treatment. The microscopic changes in other tissues, including the adrenal gland, were similar to those in controls. The finding that 9–27% of the males in the control and treated groups showed lactation and/or galactocoele may have been associated with the increased incidence of mammary fibroadenoma in males at the highest dose. The only other noteworthy neoplastic findings were increased incidences of astrocytoma in males and hepatocellular carcinoma in females at 200 and 1000 ppm. The incidences of these tumours were not, however, significantly different from those in concurrent controls (Fisher exact test). The incidence of hepatocellular carcinoma was not significantly different from that in historical controls, but that of astrocytoma in males was significantly increased at both 200 and 1000 ppm ( $p < 0.05$ ) when compared with the incidence in historical controls from seven studies. Comparison with the maximum incidence in historical controls showed no significant difference, even at 1000 ppm. An additional glioma was reportedly found in the control group by a consultant to the company who evaluated three additional brain sections from each control and treated male. No preneoplastic lesions (gliosis) were seen in the original or additional brain slides (Squire, 1984). The latency to appearance of astrocytoma was not reduced by treatment. The minimum-effect level on parameters other than tumours was 40 ppm, the lowest dose tested, equivalent to 2.0 mg/kg bw per day (Serota et al., 1981b; JMPR, 1985).

Further data on the changes in organ weights, provided subsequently by the testing laboratory, gave a NOAEL of 200 ppm for the decrease in relative and absolute weights of the adrenal glands in female rats, and a NOAEL of 200 ppm for the increase in absolute and relative liver weights in male rats. The NOAEL for the increase in absolute and relative liver weights in females was 40 ppm (JMPR, 1998)

Groups of 60 young Sprague-Dawley rats (CrI:CD BR (VAF/Plus)) of each sex were fed diets containing technical-grade dimethipin (purity, 98.5%) at concentrations of 0, 40, 1750, or 3500 ppm for males (equal to 0, 1.8, 78, or 160 mg/kg bw per day) and 0, 40, 875, or 1750 ppm

for females (equal to 0, 2.2, 50, or 100 mg/kg bw per day) for 104 weeks. The study was conducted under GLP requirements, and a signed quality assurance statement was available. Ten animals of each sex per dose were killed after 12 months for interim evaluations of clinical signs, deaths, body weight, food consumption, ophthalmological, haematological, clinical chemical, and urinary parameters, organ weights, and gross and histopathological appearance. The survival of females at the high dose was decreased but not statistically significantly. Of the 50 rats per sex allocated to the main study, only 20, 24, 19, and 21 of the males and 20, 18, 18, and 13 of the females in the control, low-, intermediate-, and high-dose groups, respectively, survived to week 104. There were no treatment-related clinical signs of toxicity, ophthalmic or haematological effects, or changes in urinary parameters in any treated group in comparison with controls. The mean body weight of males at the high dose was decreased by 13–20% during the study; females at the intermediate dose weighed  $\leq 16\%$  less than controls and those at the high dose weighed  $\leq 19\%$  less. The mean body-weight gain of males at the high dose was consistently decreased by 24%, and those of females at the intermediate and high doses by 13 and 29% in comparison with controls. No accompanying decrease in food consumption was seen.

Significantly increased serum aspartate and alanine aminotransferase activities were observed in females at the intermediate and high doses at 12, 18, and 24 months and in males at the high dose at 18 months. Animals of each sex at the intermediate and high doses also had elevated cholesterol levels at all sampling intervals, and males at these doses had increased serum concentrations of urea nitrogen and creatinine. The findings indicate renal and hepatic toxicity. Macroscopic alterations were seen in the liver and kidneys of animals that died during the study or were killed at the end. Males at the high dose and females at the intermediate and high doses had an increased incidence of liver cysts, and males at the highest dose had an increased incidence of tan discolouration of the liver and small testes. Males at the two higher doses had a slight increase in the incidence of enlarged kidneys. Tan discolouration and tan foci in the liver and a granular surface on the kidneys were seen in females at the highest dose. Significant increases in organ weights ( $p < 0.05$ ) in males at the highest dose that were considered to be related to treatment were in the absolute (115%) and relative (166%) weights of the liver and the absolute (132%) and relative (152%) weights of the kidney.

At interim sacrifice at 12 months, the incidence and/or grade of bile-duct hyperplasia was increased in treated males (4/12, 5/13, and 5/12 at the low, intermediate, and high doses, respectively) when compared with controls (2/13). In females, the incidence and/or grade was increased at both 875 ppm (70%) and 1750 ppm (43%) when compared with controls (8%). Epithelial hyperplasia of the duodenum was seen in 11/12 males and 10/14 females at the high dose and in none of the controls by 12 months. In animals killed at the end of the study, the incidence and/or grade of biliary cysts in the liver was increased in females at the intermediate (12%) and high doses (37%) and in males at the high dose (10%) in comparison with controls (2% in females and 0% in males). Bile-duct hyperplasia in the liver was increased in incidence and/or severity in males at the intermediate (25/47) and high doses (29/48) in comparison with controls (21/47), and in females at the intermediate (68%) and high doses (61%) when compared with controls (33%). Dose-related increases in the severity of the lesion were seen in animals of each sex. Male rats fed the high dose also showed a significantly increased incidence (33%) of eosinophilic foci in comparison with controls (13%).

