BENTAZONE

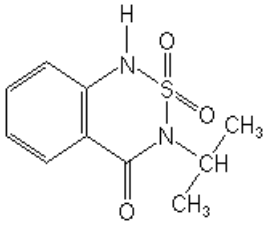
First draft prepared by
D. Kanungo[1], Vicki Dellarco[2] and Les Davies[3]

[1] Food Safety and Standards Authority of India, New Delhi, India
[2] Office of Pesticide Programs, Environmental Protection Agency, Washington, DC, United States of America (USA)
[3] Australian Pesticides and Veterinary Medicines Authority, Kingston, Australia

Explanation

Bentazone (Figure 1) is the International Organization for Standardization (ISO)–approved common name for 3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one-2,2-dioxide (International Union of Pure and Applied Chemistry), with Chemical Abstracts Service number 25057-89-0. Bentazone is a post-emergence herbicide used for selective control of broadleaf weeds and sedges in beans, rice, corn, peanuts, mint and others. It acts by interfering with photosynthesis.

Figure 1. Chemical structure of bentazone



Bentazone was first evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 1991, when an acceptable daily intake (ADI) of 0–0.1 mg/kg body weight (bw) was established on the basis of a no-observed-adverse-effect level (NOAEL) of 9 mg/kg bw per day (for increased clotting times and increased output of urine with decreased specific gravity) in a long-term study of toxicity in rats and using a safety factor of 100. In 1998, the Meeting re-evaluated bentazone and data on 6-hydroxybentazone, a metabolite of bentazone. The Meeting concluded that 6-hydroxybentazone was less toxic than bentazone and reaffirmed the ADI of 0–0.1 mg/kg bw. Because data were not evaluated to establish an acute reference dose (ARfD), the Meeting in 2004 re-evaluated bentazone and concluded that the establishment of an ARfD was not necessary.

Bentazone is being reviewed at the present meeting as part of the periodic re-evaluation programme of the Codex Committee on Pesticide Residues.

Since the 2004 JMPR review, no relevant new studies have been provided. Two published literature studies on the effects of bentazone on spermatogenesis in mice and on litter size and postnatal growth in rats were submitted. Most of the studies do not comply with good laboratory practice (GLP), as they were generated before implementation of GLP.

Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution and excretion

Mice

[^{14}C]Bentazone was administered intravenously in corn oil to C57 mice (number of animals not stated). Mice were housed in metabolism cages and provided with feed and water ad libitum. More than 95% of the administered radioactivity was recovered in the urine within 48 hours. Only traces (< 1%) were excreted in the faeces. Unchanged bentazone was the major constituent in urine; 76.83% ± 3.61% of the radioactivity recovered in the first 7 hours was identified as bentazone. The study was conducted before the implementation of GLP (Booth, 1974).

Rats

Single oral doses of 0.8 mg [^{14}C]bentazone (0.37 MBq per animal; labelled in the benzene ring) were administered by gavage in 50% aqueous ethanol to male and female Sprague-Dawley (CFY) rats (200–250 g). Urine was collected at 3, 6, 12 and 24 hours after dosing and daily thereafter. Faeces were collected daily. Expired gases from two animals were monitored for $^{14}CO_2$. Rats were sacrificed after 4 days. Young male rats were given total oral doses of 2.4 mg [^{14}C]bentazone, sacrificed at different intervals after dosing and subjected to whole-body autoradiography. Three male rats (150–175 g) with biliary cannulae were dosed as above, and bile was collected at hourly intervals for 40 hours; urine and faeces were also collected.

Oral doses of [^{14}C]bentazone were rapidly and almost quantitatively absorbed in rats. The radioactivity was quite rapidly excreted, mostly in the urine (approximately 91% within 24 hours), with only traces secreted into bile (0–2.9%). Over 4 days, less than 1% of the total dose was excreted in the faeces, and less than 0.02% in the expired air. Small amounts of radioactivity (0.54%) remained in the carcass after 4 days. Autoradiography showed that, after 1 hour, levels of radioactivity were high in the stomach, liver, heart and kidney, but undetectable in the brain or spinal cord. As determined by thin-layer chromatography, most of the radioactivity in the urine (84%) was unchanged bentazone. Two minor unidentified metabolites were detected, accounting for 2.3% and 0.8% of the radioactivity, respectively.

The study is scientifically valid, although it was conducted prior to the implementation of GLP (Hathway et al., 1971).

The bioavailability and metabolic fate of [^{14}C]bentazone free acid and [^{14}C]bentazone sodium salt were compared after oral administration to two groups of 12 adult Sprague-Dawley (CFY) rats (200–250 g). The first group received non-radioactive bentazone free acid (4 mg/kg bw dissolved in aqueous ethanol) once daily for 7 days. Twenty-four hours after the seventh dose, each rat received [^{14}C]bentazone free acid (4 mg/kg bw dissolved in aqueous ethanol), and blood samples were removed from a tail vein at intervals during 24 hours. The second group was treated in the same way, except bentazone sodium salt was used (dose equal to 4 mg/kg bw as the free acid).

For an excretion–retention study, three rats of each sex were orally intubated with [^{14}C]bentazone sodium (dose equal to 4 mg/kg bw as the free acid) in aqueous ethanol. Urine was collected from 0 to 6 hours and from 6 to 24 hours and then at 24-hour intervals over 5 days. Faeces were collected at 24-hour intervals for 48 hours and then as a single sample up to 5 days. After 5 days, the rats were killed.

After oral administration of either [^{14}C]bentazone free acid or the sodium salt to rats (4 mg/kg bw), no significant differences were detected in the maximal plasma concentrations of radioactivity or in the plasma half-lives of elimination. The mean time of occurrence of maximal concentrations of plasma radioactivity was shorter after dosing with the free acid (approximately 0.8 hour) than with the sodium salt (approximately 1.1 hours), although the difference was not statistically significant. Thus, the rate and extent of absorption were similar after administration of the sodium salt and free acid forms of bentazone.

An oral dose of [^{14}C]bentazone sodium salt (4 mg/kg bw) was well absorbed and rapidly eliminated; 6 hours after dosing, means of 63% (males) and 51% (females) of the dose had been excreted in the urine, and after 24 hours, means of 90% and 91% had been eliminated in males and females, respectively. Faecal excretion of radioactivity over 5 days accounted for 1.8% and 1.0% of the dose in males and females, respectively; no radioactivity was detected in the carcass at this time. Radioactivity excreted in the urine was mainly unchanged bentazone (85%). No significant differences were found in the pharmacokinetics or metabolism of bentazone sodium salt compared with bentazone free acid.

The study was conducted prior to the implementation of GLP, but is scientifically acceptable (Chasseaud et al., 1979).

To ascertain the extent of urinary excretion after oral dosing, a single oral dose of [U-phenyl-^{14}C]bentazone sodium salt (radiochemical purity > 97%) was administered as an aqueous solution to adult male CD rats (approximately 220 g) at a dose equal to 4 mg/kg bw as the free acid. Rats were sacrificed at different times (four per group) up to 72 hours after dosing. Radioactivity in the urine was checked during various intervals (0–6, 6–12 and 12–24 hours) post-administration. The metabolite pattern in combined urine samples collected during these intervals was investigated by thin-layer chromatography. Means of 65%, 15% and 3% of the dose were excreted in the urine as unchanged bentazone during 0–6 hours, 6–12 hours and 12–24 hours, respectively, in sum (83%) representing more than 90% of all radioactivity excreted in the urine. About 2% of the dose corresponded to 6-hydroxybentazone. Most of the remaining radioactivity (approximately 2% of the dose) was associated with polar material at the origin of the chromatogram. The reference compound 8-hydroxybentazone did not correspond to any of the radioactive components found in this experiment. These results indicate that orally administered bentazone is excreted in the urine almost entirely unchanged (83% of total dose), with traces (2%) of 6-hydroxybentazone and polar material.

The study was conducted according to the principles of GLP. A quality assurance (QA) statement was attached (Hawkins et al., 1986a).

The kinetics and metabolism of [^{14}C]bentazone were investigated in CD rats (nominal age 8 weeks). The radiochemical purity of [^{14}C]bentazone used was greater than 99%. In a preliminary experiment, two rats of each sex (205–210 g) were gavaged with a single oral high dose (198 mg/kg

bw) in order to determine whether significant amounts of ^{14}C were eliminated in the expired air. Urine, faeces and expired air were collected separately and radioassayed at 24-hour intervals for 5 days, at which time the animals were sacrificed for analysis of residual radioactivity in the carcass. By 120 hours post-dosing, less than 0.03% of the radioactivity had been eliminated in the expired air.

The details of the main experiment are shown in Table 1.

Table 1. Experimental design of a kinetics and metabolism study in rats

No.	Experiment	No. of animals	Mean body weight (g)	Mean dose (mg/kg bw)
1	Single oral low dose	5M + 5F	199 (191–209)[a]	3.8 (3.6–4.0)[a]
2	Single oral high dose	5M + 5F	200 (195–204)	205 (200–210)
3	Single oral low dose to rats pretreated for 14 days with an oral low dose of unlabelled bentazone	5M + 5F	215 (177–241)	3.6 (3.3–3.7) 4.0[b]
4	Single low intravenous dose of sodium salt	5M + 5F	202 (194–207)	4.1[c] (3.9–4.3)

From Hawkins et al. (1987)
F, female; M, male
[a] Range given in parentheses.
[b] Unlabelled bentazone.
[c] Expressed as bentazone free acid.

Urine was collected at 8 and 24 hours post-dosing and thereafter over 24-hour periods for a total of 120 hours. Faeces were collected at 24-hour intervals for up to 120 hours. The animals were sacrificed at the end of the collection period for determination of residual radioactivity in tissues. Blood and the following organs and tissues were sampled for radioactivity: liver, kidneys, thyroid, spleen, adrenals, heart, brain, lungs, pancreas, ovaries/testes, uterus, gastrointestinal tract and samples of muscle, bone marrow and fat.

In a biliary excretion study, three rats of each sex per group (average weight 200 g) were treated with a single oral low dose (3.6 mg/kg bw; range 3.3–3.9 mg/kg bw) or a single oral high dose (195 mg/kg bw; range 180–210 mg/kg bw) of bentazone. Bile was collected from bile duct cannulae at 1.5-hour intervals for up to 48 hours, at which time the animals were sacrificed to assess carcass radioactivity.

For a plasma level study, five rats of each sex per group were treated as shown in Table 2.

Table 2. Experimental design of a plasma level study in rats

No.	Type of experiment	No. of animals	Mean body weight (g)	Mean dose (mg/kg bw)
1	Single oral low dose	5M + 5F	203 (198–208)[a]	3.6 (3.6–3.7)[a]
2	Single oral high dose	5M + 5F	204 (197–208)	197 (190–200)
3	Single oral low dose (sodium salt)	5M + 5F	203 (194–209)	4.1[b] (3.9–4.2)
4	Single low intravenous dose (sodium salt)	5M + 5F	200 (194–204)	4.5[b] (4.3–4.7)

From Hawkins et al. (1987)
F, female; M, male
[a] Range given in parentheses.
[b] Expressed as bentazone free acid.

Blood samples were taken from the tail and vein at 0.25, 0.5, 1, 2, 3, 4, 6 and 24 hours post-dosing and at 24-hour intervals until plasma radioactivity had declined to the limit of detection. In addition, one sample was collected at 5 minutes from rats dosed intravenously with bentazone sodium.

For a tissue distribution study, 10 male and 5 female rats were given low oral doses (4 mg/kg bw) of [^{14}C]bentazone once daily for 7 days, and one rat of each sex was sacrificed at various time points (0.5, 6, 24, 72 and 120 hours after the last dose) for assay of radioactivity in the following organs and tissues: liver, kidneys, heart, lungs, brain, eyes, gonads (testes or ovaries), spleen, pancreas, adrenals, thyroid, gastrointestinal tract, uterus and samples of muscle, bone marrow and fat. The remaining carcass was discarded. In addition, males were also sacrificed at various time points (0.5, 6, 24, 72 and 120 hours after the last dose) for whole-body autoradiography.

Metabolite characterization studies were performed with representative urine and faecal samples collected during the first 24 hours post-dosing. Structural characterization (gas chromatography/mass spectrometry) of the major urinary radioactive component was performed. Quantification of metabolites in raw urine from animals of the various dose groups of the main study was done by high-performance liquid chromatography. Additionally, representative urine samples were treated with β-glucuronidase/sulfatase to assess the extent of conjugation of bentazone metabolites.

Single intravenous low dose: Five days after administration of a single intravenous dose of [phenyl-U-^{14}C]bentazone sodium salt (mean dose equal to 4.1 mg/kg bw as the free acid) to rats, approximately 95.4% (males) and 90.2% (females) of the dose were accounted for. Urine accounted for at least 93.87% of the dose in males and 88.96% in females. Most (91.5% and 85.8% in males and females, respectively) of the elimination in urine was complete within the first 24 hours. Faecal elimination accounted for about 1.18% (males) and 0.51% (females) of the dose.

Total radioactive residue in the carcass amounted to 0.32% of the dose in females; no data were available for males. Radioactive residues in kidneys amounted to 0.019 µg/g and 0.026 µg/g in males and females, respectively; and in the uterus, to 0.002 µg/g. Residues in other tissues were at or below the limit of measurement (twice the background radioactivity).

Single low oral dose: Five days after administration of a single oral dose of [phenyl-U-^{14}C]bentazone free acid (mean 3.8 mg/kg bw) to rats, approximately 91.95% (males) and 90.05% (females) of the dose were accounted for. Urine accounted for at least 89.8% (males) and 88.13% (females) of the dose; most of the elimination in urine (86.7% and 83.7% in males and females, respectively) was complete within the first 24 hours. Faecal elimination accounted for about 1.5% of the dose in males and 0.76% of the dose in females. Radioactive residue in the carcass amounted to 0.48% (males) and 0.69% (females) of the dose. Residues in tissues were at or below the limit of measurement (twice the background radioactivity).

Single high oral dose: Five days after administration of a single oral dose of [phenyl-U-^{14}C]bentazone free acid (mean 205 mg/kg bw) to rats, approximately 97.13% (males) and 95.78% (females) of the dose were accounted for. Urine accounted for at least 94.32% (males) and 93.03% (females) of the dose. Most of this elimination in urine (92.0% and 91.0% of the dose in males and females, respectively) was complete within the first 24 hours. Faecal elimination accounted for about 2.27% (males) and 2.00% (females) of the dose. Total radioactive residue in the carcass amounted to 0.24% and 0.17% of the dose in males and females, respectively. Residues in tissues were at or below the limit of measurement (twice the background radioactivity).

Single low oral dose with pre-dosing: One hundred and twenty hours after administration of a single oral dose of [phenyl-U-^{14}C]bentazone free acid (mean 3.6 mg/kg bw) to rats, preceded by single daily oral doses of non-radioactive bentazone free acid (4 mg/kg bw) for 14 days, approximately 96.81% and 92.5% of the dose were accounted for in males and females, respectively. Elimination in the urine accounted for at least 95.86% of the dose in males and 90.49% in females. Most (94.1% and 85.2% of the dose in males and females, respectively) of the elimination in urine was complete within the first 24 hours. Elimination in the faeces accounted for about 0.92% of the

dose in males and 1.44% of the dose in females. Total radioactive residue in the carcass amounted to 0.5% of the dose in females and was undetectable in males. Residues in tissues were at or below the limit of measurement (twice the background radioactivity).

Biliary excretion studies: Excretion of radioactivity in bile was very limited using bile duct–cannulated rats. At the high dose, biliary excretion amounted to 0.80% (females) and 1.84% (males) of the total dose. At the low dose, means of 0.2% (females) and 1.3% (males) of the radioactivity administered were excreted in the bile. Biliary excretion was essentially complete by 24 hours.