The incidence and severity of chronic progressive nephropathy was increased in 47/47 males and 37/50 females at the intermediate dose and 46/48 males and 38/46 females at the high dose in comparison with controls (33/47 in males and 18/48 in females). The incidence and grade of epithelial hyperplasia of the duodenum was increased in males at the intermediate (38%) and high doses (46%) in comparison with controls (2%), and in females at the intermediate (14%) and high doses (33%) in comparison with controls (0%). Signs of gastrointestinal tract toxicity were seen in animals of each sex at the high dose; males at this dose had an increased incidence and severity of epithelial hyperplasia of the nonglandular stomach (15%, 2% in controls), and females showed demineralization of the glandular stomach (7/46, 0/48 in controls). Males at the high dose had an increased incidence (21/47) and grade of seminiferous tubular degeneration of the testis (eight had grades of moderate and six severe; 12/47 in controls of which 0 were moderate and four severe) and hypospermia of the epididymis (13/47, 5/47 in controls). The incidence of seminiferous tubular degeneration was 13/27 (three moderate and two severe) in males at the low dose and 16/34

(four moderate and three severe) at the intermediate dose. Although the testes of all rats at the low and intermediate doses were not examined, a treatment-related increase in the incidence of testicular lesions at the intermediate dose could be discerned. The occurrence of testicular degeneration at the intermediate and high doses was considered to be related to treatment, as was the increased incidence of epididymal hypospermia, which was probably a result of the seminiferous tubular degeneration. Females at the high dose showed an increased incidence and severity of vascular mineralization of the heart (5/46, 0/46 in controls) and aortic artery (6/45, 0/48 in controls). The other histological lesions reported were considered not to be related to treatment.

The doses tested were adequate to assess the tumorigenic potential of dimethipin. The only statistically significant increase in neoplastic lesions was in the incidence of benign phaeochromocytomas (17%, 4% in concurrent controls) in the adrenal medulla of male rats at the high dose. There was no accompanying increase in hyperplasia in this tissue and no increase in the incidence of malignant phaeochromocytomas, and the combined incidence of benign and malignant neoplasms was not increased significantly. Furthermore, the incidence of benign phaeochromocytomas in historical controls ranged from about 0 to 18%, and the combined incidence of malignant and benign tumours ranged from about 0 to 20% in similar studies conducted in the same laboratory during the 5 years preceding termination of this study. The increased incidence of benign phaeochromocytomas was therefore considered not to be related to treatment. Slight but biologically insignificant increases or decreases in the incidences of other tumours were seen in treated groups in comparison with controls. The tumour incidences in this study are presented in Table 2. During the first 12 months of the study, additional fibroadenomas and adenocarcinomas were found in females in both control and treated groups and an additional fibroadenoma was found in a male at the intermediate dose. The other tumour types listed in Table 2 were not found during the first 12 months. The increased incidences of astrocytomas in males at 200 and 1000 ppm, hepatocellular carcinomas in females at 200 and 1000 ppm, and mammary fibroadenomas in males at 1000 ppm in the study of Serota et al. (1981b) were not found in this study, even though the doses and the number of rats per sex at each dose exceeded those in the earlier study. The NOAEL was 40 ppm, equal to 1.8 mg/kg bw per day (Goldenthal, 1996).

#### (d) Genotoxicity

Dimethipin had no mutagenic activity in a number of assays in microorganisms, mammalian cells, and rodents *in vitro* and *in vivo*. The only exception was the induction of forward mutation in mouse lymphoma cells in the presence of metabolic activation (Table 3).

**Table 2. Tumour incidence<sup>a</sup> in rats dying between weeks 52 and 104 in a 2-year study of dimethipin**

Tumour site	Males (dose, ppm)				Females (dose, ppm)			
	0	40	1750	3500	0	40	875	1750
Mammary gland								
Adenoma	1/7	0/3	1/2	2/7	4/48	3/41	7/46	2/46
Fibroadenoma					28/48	23/41	27/46	16/46
Adenocarcinoma					9/48	6/41	10/46	4/46
Brain								
Astrocytoma	1/44	0/24	0/28	1/48	0/48	0/31	0/33	0/46
Granular-cell tumour	1/44	0/24	0/28	1/48				
Liver								
Adenoma	1/47	0/48	3/47	2/48	1/48	0/49	1/50	3/46
Carcinoma	1/47	1/48	2/47	1/48	0/48	0/49	0/50	0/46
Interstitial-cell tumour of the testis								
Benign	2/47	1/27	2/34	5/47				
Malignant	0/47	0/27	1/34	0/47				
Combined	2/47	1/27	3/34	5/47				
Phaeochromocytoma of the adrenal medulla								
Benign	2/47	4/28	0/28	8/48*	1/48	1/32	0/35	0/46
Malignant	2/47	1/28	1/28	0/48	0/48	0/32	0/35	0/46
Combined	4/47	5/28	1/28	8/48				

<sup>a</sup> Number of rats with tumours/Number of rats examined microscopically

\* Significantly different from incidence in concurrent controls at  $p < 0.05$

(e) *Reproductive toxicity*(i) *Multigeneration reproductive toxicity*

Groups of 15 male and 25 female Charles-River CD(SD)BR rats, 5 weeks old, were fed diets containing technical-grade dimethipin (purity, 99.7%) at 0, 50, 200, or 800 ppm for 105 days before mating (one male:two females; sibling and half-sibling mating avoided). The day on which a positive vaginal smear or copulatory plug was detected was considered to be day 0 of gestation. Weanlings of the second litter ( $F_{1b}$ ) were selected to become parents of the next generation and mated after receiving the test diets for 125 days. In each generation, the second mating was allowed at least 14 days after the first litter ( $F_{1a}$  and  $F_{2a}$ ) had been weaned at 21 days of age.

In the parental generations, deaths (the incidence of which was not dose-related) occurred only among females. No compound-related behavioural abnormalities were seen.  $F_0$  and  $F_{1b}$  adult females at 800 ppm weighed less than the concurrent controls before mating, throughout gestation, and throughout most of the lactation periods. Food consumption was depressed in animals at 800 ppm, in  $F_0$  females before mating in weeks 6–10, in  $F_{1b}$  females in weeks 6–17, and in  $F_{1b}$  males in weeks, 1, 4, and 9. Fertility in males, as determined by a demonstrated ability to impregnate at least one female, mating index (% females mated), gestation index (% mated females with viable litters), the number of days required by females to mate, and the duration of gestation in treated groups were all comparable to the control values. In the progeny, the mean number of pups per litter born alive, survival of pups to days 4, 7, 14, and 21, the sex ratio, and the behaviour of pups were not adversely affected. The weights of pups in the  $F_{1a}$  and  $F_{1b}$  litters at 800 ppm were reduced on days 7, 4, and 21, and the weights of those in the  $F_{1a}$  litters at 200 ppm and in the  $F_{2a}$  litters at 800 ppm were decreased on day 21. Gross external examination of all pups, including those found dead, revealed only one abnormal pup, which was a stillborn in an  $F_{1a}$  litter at 200 ppm.

Gross pathological examination of all parental animals of the second litters killed after weaning ( $F_0$ ) or 30 days after weaning ( $F_{1b}$ ) (i.e. after 32 weeks and 39 weeks of dietary feeding, respectively) and weanlings in each generation revealed no significant difference between control and treated groups. Determinations of the weights of organs from all  $F_{1b}$  adults and five male and five female weanlings from the  $F_{1b}$  and the  $F_{2b}$  litters showed increased organ:body-weight ratios for the liver at 200 and 800 ppm and for the kidney and brain at 800 ppm in adult  $F_{1b}$  females; however, the organ:body-weight ratio of the liver in  $F_{1b}$  adult females was depressed at 50 ppm.

**Table 3. Results of assays for the genotoxicity of dimethipin (purity, > 98%)**

End-point	Test system	Dose	Result	Reference
<i>In vitro</i> Reverse mutation	<i>S. typhimurium</i> TA1538, TA1537, TA1535, TA98,	1–1000 mg/plate	Negative <sup>a</sup>	Jagannath & Brusick (1978, 1981)
Mitotic non-disjunction, recombination, and mutation	<i>S. cerevisiae</i> D4	1–1000 mg/plate	Negative <sup>a</sup>	Jagannath & Brusick (1978)
	<i>S. cerevisiae</i> D6	1–2000 mg/plate	Negative <sup>a</sup>	Bootman & Lodge (1982)
Mitotic gene conversion	<i>S. cerevisiae</i> D4	125–2000 mg/ml	Negative <sup>a</sup>	Forster et al. (1984a)
Chromosomal aberration	Chinese hamster ovary cells	5–50 mg/ml	Negative <sup>a</sup>	Sorg et al. (1983)
Sister chromatid exchange	Chinese hamster ovary cells	1.56–24 mg/ml <sup>b</sup> 3.1–200 mg/ml <sup>c</sup>	Negative <sup>a</sup>	Galloway & Brusick (1981)
Forward mutation	L5178Y <i>Tk</i> <sup>+</sup> mouse lymphoma cells	1.56–75 µg/ml <sup>b</sup> 125–200 µg/ml <sup>c</sup>	?	Myhr & Brusick (1981)
<i>In vivo</i> <i>In vivo/in vitro</i> unscheduled DNA synthesis	Wistar rat	100, 300, or 1000 mg/kg bw	Negative	McManus (1987c)
Micronucleus formation	Swiss CD-1 mouse	220 mg/kg bw	Negative	McManus (1986)
Micronucleus formation	Mouse	Two successive daily oral doses of 22, 73.3, or 220 mg/kg bw per day (males) or at 30, 100, or 300 mg/kg bw per day (females)	Negative <sup>d</sup>	Forster et al. (1984b)

Microscopic evaluation of a wide range of tissues, including the liver and kidney, from all F<sub>1b</sub> adults and five male and five female weanlings from the F<sub>1b</sub> and the F<sub>2b</sub> litters and of gross lesions and gonads from F<sub>0</sub> adults indicated no significant changes attributable to treatment.

The NOAEL was 200 ppm, equivalent to 10 mg/kg bw per day, as the finding of a decrease in pup weight on day 21 in F<sub>1a</sub> litters at 200 ppm was unlikely to be treatment-related, as it occurred in only a single generation and was not recurrent (Kehoe & Mackenzie, 1982; JMPR, 1985).