Time course of plasma radioactivity: Radioactivity in plasma reached a maximum by 15 minutes at the low oral dose (free acid or sodium salt) and by 1 hour at the high dose. Determination of the area under the plasma concentration–time curve (AUC) per unit dose revealed significantly higher values for the high-dose groups compared with the low-dose groups (Table 3). AUC values for females dosed with low oral doses were significantly lower (nearly half) than AUC values in females dosed intravenously; the corresponding values for males were not significantly different.

Table 3. Mean areas under the plasma concentration–time curves in rats dosed with [phenyl-U-^{14}C]bentazone (free acid or sodium salt)

Test group (dose in males/females, in mg/kg bw)	Form of bentazone	AUC (µg·h/ml) / dose (mg/kg bw)	
		Males	Females
Oral low (3.7/3.6)	Free acid	8.0 ± 2.6	3.5 ± 0.7
Oral high (196/198)	Free acid	15.0 ± 3.8	11.0 ± 3.9
Oral low (4.1/4.0)	Sodium salt	6.2 ± 1.8	3.5 ± 1.1
Intravenous low (4.5/4.4)	Sodium salt	6.0 ± 2.0	6.8 ± 1.2

From Hawkins et al. (1987)

Time course of tissue levels of radioactivity: Concentrations of radioactivity were highest at 0.5 hour after dosing in tissues obtained from pairs of rats sacrificed at different times after the last of seven low-level oral doses of [^{14}C]bentazone. At this time, concentrations in most tissues ranged from 0.1 to 5 µg/g. Higher concentrations were confined to the gastrointestinal tract, kidney, thyroid and plasma (5–20 µg/g). At 6 hours, concentrations of radioactivity were generally in the region of 0.05–1 µg/g, apart from higher concentrations in the gastrointestinal tract, kidney, thyroid and plasma (0.5–5 µg/g). At 24 and 120 hours after the last dose, concentrations of radioactivity in all tissues examined were below 0.1 µg/g, except in the thyroid (< 0.3 µg/g).

A comparison of the tissue distribution after a single and seven daily doses of [^{14}C]bentazone revealed no evidence of radioactivity accumulation after repeated dosing. Whole-body auto-radiography in general confirmed the distribution reported above. Some affinity of the compound to the keratinized layer of squamous epithelium of the non-fundic mucosa of the stomach was detected, as moderate levels of radioactivity were present there. In all tissues, there was a steady disappearance of label with time.

Metabolite characterization studies: Rat urine samples collected over a 24-hour period were analysed by high-performance liquid chromatography. Parent bentazone amounted to 80.63–91.02% of the dose in males and 77.37–88.95% of the dose in females. 6-Hydroxybentazone was present at up to 6.34% of the dose. The isomeric 8-hydroxybentazone was present in trace amounts (0.0–0.23% of the dose). Although the amounts of bentazone excreted in the urine appear to be slightly lower in the intravenously dosed rats, there were no major dose-dependent or pretreatment-dependent differences among groups. The level of glucuronide or sulfate conjugation was negligible or non-existent.

Characterization of metabolites in tissues was done in liver and kidney of rats sacrificed 6 hours after the last of seven daily oral doses of [phenyl-U-^{14}C]bentazone. The only compound detected was parent bentazone. The hydroxylated metabolites were not seen in tissue extracts,

presumably because the method was not sufficiently sensitive to detect metabolites that accounted for less than about 10% of the dose. Metabolites in bile and faeces were not characterized.

The results of these rat studies indicate a rapid absorption, distribution and primarily renal excretion of [^{14}C]bentazone, with no appreciable differences for the various dosing regimens applied. Biliary excretion was minimal. There was no significant difference in the pharmacokinetics of orally administered bentazone free acid and its sodium salt. However, some sex-related differences were observed with regard to AUC values for radioactivity. For females administered low doses of bentazone sodium, AUC values were significantly lower after oral administration (nearly half) than AUC values in females dosed intravenously; corresponding values for males were not significantly different. There was no evidence for accumulation of bentazone or its metabolites in tissues.

An investigation of urinary metabolites after the different dosing regimens indicated that bentazone was excreted in urine mostly unchanged (77–91% of the dose). The minor urinary metabolite was 6-hydroxybentazone, accounting for 1–6% of the dose, and was clearly distinguished from 8-hydroxybentazone, which was found only in male rats in trace amounts (ranging from 0.16% to 0.23% of the total dose). In tissues, only parent bentazone was detected using the available analytical methodology.

These studies were conducted in compliance with GLP, and a QA statement was supplied (Hawkins et al., 1987).

Rabbits

Three New Zealand albino male rabbits were administered a single oral dose of bentazone (^{14}C-labelled in the phenyl ring) at a dose of 5 mg/kg bw. Four animals were used as controls. The animals had free access to feed and drinking-water. They were housed in metabolism cages for faeces and carbon dioxide collection. Urine was collected daily via catheter. Blood was collected prior to and at 1, 2, 3, 4.5, 5.5, 9, 11, 13.5, 20 and 24 hours after dosing. The rabbits were sacrificed 6 days after treatment.

Total recovery of radioactivity averaged 93.5%, with 90.3% excreted in the first 24 hours. Most of the radioactivity was excreted in the urine (89.7%), and the remainder in the faeces (3.8%). Less than 0.1% was in the expired air. Tissue levels were below 0.02 mg/kg.

Blood levels reached a peak 2.5 hours after dosing. The elimination half-life of radioactivity in the blood was 2 hours and 12 minutes. Analytical investigation revealed that less than 1% of the radioactivity recovered could be assigned to 6-hydroxybentazone and 8-hydroxybentazone. The hydroxy compounds were eliminated in their free form in the urine, and not as conjugates with glucuronic acid.

The results after a single oral administration of [^{14}C]bentazone at 5 mg/kg bw to male rabbits were substantially similar to those obtained with rats. About 90% of the radioactivity administered was found in the urine, and nearly 4% in the faeces, within 24 hours. Six days after dosing, tissue residues were very low.

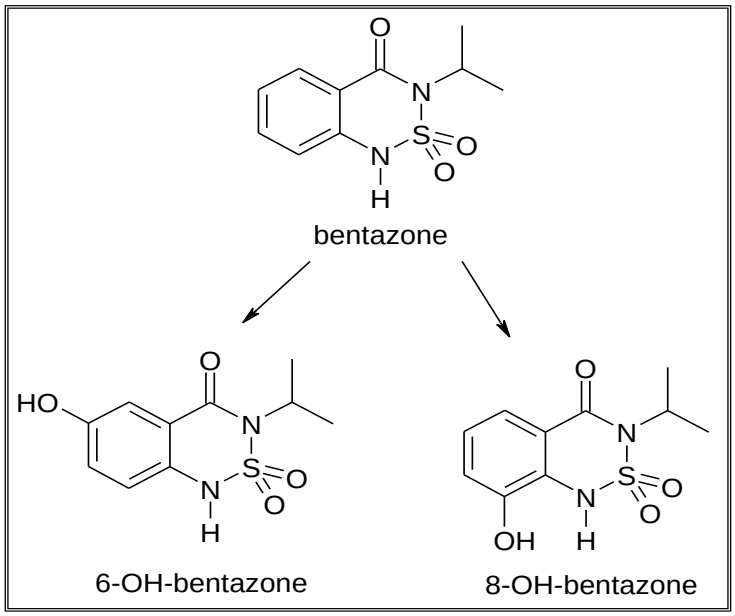
This study was performed prior to the implementation of GLP (Otto, 1974).

1.2 Metabolism

The metabolism of bentazone was investigated in a number of toxicokinetic studies following oral (rat and rabbit) or intravenous administration (mouse); these studies are described above. Bentazone was only poorly metabolized, with the parent compound being the predominant excretion product. Only small amounts of 6-hydroxybentazone and 8-hydroxybentazone could be detected. In rats, rabbits and mice, no conjugated products were found.

The metabolic pathway of bentazone is illustrated in Figure 2.

Figure 2. Metabolic pathway of bentazone



2. Toxicological studies

2.1 Acute toxicity

Acute oral toxicity studies are available for a number of species (i.e. rat, guinea-pig, cat, dog and rabbit). Acute oral median lethal doses (LD_{50}s) are between 850 and 2470 mg/kg bw. No significant differences in LD_{50} were found between the free acid and the sodium salt. Signs of toxicity included dyspnoea, apathy, cachexia, staggering and poor general state. The acute dermal toxicity LD_{50} in rat was more than 5000 mg/kg bw. The 8-hour exposure inhalation toxicity (median lethal concentration, or LC_{50}) in rat was greater than 1.2 mg/l, whereas the 4-hour exposure LC_{50} was greater than 5.1 mg/l (mass median aerodynamic diameter [MMAD] 6.4 μm); no signs of toxicity were observed at this dose. Bentazone is not a skin irritant but was a moderate eye irritant in rabbits. It is a skin sensitizer in guinea-pigs. Intraperitoneal LD_{50}s were between 316 and 975 mg/kg bw in rats and mice. Results are summarized in Table 4.

All these studies were conducted prior to the implementation of GLP.

2.2 Short-term studies of toxicity

(a) Oral administration

Short-term studies of oral toxicity in mice, rats and dogs were conducted.

Mice

In a 30-day dietary study, female B6C3F1/CRJ mice (28 days old; six of each sex per dose) were given diets containing bentazone (purity 93.9%) at a concentration of 0, 400, 2000, 5000 or 10 000 parts per million (ppm) (equal to 0, 90, 407, 905 and 1469 mg/kg bw per day for males and 0, 100, 487, 1004 and 1663 mg/kg bw per day for females, respectively). The mice were observed daily and weighed twice per week. Feed consumption and water consumption were measured twice per week. After 30 days, the animals were killed and subjected to necropsy, and selected organs were weighed and processed for histopathology. Blood was taken from some mice (three of six controls of each sex, three of six male mice at 2000 ppm and two of six female mice at 2000 and 5000 ppm) for measurement of clinical pathology parameters.

Table 4. Summary of acute toxicity studies with bentazone

Species	Strain	Sex	Route	Form, batch no. and purity (if provided)	LD_{50} (mg/kg bw) / LC_{50} (mg/l)	Result	Reference
Rat	Sprague-Dawley	M + F	Oral	Acid	~850	[a]	Zeller & Hofmann (1969)
Rat	Sprague-Dawley	M + F	Oral	Acid	1050	[b]	Hofmann (1972a)
Rat	Sprague-Dawley	M + F	Oral	Acid	1220	[c]	Hofmann (1973a)
Rat	Sprague-Dawley	M + F	Oral	Sodium salt	1480 (equal to 1356 as free acid)	[d]	Hofmann (1973b)
Rat	Sprague-Dawley (CRJ)	M + F	Oral	Acid, lot no. 270778, 94.6%	M: 2340 F: 2470	[e]	Toyoshima (1978)
Rat	Wistar	M + F	Oral	NA	1710	[f]	Kirsch & Hildebrand (1983b)
Rat	Wistar	M + F	Oral	Free acid, batch N 169, 93.9%	M: 1780 F: 1470 Combined: 1640	[g]	Kirsch & Hildebrand (1983a)
Guinea-pig	NA	M + F	Oral	Sodium salt	1100 (equal to 1000 as free acid)	[h]	Hofmann (1974)
Guinea-pig	NA	M + F	Oral	Free acid	1100	[i]	Hofmann (1991)
Rabbit	NA	M + F	Oral	—	750	[j]	Zeller & Birnstiel (1969)
Rabbit	New Zealand White	M + F	Oral	—	1139	[k]	Neuschl & Kacmar (1993)
Cat	NA	M + F	Oral	—	~500	[l]	Zeller & Magoley (1970a)
Dog	Beagle	M + F	Oral	—	—	[m]	Zeller & Magoley (1970b)
Rat	Sprague-Dawley	M + F	Dermal	—	> 2500	No toxic signs	Zeller (1969)
Rat	CRJ:SD	M + F	Dermal	Acid, batch 270778, 94.6%	> 5000	No toxic signs	Toyoshima (1978)

Table 4 (continued)

Species	Strain	Sex	Route	Form, batch no. and purity (if provided)	LD_{50} (mg/kg bw) / LC_{50} (mg/l)	Result	Reference
Rat	Sprague-Dawley	M + F	Inhalation (8 h)	—	> 1.2	[n]	Hofmann & Zeller (1969a)
Rat	SPF Wistar/ Chbb:THOM	M + F	Inhalation (dust aerosol) (4 h)	Acid, batch N 187, 97.8%	> 5.1 (MMAD 6.4 µm)	No significant toxicity	Klimisch (1986)
Rabbit	White Vienna	M + F	Skin irritation	Lot 83/5, 50% aqueous formulation	—	No skin irritation potential	Kirsch & Hildebrand (1983c)
Rabbit	White Vienna	M + F	Eye irritation	Acid, lot 83/5	—	Shows an eye irritation potential	Kirsch & Hildebrand (1983d)
Guinea-pig	Pirbright White, Dunkin-Hartley, HOE DMPK	F	Skin sensitization	Acid, batch MS 2 F 22, 94%	—	Has skin sensitizing properties	Kieczka & Kirsch (1986)
Guinea-pig	Pirbright White, Dunkin-Hartley, HOE DHPK (SPF-LAC)	F	OET for sensitizing potential	Lot WH 4976, 60%	—	[o]	Klecak (1977); Kieczka (1986); Kieczka & Hildebrand (1986)
Mouse	NMRI	M + F	Intraperitoneal	—	~400	[p]	Hofmann & Zeller (1969b)
Mouse	CRJ:ICR	M + F	Intraperitoneal	270778, 94.6%	M: 494 F: 505	[q]	Toyoshima et al. (1978b)
Mouse	CRJ:ICR	M + F	Subcutaneous	270778, 94.6%	M: 655 F: 580	[r]	Toyoshima et al. (1978b)
Rat	Sprague-Dawley	M + F	Intraperitoneal	—	344	—	Hofmann (1972b)
Rat	Sprague-Dawley (CRJ)	M + F	Intraperitoneal	270778, 94.6%	M: 403 F: 407	[s]	Toyoshima et al. (1978a)
Rat	Sprague-Dawley (CRJ)	M + F	Subcutaneous	270778, 94.6%	M: 970 F: 975	[t]	Toyoshima et al. (1978a)
Rat	Wistar	M + F	Intraperitoneal	—	> 316 < 383	[u]	Kirsch & Hildebrand (1983e)

F, female; M, male; NA, not available; OET, open epicutaneous test

[a] Signs of toxicity in the 200–1600 mg/kg bw dose groups included dyspnoea, apathy and piloerection. No abnormality was detected on days 3–5 in surviving animals.

[b] Signs of toxicity in the 1250–2000 mg/kg bw dose groups included dyspnoea and apathy. The animals of the other dose groups did not show any signs of toxicity. Necropsy findings in animals that died were cardiac dilatation and congestive hyperaemia.

[c] Signs of toxicity noted at 1000 mg/kg bw and above were dyspnoea and red incrustations in eyes. Necropsy findings of the animals that died intercurrently were acute congestive hyperaemia, acute cardiac dilatation (right chamber) and liver putty coloured with lobular pattern. No abnormalities were noted at necropsy of animals sacrificed at the end of the study. No abnormality was detected from day 7 in the 1000–1600 mg/kg bw dose groups.

[d] Clinical signs observed were dyspnoea and prostration. Necropsy findings in animals that died were congestive hyperaemia and effects on heart, stomach, intestine and liver. No abnormalities were noted at necropsy of animals sacrificed at the end of the study. No abnormality was detected in surviving animals on days 2–3. Necropsy of animals that died showed bloody gastric ulceration and haemorrhagic contents in intestine.