(ii) *Developmental toxicity*

*Rats*

Groups of sexually mature mated female rats (BLU:(SD)BR) received technical-grade dimethipin (purity, 97.5%) by intubation as a suspension in corn oil at a dose of 0, 80, 400, or 800 mg/kg bw per day on days 6–15 of gestation, the day on which a vaginal plug was observed being considered day 0. An additional group of mated female rats treated with 250 mg/kg bw per day of acetylsalicylic acid was used as the positive control. The groups at 400 and 800 mg/kg bw per day were terminated within 8 days of initiation of treatment owing to 'excessive deaths' and were not investigated further. Two new groups, at 30 and 160 mg/kg bw per day, were added 2 weeks after the study began, but no concurrent control groups were included for the two new doses. The dams were killed on day 20 of gestation and their fetuses were removed surgically for gross external, visceral, and skeletal examination.

No compound-related deaths or clinical signs were observed at doses up to 160 mg/kg bw per day, and the growth rates of dams during gestation were comparable in all groups. The number of dams in each group that became pregnant and were alive on day 20 was 20–22. The mean number of implantation sites or live fetuses, fetal weight, and sex ratio were unaffected. An increase in the mean number of resorptions per dam, with no concomitant increase in the incidence of pregnant dams with resorptions, was seen at 160 mg/kg bw per day. The incidence of skeletal or visceral malformations of fetuses did not differ significantly between control and treated groups. The positive control group had a number of fetal abnormalities, including encephalomenigocele and gastroschisis. The NOAEL for both maternal and developmental toxicity was 160 mg/kg bw per day (Knickerboker et al., 1977; JMPR, 1985).

*Rabbits*

Groups of 16 sexually mature female Dutch belted rabbits were artificially inseminated and were intubated with technical-grade dimethipin (purity, 98.3%) as a suspension in 0.5% carboxymethyl cellulose at 0, 7.5, 20, or 40 mg/kg bw per day at a constant volume of 1 ml/kg bw, on days 6–27 of gestation, day 0 of gestation being considered the day of insemination. They were killed on day 28 of gestation, and the uterine contents were examined. All fetuses, including those that were aborted or dead, were examined grossly and for skeletal and visceral abnormalities.

No deaths occurred. A slight increase in the number of females at 20 and 40 mg/kg bw per day that had a reduced amount of faeces beneath the cage was seen at various intervals during gestation as compared with concurrent controls. No data on food consumption were available. Does at 40 mg/kg bw per day showed weight loss between days 6 and 12, and maternal weight gain was depressed in a dose–response pattern in all treated groups between days 6 and 28. The fertility rate was 88–94% in control and treated groups. One doe each at 0, 20, and 40 mg/kg bw per day aborted on day 28; seven non-viable fetuses were found in does at 0 and 20 mg/kg bw per day; and three late resorptions occurred in the doe at 40 mg/kg bw per day. At terminal sacrifice, the gross pathological findings in treated does were comparable to those in the controls. No significant differences were found between controls and treated groups in the mean numbers of corpora lutea, implantations, early or late resorptions, or viable or non-viable fetuses, or in fetal weight.

A non-dose-related increase in postimplantation loss, due mainly to an increased number of early resorptions, was seen in all treated groups, although the values for this parameter were within the range of historical controls. The sex ratio of fetuses at 40 mg/kg bw per day was increased, the mean number of females being reduced. Increases in the incidence of fetuses and of litters containing fetuses with 27 presacral vertebrae and with scoliosis (with or without associated rib anomalies) were observed at 40 mg/kg bw per day, when compared with concurrent or historical control incidences, in a total of 951 fetuses in 149 litters from an unspecified number of studies

with Dutch belted rabbits over an unspecified period. There was no apparent dose- or compound-related increase in the frequency of fetal soft-tissue abnormalities. The NOAEL for maternal and developmental toxicity was 20 mg/kg bw per day (McMeekin et al., 1981; JMPR, 1985).

### Comments

After oral administration to rats, goats and hens, <sup>14</sup>C-dimethipin was extensively absorbed (69% within 24 h) and rapidly excreted (89% within 48 h), mainly in the urine. Unchanged dimethipin represented only a small fraction of the residue in animals. In one metabolic pathway, dimethipin undergoes glutathione conjugation and subsequent degradation to several metabolites, including its mercapturic acid. In another pathway, dimethipin is hydrated and then undergoes ring cleavage. Dimethipin also binds covalently to amino acids, peptides and proteins, although the extent to which this binding is catalysed by enzymes is unknown.

Dimethipin (purity, 98.5%) was moderately toxic to rats given single oral doses, with LD<sub>50</sub> values of 460 mg/kg bw in males and 550 mg/kg bw in females, or after exposure by inhalation, with LC<sub>50</sub> values of 1.5 mg/L in males and 0.88 mg/L in females. It showed little toxicity in rabbits exposed dermally, with an LD<sub>50</sub> value greater than 5000 mg/kg bw. A recrystallized form of dimethipin was severely irritating to the eye in rabbits. Technical-grade dimethipin was not irritating to rabbit skin but weakly sensitized the skin of guinea-pigs.

WHO (1999) has classified dimethipin as 'slightly hazardous'.

In 90-day and long-term tests for toxicity in rats, the liver was the main target at doses of 10 mg/kg bw per day and above. The clinical findings consisted of increased absolute and relative weights of the liver and increased serum cholesterol concentration and transaminase activity. At doses greater than 85 mg/kg bw per day, hepatocellular hypertrophy was seen in 90-day studies, whereas in long-term studies the hepatocellular effects included focal dilatation of bile ducts, biliary cysts and bile-duct hyperplasia.

The testis was identified as another target organ. In a one-year study in dogs given dimethipin, testicular changes were seen at all doses, the lowest dose being 300 ppm (equivalent to 7.5 mg/kg bw per day), but these were considered not to be related to treatment but to be a result of poor nutritional status or incidental findings, as they were similar to testicular lesions seen in other studies in dogs in the same laboratory. Additionally, no testicular lesions were seen in dogs in a 90-day study. In contrast, Sprague-Dawley rats fed diets containing technical-grade dimethipin for two years showed increased incidences and severity of seminiferous tubular degeneration at the two highest doses, 1750 and 3500 ppm (equal to 78 and 160 mg/kg bw per day), associated at the high dose with hypospermia in the epididymides. The NOAEL for testicular degeneration was 40 ppm (2 mg/kg bw per day).

In a 90-day study in dogs given dimethipin in the diet, the lowest dose of 100 ppm (equivalent to 2.5 mg/kg bw per day) was the NOAEL, on the basis of oesophageal lesions at the LOAEL of 300 ppm (equivalent to 7.5 mg/kg bw per day).

In the 1-year study in dogs described above, the effects seen at 1000 and 3000 ppm (equal to 25 and 75 mg/kg bw per day) included 'thinness' and increased relative kidney weights in animals of each sex. At this dose, males had decreased blood urea nitrogen and creatinine concentrations and females had increased relative liver weights. One male and three females at the highest dose died, and animals of each sex had decreased body weights and food consumption, hypocellularity of the bone marrow, gastritis, oedema, ulceration of the gastrointestinal tract, thymic atrophy, nephritis, centrilobular degeneration of the liver, splenic hyperplasia and lymphadenitis. The NOAEL was 300 ppm (equivalent to 7.5 mg/kg bw per day) on the basis of increased relative liver weights, increased alanine aminotransferase and alkaline phosphatase activities, and hepatocellular degeneration in females at 1000 ppm (equivalent to 25 mg/kg bw per day).

In a 78-week study of carcinogenicity in mice, a statistically significant increase in the incidence of alveolar and bronchiolar carcinomas was seen in males at the highest dose (2000 ppm, equal to 300 mg/kg bw per day). The combined incidence of lung adenocarcinoma and adenoma was significantly greater than the mean for controls in five previous studies but not when compared with that for concurrent controls or with the mean maximum incidence in controls in previous studies. As lung adenomas and adenocarcinomas occur commonly in this strain of mice, this finding was not considered to be of toxicological relevance. The NOAEL for systemic toxicity was

80 ppm (equal to 12 mg/kg bw per day) on the basis of increased erythrocyte volume fraction at the LOAEL of 400 ppm (equal to 60 mg/kg bw per day).

In two 2-year studies in rats, the NOAEL for systemic toxicity was 40 ppm (equal to 2 mg/kg bw per day) on the basis of decreased body weights, increased absolute and relative weights of the liver, an increased incidence of biliary hyperplasia, and testicular degeneration at higher doses. No increase in tumour incidence was observed in rats at any dose. The Meeting concluded that dimethipin is not carcinogenic in mice or rats and is unlikely to pose a carcinogenic risk to humans.

Dimethipin has been tested in an adequate range of tests for genotoxicity *in vitro* and *in vivo*. Negative results were obtained in most assays. It induced a weak mutagenic response in one test for forward mutation in mouse lymphoma cells in the presence of metabolic activation. The Meeting concluded that dimethipin is unlikely to be genotoxic.

In a two-generation study of reproductive toxicity in rats, the highest dose of 800 ppm (equivalent to 40 mg/kg bw per day) caused decreased body weights and food consumption in parental animals of each sex and decreased body weights in pups on days 7, 14, and 21 of lactation. The NOAEL for both systemic toxicity in the parental generation and developmental toxicity in the pups was 200 ppm (equivalent to 10 mg/kg bw per day).

In a study of developmental toxicity in rats, excess mortality occurred at doses of 400 and 800 mg/kg bw per day. The NOAEL for both maternal and developmental toxicity was 160 mg/kg bw per day. In rabbits, the NOAEL for both maternal and developmental toxicity was 20 mg/kg bw per day. Does at the maternal LOAEL of 40 mg/kg bw per day showed body-weight loss on days 6–12 of gestation and decreased weight gain on days 6–28 of gestation. The LOAEL for developmental toxicity was 40 mg/kg bw per day on the basis of an increased incidence of fetuses with skeletal malformations (scoliosis).

The present Meeting confirmed the ADI of 0–0.02 mg/kg bw established by the 1988 Joint Meeting on the basis of the NOAEL of 2 mg/kg bw per day in the 2-year study in rats conducted in 1981 and a safety factor of 100. This ADI is supported by the NOAEL of 40 ppm, equivalent to 2 mg/kg bw per day, in the 2-year study in rats conducted in 1996. The ADI provides a 1000-fold margin of safety with respect to the NOAEL of 20 mg/kg bw per day for developmental toxicity in rabbits, which showed skeletal malformations at the LOAEL of 40 mg/kg bw per day.

An acute reference dose of 0.02 mg/kg bw was established on the basis of the NOAEL of 20 mg/kg bw per day for skeletal malformations in the study of developmental toxicity in rabbits and a safety factor of 1000. This high safety factor was used because of the nature of the effect.