[e] Signs of toxicity noted in all dose groups included decreased spontaneous motility, ventral position, clonic convulsions and abdominal respiration. Necropsy did not reveal abnormalities in either animals that died or animals sacrificed at the end of the study.

[f] Signs of toxicity noted in the 825–2610 mg/kg bw dose groups were dyspnoea, apathy, cachexia, staggering and poor general state. No signs of toxicity were seen in females of the 1780 mg/kg bw dose group and all animals of the 562 mg/kg bw dose group. The expected body weight gain was observed in the course of the study. Necropsy findings in animals that died were general congestion, spot-like hyperaemia and slight emphysema in lungs, ulcers and haemorrhages in the gastrointestinal tract, and anaemic colour and slight acinar pattern of the liver. Kidneys of one male animal of the highest dose group were sand coloured, and adrenals were loam coloured. No abnormalities were noted at necropsy of animals sacrificed at the end of the study. Necropsy of animals that died showed bloody gastric ulceration and haemorrhagic contents in intestine.

[g] Clinical signs observed were dyspnoea, apathy, staggering, opisthotonus, cachexia and poor general state. Body weight development was unaffected. Necropsy of animals that died showed bloody gastric ulceration and haemorrhagic contents in intestine.

[h] Signs of toxicity noted in the 1250 and 1600 mg/kg bw groups were prostration, apathy and tachypnoea. The animals of the other dose groups did not show any symptoms.

[i] Signs of toxicity noted in the 1200, 1600 and 3200 mg/kg bw groups were abdominal lateral position, apathy, tachypnoea, atonia and dyspnoea. The animals of the other dose groups did not show any symptoms. Necropsy findings of animals that died were acute congestion, acute cardiac dilatation and acute inflation of the lung. No abnormalities were noted at necropsy of animals sacrificed at the end of the study.

[j] Signs of toxicity noted in the 100, 500 and 2000 mg/kg bw dose groups were slight giddiness, anorexia and diarrhoea. This study was not acceptable.

[k] This was a summary of published literature. For male and female adult New Zealand White rabbits, a combined LD_{50} of 1139 mg/kg bw was calculated, with respiratory, cardiac and central nervous system symptoms occurring.

[l] Signs of toxicity noted in the 500–2000 mg/kg bw dose groups comprised titubation, vomiting, transient mydriasis, dysbasia, tremors, prostration, loss of rising reflex, atony, convulsions, opisthotonus, tetanic spasm and spastic paresis. Additionally, a slight body weight loss was noted at all dose levels. Necropsy findings of animals that died were foci of fatty degeneration and necrobiosis on the cut surface of the liver. No abnormalities were noted at necropsy of animals sacrificed at the end of the study.

[m] As a result of the vomiting of the animals in the higher dose groups, it was not possible to determine the acute oral LD_{50}.

[n] The results were not used for the evaluation.

[o] A sensitizing potential that could be of significance under conditions in practice can be assumed for application concentrations higher than 10% bentazone sodium.

[p] Signs of toxicity noted in the 200–800 mg/kg bw dose groups included dyspnoea, apathy, pronation and tremors. Female mice appeared to be more sensitive than males. Necropsy findings of animals that died were intra-abdominal adhesions. No abnormalities were noted at necropsy of animals sacrificed at the end of the study.

[q] Signs of toxicity noted in all dose groups comprised decreased spontaneous motility, ventral position, clonic convulsions and abdominal respiration. Necropsy did not reveal abnormalities in organs of either animals that died or animals sacrificed at the end of the study. In animals that died, traces of unabsorbed test compound were found at the injection sites.

[r] Signs of toxicity noted in all dose groups included decreased spontaneous motility, decreased response to external stimulus, such as sound and light, ventral position, clonic convulsions and abdominal respiration. Necropsy did not reveal abnormalities in organs of either animals that died or animals sacrificed at the end of the study. In animals that died, traces of unabsorbed test compound were found at the injection sites.

Table 4 (continued)

[s] Signs of toxicity noted in all dose groups were decreased spontaneous motility, ventral position, clonic convulsions and abdominal respiration. Necropsy did not reveal abnormalities in organs of either animals that died or animals sacrificed at the end of the study. In animals that died, traces of unabsorbed test compound were found at the injection sites.

[t] Signs of toxicity noted in all dose groups included decreased spontaneous motility, ventral position, clonic convulsions and abdominal respiration. Necropsy did not reveal any abnormalities in organs of either animals that died or animals sacrificed at the end of the study. In dead animals, traces of unabsorbed test compound were found at the injection sites.

[u] Signs of toxicity noted in the 316–562 mg/kg bw groups were dyspnoea, apathy, staggering, excitation, twitching and poor general state. Animals of the 261 mg/kg bw dose group did not show any effects. The surviving animals gained weight during the observation period.

At 10 000 ppm, all mice died within 30 days, whereas at 5000 ppm, six males and four females died. At 5000 and 10 000 ppm, the mice showed depression, skin pallor and low skin temperature. At 5000 and 10 000 ppm, decreases in body weight gain, feed consumption and water consumption were seen in both sexes. At necropsy of the dead animals at 5000 and 10 000 ppm, haemorrhages were seen in subcutaneous tissue, pia, lungs, thoracic, pericardial and abdominal cavities, thymus, orbits and skeletal muscles. Prothrombin time and partial thromboplastin time were prolonged at 2000 ppm in both sexes and at 5000 ppm in females. However, no such investigation was done at 400 ppm. On histopathological examination of animals receiving bentazone at 5000 and 10 000 ppm, there were haemosiderosis and extramedullary haematopoiesis in the spleen, haemorrhage and haemosiderosis in cardiac muscle, and haemorrhages in the cerebral cortex and pia.

Repeated administration of bentazone to mice led to an impairment of blood coagulation at a dose of 2000 ppm and above. The clinical signs and the haemorrhages observed at 5000 and 10 000 ppm are attributable to this effect. As the blood coagulation parameters were not investigated at 400 ppm and as there were effects at the next higher dose level of 2000 ppm, the NOAEL cannot be set at 400 ppm. The lowest-observed-adverse-effect level (LOAEL) is suggested to be 2000 ppm (equal to 407 and 487 mg/kg bw per day for males and females, respectively) on the basis of prolonged prothrombin and partial thromboplastin times at this dose. The study was not done according to GLP, and no QA statement was attached (Anonymous, 1981; Takehara & Tajima, 1982).

Rats

In a range-finding study, bentazone (purity 93.9%) was administered to Fischer 344 rats (34 days old; eight of each sex per dose) via the diet at a concentration of 0, 600, 1800, 5000 or 10 000 ppm (equal to 0, 64, 196, 554 and 1068 mg/kg bw per day for males and 0, 71, 217, 607 and 1132 mg/kg bw per day for females, respectively) for 31–33 days. Clinical observations were made twice a day. Body weight, feed consumption and water consumption were recorded twice a week. Clinical, haematological, gross pathological and histopathological examinations were carried out at the end of the study. Clinical chemistry examinations were not included. Organ weights of spleen, heart, pituitary, adrenal glands and ovaries were determined.

At 10 000 ppm, rats showed cyanosis of the skin of distal parts of the body and fading of the pigment of the fundus of the eyeball. One male rat died on day 10. Body weight gain of male rats of the 10 000 ppm dose group was significantly suppressed. A temporary decrease in feed consumption was observed in female animals, and water consumption was slightly decreased in both sexes. No clinical signs of toxicity were observed in animals of the other dose groups. Haematological examinations revealed a decrease in haemoglobin and haematocrit in male rats of the 10 000 ppm dose group and a fall in the mean red blood cell haemoglobin, whereas white blood cell count was significantly increased in this male group. The prothrombin and partial thromboplastin times were significantly prolonged in both sexes at this dose (Table 5). Findings of the one male animal that died comprised subcutaneous bleeding and bleeding from the thorax and thymus gland. Necropsy of animals sacrificed at the end of the study showed bleeding in various tissues and organs in seven

males and three females at 10 000 ppm. Absolute weights of heart and testicles were significantly decreased in male rats of the 10 000 ppm dose group, whereas the weights of liver and left kidney were significantly increased in females of this group. Histopathological examinations revealed bleeding from the renal cortex in one male rat and from the ovaries of two female rats at 5000 ppm. In the two lower dose groups, no substance-related findings were observed.

Table 5. Selected haematological findings (group means) in rats after administration of bentazone for 4 weeks

	Males					Females				
	0 ppm (*n* = 7)	600 ppm (*n* = 2)	1800 ppm (*n* = 2)	5000 ppm (*n* = 6)	10 000 ppm (*n* = 7)	0 ppm (*n* = 7)	600 ppm (*n* = 2)	1800 ppm (*n* = 2)	5000 ppm (*n* = 5)	10 000 ppm (*n* = 8)
Thromboplastin time (s)	17.1	17.3	19.3	22.7	27.2	14.4	14.2	15.3	15.4	15.4
(% change relative to control)	—	—	—	—	(59.1)	—	—	—	—	(6.9)
Partial thromboplastin time (s)	34	28.4	33.6	44.7	153.4	29.7	26.7	30.8	25.6	47.8
(% change relative to control)	—	—	—	(31.5)	(351)	—	—	—	—	(60.9)

From Itabashi et al. (1981)

Bentazone led to an impairment of blood coagulation in rats, and bleeding observed in several organs and anaemia were assessed to be related to this. The decreased absolute weights of heart and testes were attributed to the impaired body weight gain, rather than to compound administration itself. Liver and kidney weights were increased, although there were no related histopathological findings.

Under the conditions of this study, the NOAEL was 1800 ppm (equal to 196 mg/kg bw per day for males and 217 mg/kg bw per day for females), based on toxicity apparent at the top dose and equivocal findings, such as bleeding from the urogenital system, in some animals at 5000 ppm. The study was performed prior to implementation of specific test guidelines and was not GLP compliant (Itabashi et al., 1981).

In a 90-day dietary study, bentazone (batch and purity not given) was administered to Sprague-Dawley rats at 0, 70, 200, 800 or 1600 ppm in the diet (equivalent to 0, 3.5, 10, 40 and 80 mg/kg bw per day, respectively). At the start of the trial, the average weights of male and female rats were 124 and 118 g, respectively. Each dose group had 20 animals of each sex, with a further 10 of each sex at 0, 70 and 1600 ppm kept under observation for a post-trial period of 42 days without test substance administration. Clinical signs and feed consumption were checked daily, and body weight was determined weekly. In all animals, haematological and biochemical examinations as well as urine analysis were carried out. All animals were assessed gross pathologically and subjected to a histopathological examination.

No clinical signs of toxicity were observed. Feed consumption and body weight gain of all treated male rats were comparable with those of the control. At 1600 ppm, body weight gain of the female rats was slightly retarded. There were no differences in the absolute body weights of males and females in treated and control groups. The body weights of male and female rats in the highest dose group were slightly lower than those of the other groups. No treatment-related changes could be observed in haematological and biochemical examinations in test or control animals. There were no appreciable differences in the mean absolute weights of liver, kidneys or heart. The relative kidney

weight of male rats of the 1600 ppm group and of female rats of the two highest dose groups was increased when compared with control values. The relative liver weights of all treated rats did not differ from those of the controls. At 1600 ppm, male rats showed increased relative heart weights, and the liver to heart and kidney to heart weight ratios were higher than those of the controls. Female rats of the 70 ppm group exhibited lower relative heart weights, and the liver to heart weight ratio was increased at this dose level. Females of the 70, 800 and 1600 ppm groups exhibited increased kidney to heart weight ratios. In the withdrawal trial, these increased ratios proved to be reversible. Feed consumption of both sexes and body weight gain of the male animals remained unaffected during the post-observation period. Females of the 1600 ppm group exhibited lower body weight gains. No test substance–related macroscopic changes were found at necropsy of the test animals. Two animals of each of the 200 ppm and 1600 ppm groups were found to have detectable degeneration of the testicular tissue. No further histopathological changes occurred in any of the other organs. Although no histopathological changes were observed, the liver weight changes were assessed as indicative of a slight liver adaptation process induced by the administration of the test substance. The minor and inconclusive effects on organ weights noted at 70 and 200 ppm in single animals were assessed as incidental because there was a lack of a dose–response relationship.

In view of the above, the NOAEL was 200 ppm (equal to 10 mg/kg bw per day), based on increased relative kidney weight in females at 800 ppm (equal to 40 mg/kg bw per day) and slight effects on body weight gain at the top dose level. The study was conducted prior to implementation of any specific test guidelines or of GLP (Zeller & Kirsch, 1970).

Bentazone (ZNT No. 86/48; batch N 187; purity 97.8%) was administered to rats (Wistar KFM-Han) at a dietary concentration of 0, 400, 1200 or 3600 ppm (equal to 0, 25.3, 77.8 and 243.3 mg/kg bw per day for males and 0, 28.9, 86.1 and 258.3 mg/kg bw per day for females, respectively) in a 13-week oral toxicity study. The study comprised four groups, each containing 10 male and 10 female rats about 8 weeks of age and weighing 168–206 g (males) and 150–177 g (females). The reversibility of treatment-related changes was studied using 10 additional animals of each sex at dietary concentrations of 0 and 3600 ppm over a 4-week recovery period.

No signs of toxicity were noted. There were a total of three deaths at the highest dose. Two rats were found dead in their cages during the 9th and 12th weeks of treatment. Another high-dose female rat died during anaesthesia on the day of scheduled necropsy. There was no compound-related effect on feed consumption in any group. In the high-dose group, body weight gains were slightly reduced for females, leading to a 6% decrement (significant at 5% level) in mean terminal body weight relative to female controls after 13 weeks of treatment. Body weight gains noted for males of this dose group and for both sexes of the low- and mid-dose groups were similar to those of the respective control animals. Body weight gains of animals of the high-dose recovery group during the 4-week regression period were also similar to those of the respective control animals. No compound-related effect was noted in ophthalmoscopy. Haematological examinations revealed prolonged thromboplastin and partial thromboplastin times for male animals of the high-dose group. The prolonged coagulation times may reflect an inhibitory effect on blood clotting factors. This effect was found to be reversible at the end of the recovery period. The biological meaning of a shortened prothrombin time as seen in females is equivocal (Table 6).

There was increased total cholesterol in high-dose females, as well as an increased albumin fraction and albumin to globulin ratio for mid- and high-dose males. These changes were reversible (Table 7).

Table 6. Selected haematological findings (group means) in rats administered bentazone technical for 91 days

	Males				Females			
	0 ppm	400 ppm	1200 ppm	3600 ppm	0 ppm	400 ppm	1200 ppm	3600 ppm
Thromboplastin time (s)								
- at end of treatment period	13.5	13.2	13.2	15.8*	13.2	12.5*	12.4*	12.4*
- at end of recovery period	13.3	—	—	13.4	12.9	—	—	13.0
Partial thromboplastin time (s)								
- at end of treatment period	22.5	22.3	23.9	30.2*	20.4	21.3	20.8	21.8
- at end of recovery period	21.2	—	—	21.4	19.2	—	—	18.9

From Tennekes et al. (1987)
* $P \leq 0.05$ (Dunnett)

Table 7. Selected clinical chemistry findings (group means) in rats administered bentazone technical for 91 days

	Males				Females			
	0 ppm	400 ppm	1200 ppm	3600 ppm	0 ppm	400 ppm	1200 ppm	3600 ppm
Total cholesterol (mmol/l)								
- at end of treatment period	2.36	2.49	2.32	2.49	2.30	2.45	2.44	2.65*
- at end of recovery period	2.48	—	—	2.27	2.30	—	—	2.47
Albumin (g/l)								
- at end of treatment period	34.6	34.7	35.5	36.4*	43.0	41.4	43.4	42.1
- at end of recovery period	40.6	—	—	39.4	47.2	—	—	46.2
A1 globulins (g/l)								
- at end of treatment period	10.4	9.8	9.6	9.1*	5.9	7.5*	7.5*	7.4*
- at end of recovery period	9.8	—	—	10.4	7.4	—	—	7.9
A2 globulins (g/l)								
- at end of treatment period	2.7	2.9	2.6	2.4*	2.9	2.9	2.5*	2.7
- at end of recovery period	2.7	—	—	2.9	2.2	—	—	2.1

From Tennekes et al. (1987)
* $P \leq 0.05$ (Dunnett)

An increased urinary output and a corresponding decrease in specific gravity were observed at the high dose. These findings, which were reversible, may reflect an increased fluid intake related to treatment. A slight enlargement of the kidneys was noted macroscopically for high-dose animals of both sexes. This effect was more marked in males than in females; however, it was fully reversible in male rats but not in females. The slight increment noted for absolute adrenal weights in the high-dose males as well as the slight increment in the liver to body weight ratios noted for high-dose females were considered to be incidental findings and within the range of biological variation. No further gross pathological changes were detected, and histopathology did not demonstrate any effect.