### Toxicological evaluation

#### *Levels that cause no toxic effect*

Mouse:	80 ppm, equivalent to 12 mg/kg bw per day (toxicity in a 78-week study of toxicity and carcinogenicity)
Rat:	40 ppm, equivalent to 2 mg/kg bw per day (toxicity in two 2-year studies of toxicity and carcinogenicity) 160 mg/kg bw per day (maternal and developmental toxicity in a study of developmental toxicity) 10 mg/kg bw per day (parental and reproductive toxicity in a two-generation study of reproductive toxicity)
Rabbit:	20 mg/kg bw per day (maternal and developmental toxicity in a study of developmental toxicity)
Dog:	100 ppm, equivalent to 2.5 mg/kg bw per day (toxicity in a 90-day study of toxicity)

#### *Estimate of acceptable daily intake for humans*

0–0.02 mg/kg bw

#### *Estimate of acute reference dose*

0.02 mg/kg bw

*Studies that would provide information useful for continued evaluation of the compound*

## Further observations in humans

**Toxicological end-points relevant for setting guidance values for dietary and non-dietary exposure to dimethipin***Absorption, distribution, excretion and metabolism in mammals*

Rate and extent of oral absorption	69% within 24 h, rats
Dermal absorption	Low dermal penetration, rabbits
Distribution	Widely distributed, rats

**Toxicological end-points(contd)**

Potential for accumulation	No evidence of accumulation
Rate and extent of excretion	89% within 48 h mainly via urine
Metabolism in animals	Parent < 5%; metabolites consist of glutathione conjugates and degradates, rats
Toxicologically significant compounds (animals, plants and environment)	Parent compound

*Acute toxicity*

	Oral toxicity is moderate, but only slightly toxic by dermal and inhalation routes of exposure
Rat, LD <sub>50</sub> , oral	440 mg/kg bw (males) and 600 mg/kg bw (females)
Rabbit, LD <sub>50</sub> , dermal	> 5000 mg/kg bw
Rat, LC <sub>50</sub> , inhalation	0.88 mg/L, 4 h (female) and 1.5 mg/L, 4 h (males)
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Severely irritating
Guinea-pig, dermal sensitization	Weakly sensitizing

*Short-term toxicity*

Target/critical effect	Liver: hepatotoxicity, hepatic hypertrophy, rats
Lowest relevant oral NOAEL	2 mg/kg bw per day, rats
Lowest relevant dermal NOAEL	1000 mg/kg bw per day (highest dose tested), rats
Lowest relevant inhalation NOAEL	Not determined

*Long-term toxicity and carcinogenicity*

Target/critical effect	Rat liver: increased weight, liver enzymes, bile-duct hyperplasia; rat testis: degeneration
Lowest relevant NOAEL	2 mg/kg bw per day in two 2-year studies, rat
Carcinogenicity	Not carcinogenic in mice or rats

*Genotoxicity*

Not genotoxic

*Reproductive toxicity*

Reproductive target/critical effect	None. Decreased pup body weight on days 7, 14, and 21 of lactation at maternally toxic doses, rats
Lowest relevant reproductive NOAEL	10 mg/kg bw per day
Developmental target/critical effect	Increased incidence of skeletal malformations, rabbit
Lowest relevant developmental NOAEL	Rabbit, 20 mg/kg bw per day

*Neurotoxicity/Delayed neurotoxicity*

No evidence of neurotoxicity

*Medical data*

None

<b>Summary</b>	<b>Value</b>	<b>Study</b>	<b>Safety factor</b>
ADI	0–0.02 mg/kg bw	Two 2-year studies in rats	100
Acute reference dose	0.02 mg/kg bw	Skeletal malformations in rabbits	1000