The NOAEL was 400 ppm (equal to 25.3 and 28.9 mg/kg bw per day for males and females, respectively), and a QA statement was attached (Tennekes et al., 1987).

Dogs

In a subchronic study, bentazone (purity not specified) was administered to three male and three female Beagle dogs (10–12 months of age and weighing between 9 and 10 kg [males] and

between 8.4 and 9.4 kg [females]) per group for a period of 3 months in the diet at 0, 100, 300, 1000 or 3000 ppm (equal to 0, 4.0, 12.0, 39.6 and 113.8 mg/kg bw per day for both sexes). The animals were housed singly under controlled conditions and received a daily ration of 40 g/kg bw commercial diet (Altromin H). Water was available ad libitum.

Dietary concentrations of 100 and 300 ppm were tolerated without any symptoms. At 1000 ppm, one of the six animals displayed a slight but increasing sedation during the last 2 weeks of the study. The same dog developed an ulcer on the left hind leg. The surrounding area was affected by alopecia, and the ulcer had not healed at the end of the study. No other pathological changes were noted at this dose level. At 3000 ppm, three of the six animals died in a coma, preceded by agonal spasms in two cases. Signs of toxicity noted were sedation, attacks of superactivity, ataxia, prostration, loss of rising reflex and tremors. The sedative effect was seen first between the 2nd and 4th weeks of treatment in all high-dose dogs. It appeared 10–60 minutes after feed and compound intake. Its duration increased from about 5 hours up to 24 hours at the end of the treatment period. The three male dogs in this group vomited from time to time. In the second half of the study, all animals had increasingly severe diarrhoea, in some cases with visible blood. Anorexia was observed during the whole study period. At first, the feed consumption was only retarded, but later, the amount consumed was reduced. All animals lost weight. At 3000 ppm, all six animals had bilateral haemorrhagic conjunctivitis, mostly in a mild form. Male animals exhibited ulcerative stomatitis. One male had ulcerations surrounded by areas of alopecia on the right paw, the right ear and left of the umbilicus. The recuperative powers of the animals appeared to have been diminished, as none of these inflammatory changes healed by the end of the trial. Oedema in the thoracic region was observed in one male. Erythrocyte count, haemoglobin and haematocrit were reduced in the high-dose group. Blood sedimentation and blood coagulation were retarded in this group, and the platelet count was reduced. Furthermore, there was increased activity of several serum enzymes, such as alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase, increased urea and bilirubin contents and reduced albumin and total protein concentrations in the blood. Albuminuria and ketonuria occurred more frequently in this group.

At 3000 ppm, necropsy in all dogs revealed pale liver. Pale kidneys were noted in one dog. The males also had heavily marked lobes of the liver, and one had gastric ulcers. Swelling of the thoracic region was noted in one male and one female animal. Liver, kidneys and adrenals were distinctly enlarged. Relatively high weights were also recorded for spleen, lungs, thymus, thyroid and brain. At histopathology, there were some compound-induced changes, mainly consisting of severe congestive symptoms and necrotic congestion of the liver lobe centres. Marked fatty degeneration was observed in five dogs. These liver findings were in keeping with hypoxic damage to liver parenchyma. Furthermore, extramedullary haematopoiesis in the spleen was noted. However, the bone marrow showed a normoplastic picture. Droplets of fatty degeneration in the ventricular myocardium and albuminous swelling of the renal tubules were observed. Histological examination of the organs from the animals of the lower dose groups revealed no compound-induced pathological changes.

The highest dose of 3000 ppm was severely toxic to the dogs and was lethal in three out of six animals. The maximum tolerated dose was clearly exceeded. Anaemia and disturbed blood coagulation as well as signs of kidney and liver damage were noted, confirming the findings obtained in other species. The clinical findings, such as cachexia, conjunctivitis, stomatitis and oedema, were assessed to be secondary effects of the severe intoxication. The histopathological findings were considered to reflect chronic hypoxidosis caused by treatment.

The NOAEL was 300 ppm (equal to 12.0 mg/kg bw per day), on the basis of sedation and ulceration and alopecia in the leg of one dog at 1000 ppm (equal to 39.6 mg/kg bw per day) (Leuschner et al., 1970; Leuschner & Otto, 1972, 1973).

In a 1-year study in Beagle dogs (aged 5–7 months and weighing around 7.1–9.4 kg [males] and 7.0–9.3 kg [females]), four groups of six males and six females were given diets containing bentazone (batch N 187, purity 97.8%) at a concentration of 0, 100, 400 or 1600 ppm (equal to 0, 3.2, 13.1 and 52.3 mg/kg bw per day) for 52 weeks.

Treatment-related clinical signs were restricted to a few individual dogs of the highest dose group. One male dog of the highest dose group showed slight, but persistent, weight losses during the first half of the dosing period. Body weight during the second half of the study showed only slight fluctuations. From weeks 19 to 52, this dog appeared emaciated and dehydrated, although feed consumption throughout the treatment period remained maximal. Another male dog of the same group exhibited frequent diarrhoea. This was observed an average 1–2 times per week and persisted throughout the treatment period. The highest frequency was observed between weeks 3 and 7, when diarrhoea was recorded virtually every day. On days 27, 28, 29 and 40, the faeces appeared red, probably due to the presence of blood. A moderate decrease in activity and the pale appearance of the mucous membranes were first recorded for this dog on day 42. At this time, haematological investigations revealed a marked anaemia. Treatment was withdrawn between days 44 and 49, and the animal was offered control diet. Feed consumption remained maximal at all times. During week 8, an appreciable increase occurred in the values recorded for red blood cell parameters. Between days 43 and 53, the decrease in activity was scored as slight, whereas the mucous membranes of the mouth became increasingly pale. From day 54, however, the oral mucous membranes were scored as only slightly pale, and by day 74, colour had returned to normal. A third high-dose male dog had slight to marked hyperaemia of the skin of the ear pinnae from week 7 and of the legs and paws from week 20. Slight to marked alopecia affecting the ear pinnae, paws and head was also recorded from week 8. An improvement in the condition of the skin was evident from week 25, and complete recovery was seen by the end of week 27. In a high-dose female dog, a marked reduction in feed consumption was recorded during week 3. This was associated with the appearance of diarrhoea, with the faeces thought to contain blood. A diagnosis of gastroenteritis was made, and the animal was treated with antibiotics on days 16, 17, 19, 25, 26 and 27. A subsequent improvement in the condition of this dog was observed, and normal feed consumption was recorded from week 8. No mortality was observed throughout the study.

There were no ophthalmoscopic changes that could be related to treatment. Auditory perception was also unaffected. Occult blood was not detected in the faeces of any of the dogs of the control and high-dose groups tested during week 14 of treatment. Body weight development was not impaired at any dose, based on the mean values; however, body weight development was impaired in a few individual dogs. Overall mean body weights of the dosed animals were not statistically different from those of controls.

Examination of group mean haematological data recorded at 13, 26 and 52 weeks did not reveal any findings of toxicological significance. However, some remarkable changes were noted in individual animals. During week 7, deterioration in clinical condition was apparent for a 1600 ppm male. Off-schedule haematology revealed a marked anaemia as well as thrombocytosis, reticulocytosis, leukocytosis and changes in red blood cell morphology. A slight increase in the partial thromboplastin time was also recorded (up to 50%). Further haematological investigations during week 8 (following a 6-day period without treatment) showed an increase in the erythrocyte count, haemoglobin concentration and haematocrit values over those recorded during the previous week, although all values remained lower than those recorded pretest. A reduction in platelet, leukocyte and reticulocyte counts was also noted, and the partial thromboplastin time recorded on this occasion appeared normal. At 13 weeks, slight reductions were still apparent in the haemoglobin concentration and haematocrit value, although the platelet, erythrocyte and leukocyte counts all appeared normal. The prothrombin and partial thromboplastin times recorded for this dog, however, were both longer than those seen in other animals at 13 weeks. At 13 weeks, evidence of slight anaemia was also recorded for a female from the 1600 ppm group. This was characterized by a depression of the erythrocyte count, haemoglobin concentration and haematocrit value, with increased mean cell volume and decreased mean corpuscular haemoglobin concentration. Increased number of nucleated erythrocytes, abnormal red cell morphology (slight anisocytosis), increased thromboplastin and partial thromboplastin times, an increase in segmented neutrophils and a decrease in lymphocytes were also noted at this examination. The values for these parameters were within expected limits at 26 and 52 weeks. Increasing thromboplastin and partial thromboplastin times were recorded for another male from the 1600 ppm group as the study progressed. Slightly increased partial thromboplastin time only was recorded for two 1600 ppm females at week 13 in comparison with their pretest values. The

findings for these four high-dose dogs contributed towards the significantly higher partial thromboplastin times recorded for both males and females of the 1600 ppm group at week 13. However, values for the remaining dogs in this group were similar to or lower than those recorded pretest, and therefore this effect cannot clearly be attributed to treatment (Table 8).

Table 8. Selected haematological findings in a 1-year study in dogs

Dietary concentration (ppm)	Period of observation	PT (s)		PTT (s)		RBCs (millions/ mm^3)		Reticulocytes per 1000 RBCs		HCT (l/l)		Hb (mmol/l)	
		M	F	M	F	M	F	M	F	M	F	M	F
0 (control)	Pretreatment	6.3	6.5	10.55	10.6	6.0	6.9	0.006	0.007	0.39	0.46	8.1	9.4
	Week 13	6.5	6.5	9.9	10.2	6.2	6.9	0.001	0.004	0.43	0.48	8.6	9.6
	Week 26	6.3	6.5	9.6	10.1	6.1	6.4	0.007	0.005	0.41	0.43	8.6	9.2
	Week 52	6.7	6.8	10.3	9.8	6.5	6.3	0.004	0.003	0.42	0.43	9.1	9.2
100	Pretreatment	6.4	6.2	11.0	10.9	6.4	6.8	0.009	0.005	0.43	0.45	8.6	9.3
	Week 13	6.5	6.5	10.4	10.6	6.6	6.9	0.004	0.003	0.45	0.47	9.3	9.6
	Week 26	6.3	6.3	10.7	9.8	6.5	6.3	0.004	0.005	0.42	0.42	9.2	9.0
	Week 52	6.6	6.7	10.3	10.05	6.8	6.8	0.005	0.005	0.47	0.46	9.8	9.8
400	Pretreatment	6.5	6.05	10.5	11.1	6.3	6.7	0.007	0.004	0.42	0.44	8.5	9.3
	Week 13	6.6	6.3	10.6	10.7	6.2	6.9	0.003	0.001	0.42	0.48	8.6	9.7
	Week 26	6.4	6.3	10.3	10.7	5.9	6.5	0.007	0.003	0.41	0.46	8.5	9.4
	Week 52	6.7	6.7	9.8	10.4	6.4	6.8	0.004	0.003	0.45	0.48	9.2	10.0
1600	Pretreatment	6.25	6.4	11.2	11.3	6.3	6.9	0.011	0.005	0.44	0.45	8.6	9.5
	Week 7[a]	6.3	—	14.0	—	2.4	—	0.380	—	0.19	—	—	—
	Week 8[a]	6.4	—	11.4	—	4.0	—	0.026	—	0.31	—	—	—
	Week 13	7.45	7.1	11.7	11.8	6.25	6.8	0.002	0.001	0.42	0.45	8.4	8.8
	Week 26	6.4	6.7	10.9	10.6	6.2	6.7	0.003	0.003	0.41	0.44	8.5	9.1
	Week 52	6.9	7.1	10.9	10.6	6.5	6.6	0.003	0.003	0.45	0.44	9.4	9.3

From Allen et al. (1989)

F, female; Hb, haemoglobin; HCT, haematocrit; M, male; PT, prothrombin time; PTT, partial thromboplastin time; RBC, red blood cells

[a] Only one male animal No. 21 was subjected to test because of its illness.

Examination of the clinical biochemistry and urine analysis data recorded at 13, 26 and 52 weeks did not reveal any findings of toxicological significance. Organ weights were unaffected by treatment with bentazone. No treatment-related gross pathological findings were observed. Most macroscopic findings were unremarkable and among those normally recorded in this age and strain of dog.

As a histopathological examination of the bone (sternum) was required by the protocol but omitted from the original examination, an amendment of the pathology report (Allen, 1989), including evaluation of the bone (sternum), was made. All pathological findings recorded, including some minor testicular alterations in five dogs that received the test article, were of a spontaneous nature common to dogs of this age and strain. There was no evidence of abnormal histopathological findings resulting from treatment with bentazone technical.

The NOAEL for the study was 400 ppm (equal to 13.1 mg/kg bw per day), on the basis of clinical signs, weight loss and anaemia at the highest dietary concentration of 1600 ppm (equal to 52.3 mg/kg bw per day). The study was GLP compliant, and a QA statement was attached (Allen et al., 1989).

(b) Dermal application

Rabbit

The 21-day dermal toxicity of bentazone technical (purity not given) was tested in New Zealand White rabbits (weight between 2.3 and 3.1 kg) with dermal doses of the test substance of 0, 250, 500 or 1000 mg/kg bw applied daily for a period of 8 hours to the intact and scarified skin of six animals of each sex per dose for a period of 21 days. The control animals were treated in the same way with tylose at a dose of 2 ml/kg bw. The animals with intact skin (i.e. half of the total) were kept under observation for a further 21 days after termination of treatment. Behaviour and feed and water consumption were checked daily. Body weights were determined weekly. Clinical, clinicochemical, haematological, gross pathological and histopathological examinations as well as urine analysis were carried out. Very slight, transient erythema was detected on the intact and scarified skin, the latter being slightly more affected. However, in no case did the reactions exceed those of the control animals. More extensive skin injuries, such as oedema and necrosis, were not observed. The test and control animals showed no differences during the withdrawal period. Behaviour, condition of coat, feed and water consumption, body weight gain, haematological and biochemical tests, urine analysis, gross pathological findings and organ weights at necropsy after 3 or 6 weeks of testing were similar for treated and control animals. Histological investigations carried out after 3 weeks on the animals with scarified skin and after a further 3 weeks' observation on the animals with intact skin revealed in isolated cases negligible inflammatory infiltration on the application site and on the untreated skin. No significant differences could be observed between the treated animals and the control animals. The isolated and very slight findings in other organs can be classified as spontaneous pathology.

The NOAEL for dermal toxicity was above 1000 mg/kg bw per day for male and female animals. The study was conducted prior to implementation of GLP (Leuschner et al., 1971).

In another study, bentazone (batch N 187, purity 97.8%) was applied daily for 6 hours to the clipped intact dorsal skin of New Zealand White rabbits (mean weight 2.29 kg for males and 2.27 kg for females; five of each sex per dose) over a period of 3 weeks using a semi-occlusive dressing. The doses were 250, 500 and 1000 mg/kg bw per day. A control group (five of each sex) was treated with solvent (0.5% aqueous carboxymethylcellulose). Feed consumption was determined once a week over the course of 1 day. Body weight was determined weekly. The animals were carefully inspected twice daily (before and after exposure). Skin findings were recorded daily (about 30–60 minutes after removal of the dressing). At the end of the study, clinicochemical and haematological examinations were carried out. All animals were assessed by gross pathology. Subsequently, a histopathological examination was carried out. After a thorough assessment (clinical examination, clinical chemistry, haematology and pathology), the dermal application of bentazone did not lead to any substance-related findings at the doses tested. Some changes were attributed to an infection by coccidia.

The NOAEL was greater than 1000 mg/kg bw per day for both sexes of rabbits. The study was GLP compliant, and a QA statement was attached (Schilling & Hildebrand, 1988).

Bentazone (batch N 194, purity 97.64%) was administered dermally to SPF New Zealand White rabbits (five of each sex per dose) for 21 consecutive days at a dose of 0 (solvent control), 250, 500 or 1000 mg/kg bw per day. The test material was applied for 6 hours/day as 0.5% aqueous Tylose CB 30.000 solution (cleaned sodium carboxymethylcellulose in distilled water) under semi-occlusive dressing covering at least 10% of the body surface. The animals were housed singly under controlled conditions, and each received a daily ration of about 130 g of standardized diet. A daily ration of approximately 250 ml/animal was available as drinking-water. The test substance preparations were made up each workday immediately before application. Clinical observations were made twice daily. A check for skin findings was carried out daily about 30 minutes after removal of the dressing. Feed consumption and body weight were recorded weekly. Clinicochemical, haematological (including clotting analysis for thromboplastin time), gross pathological and histopathological examinations were carried out at the end of the study.

There were no deaths during the study period. No clinical signs of systemic toxicity were observed. No treatment-related differences in feed consumption were noted during the study. Body weight in test animals was comparable with that seen in controls. No signs of irritation on the treated skin could be observed in all animals of the test groups. The treated skin of these animals was discoloured (yellow) by the test substance. Adhesive tape caused mechanical skin lesions beside the treated area. Haemorrhagic round areas with sharp margins and crateriform retractions were observed on the clipped dorsal area of all animals used as controls (solvent control). No treatment-related effects on clinical chemistry or haematology values were apparent in males or females. No pathomorphological findings considered to be treatment related were diagnosed. No treatment-related significantly different mean absolute or relative weight parameters and no treatment-related gross lesions or microscopic findings were detected. No treatment-related skin changes were detected.

The NOAEL for dermal toxicity (local and systemic) of bentazone was greater than 1000 mg/kg bw. The study was GLP compliant, and a QA statement was attached (Kirsch & Hildebrand, 1993).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

The chronic toxicity of bentazone was studied in Swiss Webster mice. Fifty animals of each sex were fed with diet containing bentazone (batch number and purity not given) at a concentration of 0, 100, 350 or 1600 ppm (equal to 0, 15, 52 and 237 mg/kg bw per day, respectively; means for males and females) for a period of 18 months. Five mice of each sex per dose group were subjected to interim sacrifice after 12 months. The animals were housed four per cage under controlled conditions and received standardized diet and water ad libitum.

More than 50% of the animals in the test and control groups died in the course of the study. Female in the highest dose group exhibited significantly reduced feed consumption and lower body weights. Males also showed a decrease in body weight and feed consumption after 18 months, but statistical significance was not reached.

Substance-induced gross pathological changes were not noted. At terminal sacrifice, mean absolute brain and liver weights in females of the 1600 ppm group were increased. In males, the absolute spleen weight was decreased. At this top dose level, the relative organ weights of brain and liver in both sexes and kidney and heart in females were increased. In males, the relative spleen weight was decreased. No substance-induced histological changes were found. No differences in the tumour incidence were observed between treated and control animals.

As the administration of bentazone led to an impairment of body weight gain and feed consumption as well as to organ weight changes at the highest dose level of 1600 ppm (equal to 237 mg/kg bw per day), the NOAEL was 350 ppm (equal to 52 mg/kg bw per day) for both sexes. The mortality rate was above 50% in all groups, and therefore no conclusive assessment of the observed effects is possible. The study was not GLP compliant, as it was generated prior to the implementation of GLP (Welsh et al., 1974).

The carcinogenicity of bentazone was studied in mice of the CFLP strain (hysterectomy-derived strain). Four groups, each with 40 animals of each sex, were fed with a diet containing bentazone (batch no. p.195.75, purity not given) at a concentration of 0, 100, 350 or 1600 ppm (equal to 0, 8.4, 29.7 and 138.4 mg/kg bw per day for males and 0, 9.5, 34.3 and 152.9 mg/kg bw per day for females, respectively) for a period of 82–95 weeks. If a survival rate of 25% was attained in a control or treated group (i.e. a minimum of 10 males and 10 females), all groups of that sex were killed and necropsied. If the high dietary group reached the 25% survival point first, this group was killed and necropsied, the remaining groups being treated as scheduled. Therefore, all surviving male mice receiving 1600 ppm were sacrificed after 82 weeks, and all the other male groups were terminated after 88 weeks of treatment. The female groups were maintained up to 95 weeks. The animals were caged under controlled conditions and had free access to water and standardized diet. Clinical

observations were made daily. Feed consumption was recorded weekly, and body weight was determined weekly for the first 3 months and every 2 weeks thereafter. Clinicochemical and haematological examinations were not carried out. At termination, all surviving animals were sacrificed and examined gross pathologically. Gross lesions as well as liver, spleen, lymph nodes, adrenals, thyroid, ovaries and pineal body were examined histopathologically. Mortality rates were compared between the groups using stratified contingency tables. Student's *t*-test was applied to assess the significance of intergroup differences in body weight, feed intake and water intake data.

No substance-induced findings in clinical parameters were found. The number of deaths among treated mice was similar to that of the controls, with the exception of males receiving 1600 ppm, for which there was an increased incidence of mortality during weeks 79–82. At week 78 (the required duration of a carcinogenicity study in mice), survival in the males had declined to 27, 21, 30 and 23 animals in the control, 100, 350 and 1600 ppm groups, respectively. Among females, 29, 31, 26 and 18 animals were still alive in the respective groups. Thus, at 1600 ppm, mortality had reached 55% in females at this time. However, as no macroscopic or microscopic changes were noted that could be attributed to treatment in the animals dying intercurrently, the higher mortality rate was not assessed as being substance induced. Feed consumption and body weight gain of treated animals were similar to those of control animals. No substance-induced changes were found in any gross pathological or histopathological parameters.

Under the conditions of this study, no carcinogenic effect was found, and the NOAEL was 1600 ppm (equal to 138.4 mg/kg bw per day for males and 152.9 mg/kg bw per day for females), the highest dose tested. However, the scientific value of this outcome is limited because of the insufficient number of animals on study and the high overall mortality. The study is not GLP compliant, as it was generated prior to the implementation of GLP (Hunter et al., 1978).

Bentazone (batch N 169; purity 93.9%) was administered to B6C3F1 mice (33 days old, weighing around 20.6 g [males, mean] and 16.9 g [females, mean]) at a dietary concentration of 0, 100, 400 or 2000 ppm (equal to 0, 12, 47 and 242 mg/kg bw per day for males and 0, 12, 48 and 275 mg/kg bw per day for females, respectively) for 6, 12 or 24 months. Bentazone was administered to groups of 70 mice of each sex at a dietary concentration of 0, 100, 400 or 2000 ppm for about 6 or 12 months (satellite groups; 10 animals of each sex per dose) and 24 months (main groups; 50 animals of each sex per dose). The animals were examined for morbidity or mortality twice daily. If animals were in a moribund state, they were sacrificed and necropsied. Animals found dead were necropsied as soon as possible. Signs (appearance and general behaviour) were checked daily. Palpations on skin and abdominal organs were performed once per week. The body weight of the animals was determined at the start of the treatment (day 0), at weekly intervals thereafter and prior to necropsy. At the end of the administration period, the animals were sacrificed.

Feed consumption was determined for 10 mice of each sex per group of the main test group. From week 41 onward, measurements were done for 20 mice of each sex per group to avoid group sizes of less than 10 due to death of mice. The eyes of all surviving animals were examined at the end of their administration period for any changes using the naked eye, an ophthalmoscope and a funduscope. Atropine was dropped into the eye 5–10 minutes before the examination. The haematological and clinical chemistry parameters were determined for 10 animals (8 or 9 in some groups after 6 or 12 months) per test group after their respective dosing period (17 for haematology after 24 months). Urinary parameters were determined in 10 animals (8 or 9 in some groups after 6 or 12 months) of each sex per test group after 6, 12 and 24 months of administration.

All animals—if not found dead—were sacrificed, and exsanguinated animals were necropsied and assessed by gross pathology. Animals that died intercurrently were necropsied as soon as possible after death and assessed by gross pathology. The organs were sampled, weighed and examined histopathologically. Tumours in mice examined were classified according to IARC (1979).

There were no remarkable findings in the 6- and 12-month groups. In the 24-month main group, various findings common to both sexes were noted. They comprised palpable masses in the

abdomen and dyspnoea considered to be caused by tumours in the liver, lung and haematopoietic tissues. Signs common in agony, such as lack of vigor, emaciation, reduced skin temperature, pallor in the auricles and limbs, tachypnoea, systemic cyanosis and abdominal inflation, were also observed. However, those incidences did not indicate an effect of treatment.

No deaths occurred in the 6-month test, except for a male of the control group. In the 12-month group, mortality was noted in one male in each of the control and 2000 ppm groups and in one, two and one female of the 100, 400 and 2000 ppm groups, respectively. The cumulative numbers of deaths in the 24-month test were 14 (28%), 14 (28%), 15 (30%) and 20 (40%) in the control, 100, 400 and 2000 ppm groups, respectively, for males and 10 (20%), 9 (18%), 13 (26%) and 15 (30%), respectively, for females. The mortality rates of the treated groups are not considered to be different from control values.

No treatment-related ophthalmoscopic findings were noted in the animals sacrificed after 6, 12 or 24 months.

Body weight development in the 100 and 400 ppm animals was unaffected. In the 2000 ppm males, a transient minor but statistically significant suppression of body weight gain was noted up to week 23 and again at weeks 69 and 73. No effects were noted in the females of the high-dose group.

No differences relative to controls in feed consumption or feed efficiency or water intake were seen in any of the treated groups. Occasional variations in a single week were not considered to be of relevance. There were changes in the haematological parameters, namely reduced red blood cells and increased mean corpuscular volume in the treated females after 6 months and reduced white blood cells in females after 12 months. However, there was no trend that confirmed these findings on other occasions, and therefore they are not considered to be treatment related. There was a prolonged prothrombin time for the males of the 400 and 2000 ppm groups in the 24-month test, which was taken to be the result of the toxic effect of the test substance.

Some statistically significant clinical chemistry findings were observed, notably changes in the total cholesterol concentration at months 6 and 24 and in the albumin to globulin ratio in high-dose males after 24 months. However, these deviations did not show a consistent trend, and therefore they are considered to be incidental and not treatment related.

The only notable finding in urine analysis was the increase in specific gravity after 12 months in the males fed 400 and 2000 ppm in the diet. A few other spurious findings were not considered to be treatment related.

On necropsy, no obvious changes in the organ weights and ratios in the animals sacrificed after either 6 or 12 months could be noticed. However, several significant changes (increase or decrease) were noted in the organ weights of animals sacrificed after 24 months, but none of them could be attributed to an effect of the treatment.

There were only a few gross pathological findings in the animals of the 6- and 12-month sacrifices, and they were mainly limited to single incidences. Exceptions are the hair loss on truncus or head and neck and the observation of cyst in the uterus. However, there is no consistent pattern that would indicate a relationship to treatment. There were various gross pathological findings in the 24-month sacrifice group. The findings that reached any statistical significance (from either the scheduled kill subgroup or moribund/spontaneous death) are listed in Table 9. None of them indicate a relationship with treatment.

There were several histological non-neoplastic findings in the animals sacrificed after either 6 or 12 months. Most of them were of single occurrence. Of those found with higher incidences, none appeared to indicate a treatment-related effect. They were considered to be due to physiology and ageing. The pancreas islet cell hyperplasia seen in males after 12 months was also noted after 24 months, but did not develop into neoplasms. A number of non-neoplastic findings were present in the animals of the 2-year group either killed by design or at moribund/spontaneous death; however, very few reached statistical significance. Noticeable findings were the hyperplasia of Langerhans islet cells of the pancreas in the mid- and high-dose males, an effect also seen at 12 months. However, no

neoplastic lesions developed. Ioannou (1989) reported historical control data from the Nippon Institute of Biological Sciences of 22/60 (36.7%) for males and 1/60 (1.7%) for females.

Table 9. Incidence of selected gross pathological findings (with potentially relevant statistical significance) in mice administered bentazone for 24 months (moribund and scheduled sacrifices added)

	Incidence of finding							
	Males				Females			
	0 ppm	100 ppm	400 ppm	2000 ppm	0 ppm	100 ppm	400 ppm	2000 ppm
No. of animals examined	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*
Abdominal cavity								
- retention of blood	0	5*	1	7*	3	2	4	4
Liver								
- discoloured	0	1	5*	5	3	7*	8*	4
- greyish foci	2	4	9	4	0	1	1	5*
- nodule	21	25	22	25	11	7	11*	7
- mass	6	6	8	9	2	0	3	2
Spleen								
- distinct follicles	1	3	9*	1	9	10	8	8
Thymus								
- atrophy	13	16	13*	19	7	4	9	7

From Takehara (1984b, 1985)
* $P < 0.05$ (statistical significance from either scheduled kills or moribund/spontaneous deaths; Fisher's direct computation for probability)

Calcification of the testicular tunica albuginea and deferent canals was significantly increased in males of the 400 and 2000 ppm groups after 24 months (Table 10). Ioannou (1989) reported historical control data from the Nippon Institute of Biological Sciences of 5/50 (10%) and mentioned another report with higher incidences of slight severity. The lesion was not found after 6 and 12 months. Although its pathological development is unknown, it is considered to be treatment related in view of the dose–effect relationship. Spermatogenesis of these calcified testes was normal. Besides these lesions, there were lesions that increased or decreased significantly in each treated group compared with the controls, but all of them were either secondary lesions caused by tumours or ageing lesions unrelated to the test substance.

Additional histopathological investigation into the salivary gland and mammary gland was taken from the long-term feeding study by the Nippon Institute of Biological Sciences. All of the tissues from the main study were available for evaluation. These findings were reported in detail in Yamate (1988).

Bentazone was not carcinogenic, nor did it produce any other adverse effects on the mammary gland or salivary gland. One adenocarcinoma of the mammary gland was found in a female receiving 400 ppm, which was killed at the end of 24 months. Leukaemic cell infiltration was found sporadically in salivary glands and mammary glands; there was a non-significant increase in dosed females, which was considered to be spontaneous or incidental. Lymphocytic aggregation (perivascular) in the salivary glands was frequent; lymphocytes appeared normal. For male mice sacrificed moribund or found dead, the incidence of lymphocytic aggregation (perivascular) in the salivary glands in the 2000 ppm group (17/21) was significantly ($P < 0.05$) greater than in comparable

controls (6/14), but for mice sacrificed at termination, there was no increase. This finding was not considered to be of biological importance.