## References

- Babish, J.G. (1977) Harvade® (N252) Tech. C-8-1162-00. Acute inhalation study in rats. Unpublished report from Food and Drug Research Laboratories, Inc. Submitted to WHO by Uniroyal Inc., USA.
- Benson, B.W. (1981) 1-Year dietary toxicity study in dogs with N252. Unpublished report from International Research and Development Corp., USA. Submitted to WHO by Uniroyal Chemical Co., Inc., Bethany, Connecticut, USA
- Billings, T.J. (1987) A (C14)-radiolabeled pharmacokinetics and metabolism study in the rat using Harvade. Southwest Bio-Labs, Inc. (Project No. 8656r). Submitted to WHO by Uniroyal Inc., USA.
- Blaszczak, D.L. (1992a) Acute oral toxicity study in rats with Harvade technical (Project No. 6169-91), unpublished report from Bio/dynamics, Inc. Submitted to WHO by Uniroyal Inc., USA.
- Blaszczak, D.L. (1992b) Acute dermal toxicity study in rabbits with Harvade technical (Project No. 6170-91), unpublished report from Bio/dynamics, Inc. Submitted to WHO by Uniroyal Inc., USA.
- Blaszczak, D.L. (1992c) Primary dermal irritation study in rabbits with Harvade technical (Project No. 6171-91), unpublished report from Bio/dynamics, Inc. Submitted to WHO by Uniroyal Inc., USA.
- Bootman, J. & Lodge, D.C. (1982) ARS7728 (technical grade Harvade®): Assessment of its ability to induce genetic damage in *Saccharomyces cerevisiae*. Unpublished report from Life Science Research, United Kingdom. Submitted to WHO by Uniroyal Inc., USA.
- Byrd, J.W. (1992), Nature of the residue of radiolabeled Harvade in lactating goat; Part I: Dosing, specimen collection, and quantitation of Harvade residues in lactating goats (Project No. 9202), unpublished study. Submitted to WHO by Uniroyal Chemical Co., Inc., Bethany, Connecticut, USA.
- Burtner, B.R., Kennedy, G.L., Kinoshita, F.K. & Keplinger, M.L. (1976) 90-Day sub-acute oral toxicity study with UBI-N252 technical in dogs. Unpublished report (IBT No. 61108063) from Industrial Bio-Test Laboratories, Inc., USA (validated by the Canadian Health Protection Branch). Submitted to WHO by Uniroyal Inc., USA.
- Bushby, S.R.M. (1970) Hematological studies during toxicity tests. In: Paget, G.E., ed., *Methods in Toxicology*, Oxford, Blackwell Scientific Publications.
- Caplan, J. & Merricks, D.L. (1978) <sup>14</sup>C-Harvade® radiocarbon study in rats. Unpublished report from Biospherics, Inc., USA. Submitted to WHO by Uniroyal Chemical Co., Inc., Bethany, Connecticut, USA.
- Forster, R., Edwards, N. & Nunziata, A. (1984a) Mitotic gene conversion in *Saccharomyces cerevisiae* D4. Test substance: Dimethipin tech. 131001-M-00284. Final report. Unpublished report from Life Science Research, Roma Toxicology Centre, Italy. Submitted to WHO by Uniroyal Chemical Co., Bethany, Connecticut, USA.
- Forster, R., Mosesso, P. & Nunziata, A. (1984b) Micronucleus test. Test substance: Dimethipin tech. Final report. Unpublished report from Life Science Research, Roma Toxicology Centre, Italy. Submitted to WHO by Uniroyal Chemical Co., Inc., Bethany, Connecticut, USA.
- Galloway, S.M. & Brusick, D.J. (1981) Mutagenicity evaluation of Harvade® (N252) 90% D-11401 in the sister chromatid exchange assay with Chinese hamster ovary (CHO) cells. Final report. Unpublished report from Litton Bionetics, Inc., USA. Submitted to WHO by Uniroyal Chemical Co., Inc., Bethany, Connecticut, USA.
- Gay, M.H. & Lau, R.C.M. (1996) Nature of the residue of dimethipin in lactating goat (Project No. 9202), unpublished study. Submitted to WHO by Uniroyal Chemical Co., Inc., Bethany, Connecticut, USA.
- Goldenthal, E.I. (1991) 21-Day dermal toxicity study in rats with Harvade technical (Report No. 399-116), unpublished study from International Research and Development Corp. Submitted to WHO by Uniroyal Inc., USA.
- Goldenthal, E.I. (1993) 13 Week dietary toxicity study in rats with Harvade technical (Project No. 399-133), unpublished study from International Research and Development Corp. Submitted to WHO by Uniroyal, Inc. USA.
- Goldenthal, E.I. (1996) Two year dietary chronic toxicity and oncogenicity study in rats (with) Harvade technical (dimethipin) (Project No. 399-134), unpublished study from MPI Research. Submitted to WHO by Uniroyal Chemical Co., Inc., Bethany, Connecticut, USA.
- Griffiths, J.T. & Koschier, F.J. (1980) Rabbit eye irritation study with Harvade technical (recrystallized) (Project No. 6413a), unpublished report from Food and Drug Research Laboratories, Inc. Submitted to WHO by Uniroyal Inc., USA
- Hoffman, G.M. (1992) An acute nose-only inhalation toxicity study of Harvade technical in the rat (Project No. 91-8376), unpublished study from Bio/dynamics, Inc. Submitted to WHO by Uniroyal Inc., USA.
- Jagannath, D.R. & Brusick, D.J. (1978) Mutagenicity evaluation of N-252, technical lot D10406 BL8998 CC0005 in the Ames *Salmonella*/microsome plate test. Final report. Unpublished report from Litton Bionetics, Inc., USA. Submitted to WHO by Uniroyal Chemical Co., Inc., Bethany, Connecticut, USA.
- Jagannath, D.R. & Brusick, D.J. (1981) Mutagenicity evaluation of Harvade® (N252) 98% D-11401 in the Ames *Salmonella*/microsome plate test. Final report. Unpublished report from Litton Bionetics, Inc., USA. Submitted to WHO by Uniroyal Chemical Co., Inc., Bethany, Connecticut, USA.
- Kehoe, D.F. & Mackenzie, K.M. (1982) Final report. Two-generation rat reproduction study with N252. Unpublished report from Hazleton Raltech, Inc., USA. Submitted to WHO by Uniroyal Inc., USA.