Table 10. Incidence of selected non-neoplastic histopathological findings (with potentially relevant statistical significance) in mice administered bentazone for 24 months

	Incidence of finding							
	Killed by design				Terminated or found dead			
	0 ppm	100 ppm	400 ppm	2000 ppm	0 ppm	100 ppm	400 ppm	2000 ppm
24-month sacrifice males								
No. of animals examined	*36*	*36*	*35*	*29*	*14*	*14*	*15*	*21*
Liver								
- haemorrhage	0	1	0	2	1	3	2	10*
Spleen								
- extramedullary haematopoiesis, slight	1	0	0	1	0	5*	1	2
Heart								
- haemorrhage	0	0	0	0	0	0	0	6*
Pancreas								
- hyperplasia of islet cells	7	8	15*	12*	2	3	4	10*
Brain								
- vacuolization in white matter	1	32***	2	2	0	1	0	0
Testis								
- calcification	2	5	12**	24***	0	1	0	11***
24-month sacrifice females								
No. of animals examined	*40*	*41*	*37*	*35*	*10*	*9*	*13*	*15*
Lung								
- haemorrhage	0	1	1	1	0	0	5*	1
Lymph nodes, mesenteric								
- red blood cell infiltration in sinus	0	0	5*	0	0	0	1	0

From Takehara (1984b, 1985)
* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$ (Fisher's direct computation for probability)

The tumour incidence in most organs was low and/or did not reach statistical significance or did not suggest a treatment-related effect due to a lack of a dose–effect relationship. The notable exception is the liver. In the original assessment, the incidence of liver tumours (neoplastic nodules and hepatocellular carcinoma) in each group was 52–70% for male mice and 10–26% for female mice. The corresponding historical control incidence is quoted as approximately 80% for males and 13% for females (Ward et al., 1978), and ranges from the literature are given as 7–55% for males and 0–21% for females (Tarone, Chu & Ward, 1981). Further, it is noted that the sums of neoplastic nodules and hepatocellular carcinomas for each group were not statistically significantly different from the controls. Further, in the 6- and 12-month tests, swelling of hepatocytes, degenerative focus or hepatocellular tumours were not observed in the higher dose groups. The conclusion is that the hepatic tumours observed are not due to the treatment with bentazone. In Ioannou (1989), slightly different control data are quoted, yet the conclusion that bentazone is not carcinogenic in the livers of

B6C3F1 mice is supported. It is also mentioned that these control data are higher than data published by Haseman, Huff & Boorman (1984) based on 2300 mice.

The hepatic tumours were reviewed after completion of the report and reassessed according to Vesselinovitch, Mihailovich & Rao (1978). Hyperplastic and adenomatous nodules were classified as non-carcinomatous nodules, and trabecular nodules were classified as carcinomas. There were no significant differences in the incidences of non-carcinomatous nodules and carcinomas between the controls and any of the treated groups, except non-carcinomatous nodules in the female mice receiving 2000 ppm. Thus, the conclusion regarding the absence of an oncogenic effect in the liver was confirmed.

Other tumour lesions found in various other organs (e.g. lung and haematopoietic system) did not show a pattern suggestive of a treatment-related effect, and the incidences were not significantly different from controls. The total number of tumours, number of tumour-bearing mice and number of tumours per mouse in the treated groups were all similar to those of the controls and without any significant difference. Thus, no oncogenic potential was found in this study.

Bentazone administered to mice at dietary concentrations of 0, 100, 400 and 2000 ppm for 2 years resulted in a transient impairment of body weight development in males at 2000 ppm. Bentazone caused a prolongation of the prothrombin time in males at 400 and 2000 ppm, which is in agreement with similar findings in the short-term studies of toxicity in mice and rats (see above). The gross pathological examination showed various lesions in the liver, spleen and thymus in animals of all the treatment groups and the control group. These lesions occurred in some cases significantly more frequently only in the two intermediate dose groups and showed no dose–response relationship. They were assessed as being age induced and not related to the test substance.

Testis histology revealed an increased incidence of calcification of the testicular tunica albuginea and deferent canals in the 400 and 2000 ppm males. In view of historical control data, this change is a very common lesion in aged mice, including the strain used in this study. There was, in addition, no evidence of any other adverse effect on testes or on male reproductive performance obtained in any study on bentazone.

There were no neoplastic changes indicative of an effect of bentazone; however, bentazone may have slightly increased proliferative lesions in the liver of female mice. Thus, the compound did not show an oncogenic potential.

The NOAEL was 100 ppm (equal to 12 mg/kg bw per day in both sexes), based on prolongation of prothrombin time and increased incidence of calcification of the testicular tunica albuginea and deferent canals in males at 400 ppm (equal to 47 mg/kg bw per day). The study was conducted according to the principles of GLP, and a QA statement was attached (Takehara, 1984b, 1985; Carlton et al., 1987; Millar, 1987; Butler, 1988; Yamate, 1988; Ioannou, 1989).

Rats

In a chronic toxicity study, bentazone (batch and purity not given) was administered to Sprague-Dawley rats (50 of each sex per group) via a diet containing 0, 100, 350 or 1600 ppm (equal to 0, 5, 17 and 76 mg/kg bw per day, respectively; means for males and females) for 2 years. The animals were housed singly under controlled conditions and received standardized diet and water ad libitum. Clinical observations were made daily. Feed consumption was recorded weekly for the first 2 months and then every 2 weeks. Body weight was determined weekly for the first 2 months and monthly thereafter. Ophthalmoscopy was carried out prior to the beginning of the study and after 6, 9, 12, 18 and 24 months. Haematological, clinical chemistry and urine analysis parameters were determined in 5 animals of each sex per dose group after 3, 6, 9 and 12 months and in 10 animals of each sex per dose group after 18 and 24 months. After 12 months, five animals of each sex per dose group were sacrificed and necropsied. All the sacrificed animals were subjected to a gross pathological examination. All sacrificed animals of the control group and of the highest dose group and 10 animals of each sex for each of the other dose groups were examined histopathologically. However, blood coagulation parameters were not investigated. For statistical calculations, various

methods were applied, including Bartlett's test for homogeneity of variances, analysis of variance, Duncan's multiple range test and Wilcoxon or Mann-Whitney rank sum test.

Mortality was not affected by the substance administration. Statistical evaluation of body weight revealed a significant decrease at 1600 ppm in both sexes due to a diminished body weight gain in the 2nd year of the study. Similarly, mean feed consumption was reduced at the top dose level in the 2nd year. No treatment-related findings in haematology, clinical chemistry or ophthalmoscopy were noted. At 1600 ppm, the organ weights of kidney, liver and spleen in both sexes and brain and heart in females were increased. The organ to body weight ratios of kidney, liver and spleen were increased in both sexes. Females exhibited an increased brain and heart to body weight ratio. No statistical significance was shown in any of the other three dose groups. Tumours that occurred were examined histologically and did not reveal any signs of malignancy. Statistical analysis of tumour incidence did not reveal any significance among the groups tested. No substance-induced histopathological changes were noted.

In view of the above observations, the NOAEL for chronic toxicity was 350 ppm (equal to 17 mg/kg bw per day for both sexes), based on decreased feed consumption and body weight gain and increased organ weights at the highest dose level. The study is not GLP compliant, as it was generated prior to the implementation of GLP (Cannon et al., 1974).

Bentazone (batch N 169; purity 93.9%; free acid) was administered to groups of 70 Fischer F344 Du/Crj (SPF) rats (34–35 days old, weighing between 77 and 108 g [males] and 70 and 89 g [females]) of each sex at a dietary concentration of 200, 800 or 4000 ppm for about 6 or 12 months (satellite groups; 10 animals of each sex per dose) and 24 months (main groups; 50 animals of each sex per dose). At the end of the respective administration period, the animals were sacrificed.

The mean compound intakes corresponded well with the nominal dose levels and were calculated for the periods of 1–26, 1–52 and 1–104 weeks (Table 11). Both sexes ingested more of the compound when they were younger, and the intakes decreased with age.

Table 11. Mean compound intake over the duration of the 2-year study in rats

Sex	Period (weeks)	Compound intake (mg/kg bw per day)		
		200 ppm	800 ppm	4000 ppm
Males	1–26	12	47	233
	1–52	9	39	197
	1–104	9	35	180
Females	1–26	14	55	274
	1–52	12	48	249
	1–104	11	45	244

From Takehara (1984a)

The animals were examined for their general state (appearance and behaviour), morbidity or mortality twice daily on working days and once daily on weekends and public holidays. If animals were in a moribund state, they were sacrificed and necropsied. Animals found dead were necropsied as soon as possible. Palpations on skin and abdominal organs were performed once per week. The body weight of the animals was determined at the start of the treatment (day 0), at weekly intervals thereafter and prior to necropsy. Feed consumption was determined on 10 rats of both sexes in each group of the main test group. From week 28 onward, measurements were done on 20 rats of each sex per group to avoid group sizes of less than 10 due to death of rats. Water intake was determined for 10 rats of both sexes in each group of the main test group. From week 28 onward, measurements were done on 20 rats of each sex per group to avoid group sizes of less than 10 due to death of rats.

The eyes of all surviving animals were examined at the end of their administration period for any changes using the naked eye, an ophthalmoscope and a funduscope. Atropine was dropped into the eye 5–10 minutes before the examination. Blood samples were withdrawn under light anaesthesia from the descending aorta by laparatomy. The haematological and clinical chemistry parameters were determined for 10 animals (9 at 800 ppm for females after 12 months) per test group after their respective dosing period (17 for haematology after 24 months). The urine analyses were conducted for 10 animals of each sex per group after 6, 12 and 24 months of administration. All animals—if not found dead—were sacrificed by alcohol, chloroform and ether vapour anaesthesia and exsanguination. The exsanguinated animals were necropsied and assessed by gross pathology. Animals that died intercurrently were necropsied as soon as possible after death and assessed by gross pathology. The organs were sampled, weighed and examined histopathologically.

No specific condition caused by the administration of bentazone was noted in any of the groups in either sex over the 6-, 12- or 24-month period. In both treated and control groups, almost all rats survived until month 12. Mortality increased from week 81 onwards, but none of the groups showed any early deaths or high rate of death. In the 24-month group, mortalities for the 200, 800 and 4000 ppm groups ranged from 18% to 44% for males and from 30% to 46% for females, respectively. The mortalities for treated males and females were not significantly different from those of the respective controls.

No treatment-related ophthalmoscopic findings were noted in the animals sacrificed after 6 or 12 months. In the 24-month animals, cataracts were observed in males receiving 4000 ppm, but also in control males. The incidences were low in the 200 and 800 ppm groups. Retinal changes and cataracts are frequent, age-induced manifestations in the strain of rats used, with a widely variable incidence. All cataracts except one were unilateral, and all except one appeared after 24 months. Two points argue against a cataractogenic substance effect. In the case of a substance-related cataractogenic effect, bilateral cataracts would be expected. Furthermore, cataractogenic substances have been shown to predominantly affect younger animals. This is attributed, in part, to differences in the penetrability of drugs from the bloodstream through the blood–aqueous barrier into the eye, as well as to innate differences in the susceptibility of the lens itself to a cataractogenic effect of a drug. Accordingly, it seems unlikely that the unilateral cataract noted in males receiving 4000 ppm was caused by the test substance. This position was verified after a re-examination of the above-mentioned clinical and pathological findings and reinforced in the supplemental report (Takehara, 1986, based on a review performed by Butler, 1985), which states that "the age-related susceptibility of the lens to the test substance could not be demonstrated". Furthermore, significant differences in the incidence of retinal degeneration and atrophy between treatment and control animals were not detected when these findings were regarded as one type of lesion. The toxicological significance of the degenerative changes in the optic nerve remains equivocal, in particular, as a detailed examination revealing atrophy was confined to high-dose males. Furthermore, it should be taken into account that such eye findings were not noted in any other long-term or subchronic study with bentazone.

Body weight gain in the 200 ppm animals was unaffected. In the 800 ppm animals, a transient suppression of body weight gain was noted in the period between weeks 19 and 36 in males and in the separate weeks 60 and 65 in females.

The weekly body weight gains were frequently suppressed from weeks 5–6 onward in both sexes treated with 4000 ppm.

The overall body weight gain after week 104 was reduced only in high-dose males and females by about 4.7% and 7.7%, respectively. After week 12, body weight gain was suppressed in the high-dose males by about 4% and in females by about 2.6%.

No differences in feed consumption relative to controls were seen in the animals in the 200 ppm group. In the 800 and 4000 ppm groups, feed consumption tended to decrease in males and was approximately the same as in controls in females. There was no significant difference in the total average feed efficiencies for males and females between every treated group and the control group.

The water consumption of the 200 ppm males was comparable to that of controls over the whole test period. In the 800 ppm males, water consumption was increased occasionally between weeks 29 and 77. In the females of this dose group, water consumption was increased from week 29 until the end of the study. In the 4000 ppm group, water intake was increased from week 6 onwards in males and from week 17 onwards in females. Over the complete period, the mean weekly water intake in these high-dose animals was increased by about 40%.

There were a number of variations in the red and white blood cell parameters in either direction (Table 12). These changes remained within the normal range and were not considered to be treatment related. Blood platelet counts were reduced in both sexes of the 4000 ppm group as well as in males in the 800 ppm group at 6 months. The reduction in platelet counts in the low-dose group after 12 months in females was not considered to be treatment related due to the lack of a dose–response relationship.

Prothrombin time and activated partial thromboplastin time were prolonged in males receiving 4000 ppm at months 6 and 12. Activated partial thromboplastin time was prolonged in males receiving 800 ppm at month 12 and in males receiving 4000 ppm at month 24 (Table 12). Females showed a prolongation of activated partial thromboplastin time in the high-dose group after 12 months. The changes in prothrombin time in females after 24 months at the middle dose and in activated partial thromboplastin time in females in the 800 ppm group after 12 and 24 months were discussed in the report by Takehara (1984a) as not being substance related. This conclusion was further supported by Ioannou (1989), in which a statistical re-evaluation revealed no significance. Therefore, prolongations in prothrombin time and/or activated partial thromboplastin time in combination with reduced platelet counts within 6 months and prothrombin time and/or activated partial thromboplastin time within a 1-year interval suggest the presence of haemorrhagic diathesis and are considered to be the result of test substance administration. This is further supported by the observations in one dead male (#232) in the 4000 ppm group showing haemorrhagic lesions in the intraperitoneal adipose tissue and pia mater of the rhinencephalon. No female died in the first 6 months, but in the second 6-month period up to 12 months, one female in the 800 ppm group (#756) died with haemorrhage in the thoracic cavity.

A number of statistically significant clinical chemistry findings were observed; however, many of them were considered as either not relevant/adverse or not treatment related. For example, the decrease in lactate dehydrogenase level in females was considered to fall within the physiological range of values and was not considered to be a morbid change.

At month 6, blood urea nitrogen concentration was increased in males in all treated groups and in the high-dose females. In the 4000 ppm group males, the albumin to globulin ratio was increased, total cholesterol concentration was decreased and glucose concentration and aspartate aminotransferase activity tended to decrease, whereas in 4000 ppm females, alanine aminotransferase activity was decreased. At month 12, in the 4000 ppm group, glucose concentration, aspartate aminotransferase activity and sodium ion concentration were decreased in males, whereas the albumin to globulin ratio and creatinine and blood urea nitrogen concentrations were increased in females. The blood urea nitrogen concentration was also increased in females receiving 800 ppm. At month 24, glucose and total cholesterol concentrations were decreased in males and females receiving 4000 ppm.

At 6 and 12 months, the urine volume was increased in both sexes within the 4000 ppm group, whereas the specific gravity decreased in parallel. In both sexes in the 800 ppm group, a volume increase coupled with a decrease in specific gravity was limited to the 6-month analysis. After 24 months, the specific gravity was decreased in both sexes in the 4000 ppm group and in females in the 800 ppm group. None of the corresponding urine volumes were increased; on the contrary, the high-dose females showed 47% reduced urine volume. A urine volume increase was apparent only in 800 ppm males, but without changes in specific gravity. The colour of the urine ranged from yellow in the controls to light yellow in the dosed groups. In the report, it was suggested that the increase in urine volume coupled with the decrease in specific gravity in the 4000 and 800 ppm groups was related to an increase in water intake. However, the decrease in urinary specific gravity in the absence

of an increase in urinary volume in females in the 200 ppm group at month 6 and in males in the 200 and 800 ppm groups at month 12 was not considered to be related to the treatment.

Table 12. Selected haematological findings (group means) in rats administered bentazone for 6, 12 or 24 months

	Month	Males				Females			
		0 ppm	200 ppm	800 ppm	4000 ppm	0 ppm	200 ppm	800 ppm	4000 ppm
RBC (10^4/mm^3)	6	845	800	808	823	842	815	821	813
	12	968	934	926	968	903	918	925	896
	24	761	619	865	857	841	859	904	834
Hb (g/dl)	6	15.0	15.2	15.4**	15.5**	14.8	15.2**	15.2*	14.9
	12	15.5	15.2	15.3	16.0*	15.2	15.3	15.5	15.6***
	24	13.5	11.3	13.6	14.5	14.4	13.7	14.3	14.4
HCT (%)	6	47.2	47.3	47.7	47.8	45.7	46.4	46.3	45.0
	12	47.9	47.4	47.5	49.0***	46.9	46.5	47.5	47.3
	24	43.3	37.5	45.0	46.8	45.1	44.6	46.4	46.3
MCV (µm^3)	6	56.1	59.3	59.1*	58.1	54.4	56.9*	56.6	55.5
	12	49.5	50.8	51.5	50.7	52.1	50.7	51.5	52.9
	24	60.1	62.0	52.2	55.0	54.3	53.9	51.7	55.7
MCH (µg)	6	17.8	19.0*	19.1*	18.8*	17.6	18.7**	18.6*	18.3
	12	16.0	16.4	16.6	16.5	16.9	16.7	16.8	17.5
	24	18.3	18.4	15.6*	17.1	17.3	16.5	15.9	17.4
MCHC (%)	6	31.9	32.1	32.3**	32.4**	32.3	32.8*	32.9***	33.0***
	12	32.3	32.2	32.2	32.6	32.4	33.0*	32.6	33.1**
	24	31.6	29.8	29.9	31.1	31.8	30.7***	30.8***	31.2*
WBC (10^2/mm^3)	6.0	47.0	56**	56**	50.0	34.0	37,0	35.0	34,0
	12.0	57.0	57.0	61.0	57.0	30.0	28,0	33.0	32,0
	24.0	121.0	121.0	98.0	48.0	56.0	69,0	37.0	31,0
Platelets (10^4/mm^3)	6	69	66	64**	61***	67	70	64	59***
	12	63	65	61	61	57	51***	58	58
	24	82	63	89	100	64	55	66	69
PT (s)	6	19.0	18.5	19.8	22.4***	15.8	15.6	15.8	15.3
	12	19.0	18.4	18.8	23.4***	16.0	16.6	16.0	16.5
	24	16.1	16.5	16.4	15.9	15.7	16.0	16.3[a]	15.2
APTT (s)	6	19.8	20.2	19.7	24.0**	16.7	15.9	16.3	17.4
	12	17.8	19.1	20.2**	22.5***	17.3	17.4	18.0[a]	18.6*
	24	15.9	16.7	17.3	18.7*	16.7	16.9	15.4[a]	17.3

From Takehara (1984a)

APTT, activated partial thromboplastin time; Hb, haemoglobin; HCT, haematocrit; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; PT, prothrombin time; RBC, red blood cells; WBC, white blood cells

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$ (Student's *t*-test)

[a] Including corrections of Ioannou (1989).

At 6, 12 and 24 months, the pH and protein level in the urine tended to decrease in the treated rats of both sexes dose dependently. This might reflect a modification of the excretion pattern in a

dose-dependent matter, which is not solely associated with higher urinary output. A few other spurious findings were considered to be not treatment related or not adverse. These were an increase in bilirubin level in the low-dose animals of both sexes at 6 months and the tendency for a decrease in urobilinogen and ketone bodies in male and female rats at 24 months.

Organ weights, satellite groups (6-month sacrifice): A decrease in terminal body weights was observed in the high-dose animals. This decrease was statistically not significant in the males (approximately 4.7%) but significant (approximately 6.4%) in the females.

The kidney weight and the kidney to body weight ratio were increased in males and females receiving 4000 ppm, but without histopathological correlate. This effect is probably related to the higher urinary output and the reduction of body weight in the top-dose animals. The weight of the thyroid and the thyroid to body weight ratio were decreased in 800 and 4000 ppm males, and the thymus weight and thymus to body weight ratio were significantly decreased in 4000 ppm females, both organs without histopathological correlates.

Both the absolute and relative weights of the pituitary were significantly decreased in males of all treated groups at month 6, but these decreases were considered to be incidental rather than treatment related. The changes in absolute or relative weights of liver, spleen, heart, lung, testes and brain in the high-dose females or males were not accompanied by corroborative histomorphological changes, were not found in both sexes and were not found to be dose dependent. Thus, these changes were not considered to be treatment related but rather were attributed to body weight changes or biological variation. The adrenals were not affected in the interim kill groups but are listed here for better comparability with the main group animals and to put the data of the 90-day study (see above), which showed a significant increase in absolute adrenal weight in the high-dose males without histopathological correlate, into perspective. In respect of this study, these effects on adrenal weight are considered to be not substance related.

Organ weights, satellite groups (12-month sacrifice): A decrease in terminal body weights by about 7.3% was observed in the high-dose males. The kidney weight and kidney to body weight ratio were increased in males and females receiving 4000 ppm, again without histopathological correlate. It is assumed that this result is probably related to the higher urinary output and the decreased body weight. The weight of the thyroid and thyroid to body weight ratio were decreased in males receiving 800 and 4000 ppm, and the absolute thyroid weight was decreased in females receiving 4000 ppm. Histopathological examinations revealed a non-significant increase in hyperplasia of C-cells. The decrease in pituitary weight seen in males at month 6 was no longer present at 12 months. Decreases in relative and/or absolute weights of liver, spleen, heart, lungs and brain in high-dose males were considered to be secondary to the significantly lower terminal body weights. The decrease in absolute brain weight in females in all dose groups was not dose dependent or correlated with morphological changes. The weight of thymus and the thymus to body weight ratio were reduced, predominantly in males and marginally in females, each without statistical significance or dose dependency. Additionally, no histopathological change was seen in the thymus, so the significance of the weight decrease in females after 6 months is questionable. Adrenals and testes/ovaries were not affected after 12 months.

Organ weights, main group (2-year sacrifice): A decrease in terminal body weight was observed in the high-dose animals, significantly for females.

Absolute kidney weight was reduced in males at 800 ppm without a dose–response relationship. The relative kidney weight was increased in males and females receiving 4000 ppm and decreased in males receiving 800 ppm (according to the statistical re-evaluation performed on request of the United States Environmental Protection Agency [USEPA]; see Ioannou, 1989). Histopathological evaluation revealed a reduction in the severity of chronic nephropathy at higher dose levels in females, so the relevance of this effect is questionable. The absolute and relative weights of the liver were decreased in males receiving 800 and 4000 ppm, and the weight of the spleen was also decreased in males receiving 4000 ppm. These changes were discussed in the report (Takehara, 1984a) as reflecting the reduction in body weight. This is also valid for the changes in heart and brain weights in males. Weight decreases in left-side adrenals in all treated females were not

reflected in the sum of the means, were not dose related and were completely opposite to the weight increase in the low-dose males. The increases in absolute and relative testes weights in the mid-dose males as well as the absolute weight increase in the ovaries were not dose related. No significant weight effects were seen in the pituitary, thyroid or lungs. The thymus was not weighed in the 24-month group because of general age-related atrophy.

Gross pathology: There were only a few gross pathological findings in the animals of the 6-month sacrifice, and they were mainly limited to single incidences without statistical significance. Two males died prematurely; one from the 200 ppm group was killed in a moribund state, and the other from the 4000 ppm group died (#323) showing signs of bleeding, with haemorrhagic lesions in the intraperitoneal adipose tissue and pia mater of the rhinencephalon as well as anaemic appearance of major organs. In the following 6 months, spontaneous mortality occurred in a female of the control group, a male of the 200 ppm group and a female of the 800 ppm group. No findings that could be related to treatment were made. There were various gross pathological findings in the 24-month sacrifice group. Although certain findings reached statistical significance (from either the scheduled kill subgroup or moribund/spontaneous deaths), none of them indicated a relationship to treatment except for the atrophy of the optic nerve in the 4000 ppm males.

Histopathology, non-neoplastic lesions, 6- and 12-month groups: There were several histological non-neoplastic findings in the animals sacrificed after either 6 or 12 months. Most of them were of single occurrence, showed no dose–response relationship and were equally distributed between control and treated groups. Of those with higher incidences, none appeared to indicate a treatment-related effect.

Histopathology, non-neoplastic lesions, 2-year group: A number of non-neoplastic findings were present in the animals either killed by design or at moribund/spontaneous death. Histological examination (and necropsy) performed at month 24 disclosed atrophy of the optic nerves and retinal degeneration in males in the 4000 ppm group, and these lesions are deemed to be associated with cataract and the tendency of Fischer rats to develop retinal degeneration as an age-related change.

In the supplemental report (Takehara, 1986, based on a review performed by Butler, 1985), cataracts, atrophy of the optic nerve and retinal degeneration and atrophy were readdressed based on the re-evaluation of 19 control and 21 high-dose animals. The differences in numbers found in histological examination, gross postmortem examination and ophthalmoscopic examination were acknowledged, and the ophthalmoscopic examination was considered as most relevant. It was concluded that the observations in this study do not suggest a compound-related effect, although more cataracts are observed in the top-dose group (7 in control versus 18 in top-dose males).

The facts that the cataracts were all, except one, unilateral and that all, except one, appeared above 1 year of age add weight to the opinion that the effect is not substance induced. In the case of substance-induced cataracts, they would be expected to be bilateral and to occur in younger animals, in which the lens is due to a greater penetrability of drugs from the bloodstream through the blood–aqueous barrier into the eye and to an innate higher susceptibility of the lens itself to a cataractogenic effect of a drug.

By examination of rats having cataracts with or without apparent degeneration of the optic nerve, it was found that "the optic nerve atrophy coexisted with other abnormalities in the same eyeball, suggesting that there might be a relationship between optic nerve atrophy and the other lesion in the eyeball". With regard to retinal atrophy and retinal degeneration, it is argued that these should be grouped together as a single entity of retinal disorders. No statistically significant differences between incidences are then evident. The conclusion of the amendment (Butler, 1985) is that cataracts, atrophy of the optic nerve and retinal degeneration and atrophy are not related to the administration of bentazone.

Lesions observed in the liver, spleen, kidneys, lungs, adrenals and other organs could be attributed to ageing. The occurrence of these changes was more frequent in rats that died than in rats killed by design. An influence of test substance administration was not apparent in any of the organs.

The incidence of pulmonary lesions was not related to dose, and some pulmonary lesions were interpreted to have been caused by inhalation of powdered diet, because there were abundant vegetable fibres in the foci.

Histopathology, neoplastic lesions: In the initial 12 months of the study, one mesenchymal tumour was noted in one female in the 800 ppm group, an interstitial cell tumour of the testicle in two males in the 4000 ppm group and hyperplasia of chromophobic cells of the pituitary in one female in the 4000 ppm group. In the 24-month group, there were higher incidences of tumours; however, none could be associated with the treatment with bentazone. Most of the tumours occurred in the testicles, liver, adrenals, thyroids, skin, mammary glands, pituitary and uterus. In addition to tumours in these organs, atypical monocytic leukaemia showing generalized infiltration of tumour cells into various organs was noted. These manifestations could also not be related to the treatment. In the kidneys, as the primary target organ, with an organ weight increase at the top dose, no increased tumour formation was found in males or females.

Ioannou (1989) requested, in addition to the referenced historical control data in the literature, laboratory historical control data for phaeochromocytoma and uterine endometrial polyps. For phaeochromocytoma, the laboratory historical control values are stated as being 37/257 (14%) in males and 26/203 (13%) in females, which are higher than the values from two other references mentioned in the Ioannou (1989) study report. In the study, the combined incidence of phaeochromocytoma was 6, 9, 11 and 9 in males at 0, 200, 400 and 8000 ppm, which is equal to 12%, 18%, 22% and 18%, respectively. In females, the combined incidence was 0, 2, 3 and 4, which is equal to 0%, 4%, 6% and 8%, respectively. The incidence in male animals was higher than the historical control incidence, but showed no clear dose dependency, whereas the incidence in females showed dose dependency, but stayed below the historical control values. Ioannou (1989) confirmed that the observed increased incidence of phaeochromocytoma in high-dose females was not of biological importance.

For uterine polyps, the laboratory historical control incidence is 45/203 (22%), which is slightly higher than the values given in two other references. In the study, the combined incidence of uterine polyps in females was 10, 9, 18 and 12 at 0, 200, 400 and 8000 ppm, which is equal to 20%, 18%, 36% and 24%, respectively. Nevertheless, according to Iannou (1989), the data suggest that the increased incidence of uterine polyps in mid-dose females is not of biological importance.

All other neoplastic findings either were observed in single or low incidences without a relationship to treatment or were evenly distributed among all groups, including controls. They were therefore considered to be of spontaneous origin and not related to treatment. In male and female rats, the total number of tumours, number of tumour-bearing animals and number of tumours per rat were approximately the same in all groups, including controls. Thus, no oncogenic potential was found in this study.

Histopathology of decedents: Both males and females tended to die increasingly from month 13 onward, and the incidence of death for both males and females was elevated from month 19 onward. Major lesions noted in dead males include pituitary tumours, enlargement of the spleen and liver, generalized yellowish pigmentation, subcutaneous tumours in the thoracic and abdominal regions, emaciation and purulent pneumonia. In females, emaciation and purulent pneumonia were predominant causes of death, followed by pituitary tumours, enlargement of the spleen and liver, and atypical monocytic leukaemia.

Conclusions: The administration of bentazone led to a reduction in body weight gain at 4000 ppm. Although histopathological examination revealed no substance-induced changes, indications for an impairment of liver and kidney function were noted at 800 and 4000 ppm by changes in clinical chemistry and urine analysis parameters and by increased organ weights, as well as by increased water consumption at the top dose level. Blood coagulation was affected at 800 and 4000 ppm. This finding is in agreement with the results of the shorter-term studies with bentazone (see above). Decreased organ weights were assessed as being related to the decreased body weights.

Under the conditions of this study, the NOAEL was 200 ppm (equal to 9 mg/kg bw per day in males and 11 mg/kg bw per day in females), on the basis of prolonged blood coagulation and impairment of liver and kidney function at 800 ppm (equal to 35 mg/kg bw per day in males and 45 mg/kg bw per day in females). No carcinogenic effect was observed in this study.

Although the study was performed when GLP was not compulsory, it is stated in the report (Takehara, 1984a) that the study was conducted according to the principles of GLP (Takehara, 1984a; Butler, 1985, 1986, 1988; Ioannou, 1989).