- Knickerbocker, M., Re, T.A. & Babish, J.G. (1977) Teratologic evaluation of N252 (Harvade®) technical in Sprague-Dawley rats. Unpublished report from Food and Drug Research Laboratories, Inc., USA, submitted to WHO by Uniroyal Chemical Co., Inc., Bethany, Connecticut, USA
- Lau, R.C.M. & Gay, M.H. (1993) Nature of the residue of <sup>14</sup>C-Harvade in laying hens (Uniroyal Chemical Co. Project No. 91121), unpublished stud. Submitted to WHO by Uniroyal Chemical Co., Inc., Bethany, Connecticut, USA.
- Madison, W.A. (1983) Technical grade Harvade®. Dermal sensitization in guinea pigs (modified closed patch technique). Unpublished report from Hazleton Raltech, Inc., USA. Submitted to WHO by Uniroyal Inc., USA.
- Marias, A.J., Kennedy, G.L., Kinoshita, F.K. & Keplinger, M.L. (1976) 90-Day sub-acute oral toxicity study with UBI-N252 technical in albino rats. Unpublished report (IBT NO. 622-08070) from Industrial Bio-Test Laboratories, Inc., USA (validated by the Canadian Health Protection Branch). Submitted to WHO by Uniroyal Chemical Co., Inc., Bethany, Connecticut, USA.
- McGee, D.H. (1983) Unpublished letter from International Research Development Corp., USA, to Uniroyal Chemical Co. Submitted to WHO by Uniroyal Chemical Co., Inc., Bethany, Connecticut, USA
- McManus, J.P. (1984) Metabolism of Harvade® in goats. Unpublished report from Uniroyal Chemical Co. Submitted to WHO by Uniroyal Chemical Co., Inc., Bethany, Connecticut, USA.
- McManus, J.P. (1986) Mouse micronucleus test with dimethipin technical, Life Sciences Research, Rome (Report 180001-M-06886). Submitted to WHO by Uniroyal Chemical Co., Inc., Bethany, Connecticut, USA
- McManus, J.P. (1987a) Analysis of urine samples from dimethipin (Harvade) rat pharmacokinetic study. Submitted to WHO by Uniroyal Chemical Co., Inc., Bethany, Connecticut, USA.
- McManus, J.P. (1987b) Metabolism of (C14) Dimethipin (Harvade) in the Rat - Urinary Metabolite Identification. Uniroyal Project No. 85125. Submitted to WHO by Uniroyal Chemical Co., Inc., Bethany, Connecticut, USA.
- McManus, J.P. (1987c) *In vivo/in vitro* UDS study in rats, Robens Institute (Report No. 4/86/TX). Submitted to WHO by Uniroyal Chemical Co., Inc., Bethany, Connecticut, USA.
- McMeekin, S.O., Schardein, J.L. & Blair, M. (1981) N252 (Harvade® technical). Teratology study in rabbits. Unpublished report from International Research and Development Corp., Inc., USA. Submitted to WHO by Uniroyal Chemical Co., Inc., Bethany, Connecticut, USA.
- Myhr, B.C. & Brusick, D.J. (1981) Mutagenicity evaluation of Harvade® (N252) in the mouse lymphoma forward mutation assay. Revised final report. Unpublished report from Litton Bionetics, Inc., USA. Submitted to WHO by Uniroyal Chemical Co., Inc., Bethany, Connecticut, USA.
- Reagan, E.L. & Becci, P.J. (1982) Acute dermal toxicity study (LD<sub>50</sub>) in albino rabbits of Harvade® (N252). Unpublished report from Food and Drug Research Laboratories, Inc., USA. Submitted to WHO by Uniroyal Chemical Co., Inc., Bethany, Connecticut, USA.
- Serota, D.G., Alsaker, R.D. & Banas, D. (1981a) 18-month toxicity and oncogenicity study in mice. N252 technical. Final report. Unpublished report from Hazleton Laboratories America, Inc., USA. Submitted to WHO by Uniroyal Chemical Co., Inc., Bethany, Connecticut, USA.
- Serota, D.G., Alsake, R.D., Dawkins, K.K. & Kunalzins, W. (1981b) 104-Week chronic toxicity study in rats. N252 (Harvade® technical) final report. Unpublished report from Hazleton Laboratories America, Inc., USA, submitted to WHO by Uniroyal Chemical Co., Inc., Bethany, Connecticut, USA.
- Shapiro, R. (1977a) Untitled and unpublished report from Product Safety Labs, USA. Submitted to WHO by Uniroyal Chemical Co., Inc., Bethany, Connecticut, USA.
- Shapiro, R. (1977b) Untitled and unpublished report from Product Safety Labs, USA. Submitted to WHO by Uniroyal Chemical Co., Inc., Bethany, Connecticut, USA.
- Smilo, A.R., Fuller, G.B., Tortora, N.J., Curtiss, K. & Cardona, R.A. (1978) Characterization of excretory metabolites from <sup>14</sup>C-Harvade® rat balance study. Unpublished report from Uniroyal Chemical. Submitted to WHO by Uniroyal Chemical Co., Inc., Bethany, Connecticut, USA.
- Sorg, R.M., Naismith, R.W. & Matthews, R.J. (1983) CHO metaphase analysis *in vitro* chromosome aberration analysis in Chinese hamster ovary cells (CHO). PH320-UN-00183 Harvade®, unpublished report from Pharmakon Research International, Inc., USA. Submitted to WHO by Uniroyal Chemical Co., Inc., Bethany, Connecticut, USA.
- Squire, R.A. (1984) Unpublished letter to Uniroyal Chemical. Submitted to WHO by Uniroyal Chemical Co., Inc., Bethany, Connecticut, USA.
- Varner, L.L. & Matthews, R.J. (1977) Acute oral LD<sub>50</sub> in rats. Uni-N-252 CND-9801 Lot No. BL-6731. Revised report. Unpublished report from Pharmakon Laboratories, USA. Submitted to WHO by Uniroyal Chemical Co., Inc., Bethany, Connecticut, USA.
- WHO (1999) *Recommended Classification of Pesticides by Hazard and Guidelines to Classification 1998–1999* (WHO/PCS/98.21/Rev. 1), Geneva, International Programme on Chemical Safety.