2.4 Genotoxicity

Bentazone was tested for genotoxicity in 16 studies, including 10 in vitro studies and 6 in vivo studies (Table 13). There are also six genotoxicity studies in the published literature on bentazone. Bentazone gave negative results in all the studies. In one in vitro forward mutation assay in mammalian cells (HRPT test), bentazone gave a weakly positive result with mice using the S9 mix. Only two studies complied with GLP, as most of the studies were generated before implementation of GLP. In three studies, QA statements are attached. On the basis of these studies, it is concluded that bentazone is unlikely to be genotoxic. A summary of the studies described is given in Table 13.

2.5 Reproductive and developmental toxicity

(a) Multigeneration studies

In a multigeneration study, bentazone (batch number and purity not given) was administered via the diet at a concentration of 0, 20, 60 or 180 ppm to three generations of Sprague-Dawley rats (F_0, F_1 and F_2, each with two litters). Twenty rats of each sex were used in each case. The control group received untreated diet only. Initially, all rats were housed three per cage under controlled conditions. For mating, one male and one female were placed together during the 12-hour dark period for 7 days. Once copulation had occurred, the females were separated and kept alone. The animals had free access to standardized diet and water throughout the study. The substance intake at the low dose level of 20 ppm ranged between 1.6 and 2.2 mg/kg bw per day for males and between 2.0 and 2.5 mg/kg bw per day for females. At the middle dose level (60 ppm), males received 4.5–6.4 mg/kg bw per day and females 6.4–7.3 mg/kg bw per day. High-dose (180 ppm) males received 14.1–19.4 mg/kg bw per day, and females, 19.8–21.9 mg/kg bw per day.

After 8–18 weeks of pretreatment, the F_0 animals were mated. The pups of the first litter (F_{1a}) were reared until they were 4 weeks old and then sacrificed and necropsied. The parental animals were mated again, and, at an age of 4 weeks, 20 animals of each sex per dose group were selected from the pups of the second litter and reared while being given further treatment. The remaining pups were sacrificed and necropsied. At an age of 18–29 weeks, these F_{1b} animals were mated twice, and the same procedure was carried out with the F_2 pups as with the F_1 pups.

No clinical signs of toxicity were noted. Body weight remained unaffected. Fertility and rearing behaviour of the animals were not affected. The development of the pups was comparable in all the groups. No substance-induced gross pathological or histopathological changes occurred.

Under the conditions of this study, the NOAEL for parental toxicity as well as reproductive and developmental toxicity was 180 ppm (corresponding to about 21.9 mg/kg bw per day), the highest dose tested. The study was not GLP compliant (Leuschner et al., 1973).

In a two-generation reproductive toxicity study, bentazone technical (batch no. N 187; purity 97.8%) was administered to groups of Wistar/HAN (Kfm: WIST, outbred, SPF Quality) rats (25 of each sex per group; 8 weeks old and weighing 176–224 g [males] and 138–178 g [females]) at a dietary concentration of 0, 200, 800 or 3200 ppm, corresponding to a lowest average intake of around 0, 14, 59 and 238 mg/kg bw per day, respectively, during the premating and gestation periods.

Table 13. Summary of genotoxicity studies on bentazone

Study	Strain/species	Substance; concentration/dose	Purity (%)	Result	Reference
In vitro					
Bacterial reverse mutation assay (Ames test)	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, TA1538; *Escherichia coli* WP2 hcr	Bentazone; 0, 10, 50, 100, 500, 1000 µg/plate	94	Negative (±S9)	Shirasu et al. (1976)
Bacterial reverse mutation assay (Ames test)	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538; *E. coli* WP2 hcr	Bentazone; 0, 1000, 2500, 5000, 10 000 µg/plate	94	Negative (±S9)	Moriya (1984)
Bacterial reverse mutation assay (Ames test)	*S. typhimurium* TA98, TA100, TA1537	Bentazone (77/357); 0, 3.1, 10, 31, 100, 310, 1000, 2000 µg/plate	92.5	Negative (±S9)	Oesch (1977)
Bacterial reverse mutation assay (Ames test)	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	Bentazone (83/3); 0, 20, 100, 500, 2500, 5000 µg/ml	96.7	Negative (±S9)	Engelhardt & Gelbke (1983)
Bacterial reverse mutation assay (Ames test and *E. coli* reverse mutation assay)	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538; *E. coli* WP2 uvrA	Bentazone; 0, 20, 100, 500, 2500, 5000 µg/ml	92.6	Negative (±S9)	Engelhardt & Gelbke (1985a)
Bacterial reverse mutation assay (Ames test)	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	Bentazone sodium (pure and technical grade); 0, 500, 1000, 2500, 5000, 7500, 10 000 µg/ml	Pure: 99.5 Technical grade: 47.7	Negative (±S9)	Engelhardt & Gelbke (1985b)
Bacterial reverse mutation assay (Ames test)	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538; *E. coli* WP2 hcr	Bentazone; up to 5000 µg/ml	Not given	Negative (−S9)	Moriya et al. (1983)
Bacterial reverse mutation assay (Ames test)	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538; *E. coli* WP2 hcr	Bentazone	99.9	Negative (±S9)	Jeang & Li (1978)
Bacterial reverse mutation assay (Ames test and *E. coli* reverse mutation assay)	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538; *E. coli* WP2 hcr	Bentazone	Not given	Negative (±S9)	Shirasu et al. (1982)
DNA damage and repair (SOS chromotest)	*E. coli*	Bentazone	Not given	Negative (±S9)	Xu & Schurr (1990)
DNA damage and repair (mitotic gene conversion assay)	*Saccharomyces cerevisiae* D4	Bentazone	Not given	Negative (−S9)	Siebert & Lemperle (1974)
DNA damage and repair (mitotic gene conversion assay)	*S. cerevisiae* D4	Bentazone	Not given	Negative (−S9)	Zimmermann et al. (1984)

Study	Strain/species	Substance; concentration/dose	Purity (%)	Result	Reference
Chromosomal aberration assay in mammalian cells	CHO cells	Bentazone 0, 500, 1000, 2000, 3000 µg/ml (−S9) 0, 2000, 3000, 4000, 5000 µg/ml (+S9)	Not given	Negative (±S9)	Taalman (1987)[a]
Forward mutation assay in mammalian cells (HPRT test)	CHO cells	Bentazone technical; 0, 100, 464, 1000, 2150, 4640, 10 000 µg/ml	93.9	Negative (−S9) Negative with rat S9 mix Weakly positive with mouse S9 mix	Jaeckh & Gelbke (1985)
Forward mutation assay in mammalian cells (HPRT test)	CHO cells	Bentazone technical (84/140); 1250, 2500, 5000, 7500, 10 000, 12 500, 15 000 µg/ml	93.9	Negative (−S9) Negative with rat or mouse S9 mix	den Boer (1985)[b]
Forward mutation assay in mammalian cells (HPRT test)	CHO cells	Bentazone technical; 0, 100, 600, 1200, 2500, 5000 µg/ml	97.6	Negative (±S9)	Muellerschoen (1991)[a]
In vivo					
Chromosome analysis (micronucleus test)	NMRI mouse	Bentazone technical; 0, 200, 400, 800 mg/kg bw; single application	95.6	Negative	Engelhardt & Gelbke (1985c)[b]
Chromosome analysis (micronucleus test)	Wistar rat	Bentazone technical; 0, 27.5, 55, 110, 220, 700 mg/kg bw; two oral administrations with 24 h time lag	Not given	Negative	Postica et al. (1982)
Unscheduled DNA synthesis	B6C3F1 mouse	Bentazone technical; 40–360 mg/kg bw; single application	Not given	Negative	Cifone (1985a)[b]
Mutation assay in germ cells (dominant lethal test)	Sprague-Dawley rat	Bentazone; dietary concentrations of 20, 60, 180 ppm over 13 weeks	Not given	Negative	Leuschner (1971)
Mutation assay in germ cells (dominant lethal test)	NMRI mouse	Bentazone technical; single intraperitoneal application of 195 mg/kg bw	Not given	Negative	Hofmann & Peh (1973)
Unscheduled DNA synthesis	B6C3F1 mouse primary hepatocytes	Bentazone; 0.05–1004 µg/ml in Williams' medium E	Not given	Negative	Cifone (1985b)

CHO, Chinese hamster ovary; DNA, deoxyribonucleic acid; S9, 9000 × *g* supernatant fraction of rat liver homogenate

[a] Complied with GLP.

[b] QA statement attached.

After the acclimatization period, F_0 parental animals continuously received the test substance throughout the entire study. At least 70 days after the beginning of treatment, male and female rats of the same dose groups were mated overnight. Females were allowed to deliver and rear their pups (F_1 generation pups) until day 4 or 21 after parturition. After weaning of the F_1 pups, the F_0 generation parental animals were sacrificed, and 25 male and 25 female F_1 pups of each treatment group were randomly selected as F_1 generation parental animals. All selected animals were treated with the test substance at the same dose level as their parents from post-weaning through adulthood up to about 1 day before they were sacrificed. At least 123 days after assignment of the F_1 generation parental animals, the males and females were paired one male to one female for a maximum of 19 days. No siblings were paired.

Like F_0 females, F_1 females were allowed to litter and rear their pups (F_2 generation pups) until day 4 (standardization) or day 21 after parturition. Shortly after weaning of the F_2 pups, the F_1 parental animals were sacrificed.

Males and females were paired at a 1:1 ratio for a maximum of 3 weeks. Vaginal smears were taken daily and examined for the presence of sperm and/or appearance of a vaginal plug. If evidence of mating was detected, pairing of the animals was discontinued. The day on which sperm were detected was denoted gestation day (GD) 0, and the following day, GD 1.

On postnatal day (PND) 4, the size of each litter was adjusted by eliminating extra pups by random selection to yield, as near as possible, four males and four females per litter. The surplus pups were sacrificed and examined macroscopically.

The average compound intakes for the females in the respective study periods are given in Table 14.

Table 14. Average bentazone intake in female parental rats

Group	Average bentazone intake (mg/kg bw per day)					
	200 ppm		800 ppm		3200 ppm	
	Average	Min/max	Average	Min/max	Average	Min/max
F_0 females (premating)	17.0	13/21	66.9	53/81	269	209/327
F_0 females (gestation)	14.7	13/16	60.7	53/67	247	226/264
F_0 females (lactation)	29.7	22/38	111	80/141	473	356/579
F_1 females (premating)	15.9	13/24	64.4	50/98	2261.6	209/372
F_1 females (gestation)	14.3	14/15	59.3	55/63	238.7	230/249
F_1 females (lactation)	29	22/36	121.3	91/152	492	357/590

From Suter et al. (1989)
max, maximum; min, minimum

The animals (i.e. parental animals and pups) were examined for mortality and evident signs of toxicity twice daily. Towards the end of the gestation period, females were examined twice daily for signs of parturition.

Females without litters and dams that lost their litters were killed together with the dams after weaning the pups and necropsied for the examination of the organs, including ovaries, uterus and uterine contents.

Body weight of parental animals was determined at weekly intervals with the exception of mating periods. The F_0 and F_1 generation parental females were weighed on the day of positive evidence of sperm (GD 0) and on GDs 7, 14 and 21, and females with litters were weighed on the day after parturition (PND 1) and on PNDs 4, 7, 14 and 21.

Pup body weights were determined on the day after birth (PND 1) and on PNDs 4 (before standardization), 7, 14 and 21. Feed consumption was recorded weekly for parental animals. On the day of birth (PND 0), the sex of the pups was determined.

All F_0 and F_1 adult animals selected for breeding were sacrificed when they were no longer necessary for assessment of reproductive effects. Excess F_1 and F_2 pups after standardization of litter sizes were sacrificed on day 4 postpartum, examined for possible defects and discarded. F_1 pups not selected for mating and all F_2 pups were sacrificed and examined for defects after weaning.

No treatment-related clinical signs of toxicity were observed. In the F_0 generation, there were a few transient signs in single animals of the control, 200 and 800 ppm groups. In the F_1 generation, signs were rare and mostly transient. In the 3200 ppm group, one female (#394) showed slight sedation and heavy body weight loss on day 4 postpartum. No treatment-related mortality was observed throughout the study.

Body weight development was impaired in high-dose parental F_0 and F_1 animals. For the males, test article–related decreased mean body weights were noted in the 3200 ppm group of the F_1 generation during both the prepairing and post-pairing periods. Similar mean body weight gain was noted in all groups of both generations during the post-pairing period.

For the females of the F_0 and F_1 generations, test article–related slightly decreased mean body weights (with statistical significance in both the F_0 and F_1 generations at isolated intervals) were noted in the 3200 ppm group during the prepairing, gestation and lactation periods. Mean body weight gain was slightly decreased during the prepairing period, similar during the gestation period and slightly increased during the lactation period when compared with the other treated groups (200 or 800 ppm) and the control group. Similar mean body weight gain and mean body weights were noted for the females of group 1 (control, 0 ppm), group 2 (200 ppm) and group 3 (800 ppm) at all periods for both generations. No test article–related changes in mean feed consumption were noted at any period for the F_0 or F_1 females of any dosed group when compared with the corresponding controls. The slight and, at some time points, statistically significant differences from the control values that were noted, mainly in the 3200 ppm group, but also in the other treated groups of the F_1 females, were considered to be of incidental origin. No clear dose–response relationship was evident.

Relative feed consumption corresponded to the feed consumption and body weights; no test article–related differences between the dose groups and the control group in either the F_0 or the F_1 generation were noted at any time point of any period.

As regards the effect on mating, gestation and rearing, for the parent females of both generations (F_0 and F_1), none of the parameters—numbers of females paired, mated, pregnant, bearing or rearing young, mating performance, fertility, duration of gestation and parturition or nursing ability—were impaired. The mean number of implantation sites, mean number of pups, postimplantation loss and breeding loss were similar in all groups.

Mean body weights of the pups were decreased and significantly different from controls during the lactation period in groups 3 (800 ppm) and 4 (3200 ppm) in both F_1 and F_2 generations. For the pups of the F_1 generation, decreased mean body weight was noted in groups 3 (800 ppm) and 4 (3200 ppm) from day 1 postpartum, but no clear dose–response relationship was evident.

In the F_2 generation, mean body weights for groups 3 (800 ppm) and 4 (3200 ppm) were similar to that of the control group on day 1 postpartum. Dose-dependent, slightly decreased body weight gain was noted thereafter during the lactation period.

The sex ratios of pups, the pup loss during the lactation period (before and after culling) and the physical development, health condition, viability and behaviour of the pups were similar in all dose groups up to the highest dose level of 3200 ppm, when compared with that of the control group.

No test article–related macroscopic changes were observed in either the F_0 or F_1 parent animals or the F_1 or F_2 pups of any group. The isolated findings described above were considered to be incidental in origin and not related to treatment with the test article.

In F_1 litters, no test article–related findings were evident in any dose group. No malformed or anomalous pups were noted in any group. Stray findings were observed in single cases in each group, which were considered to be incidental and not related to treatment with the test article.

In F_2 litters, no test article–related findings were noted in any dose group. Slight oedema in the upper region of the body, cheilognathoschisis, nose and nasal opening missing, breathing through mouth, microphthalmia or anophthalmia, encephalocele, missing tail, absence of external sex organs, and anal and uretal openings closed were observed at external examination.

No evidence of a teratogenic effect was observed in any group in either generation.

Based on the results of this two-generation reproductive toxicity study with bentazone technical in Wistar rats, the NOAEL for parental and offspring toxicity was considered to be 200 ppm (equal to about 14 mg/kg bw per day), based on the effects on body weight gain noted in F_1 generation males and females, reduced parental feed consumption and reduced pup body weight resulting from parental toxicity at 800 ppm (equal to about 59 mg/kg bw per day) in F_0 generation females that were dosed with the highest dietary concentration of 3200 ppm (equal to about 240 mg/kg bw per day) and in the F_1 and F_2 pups treated with 800 ppm and 3200 ppm, not at delivery, but during the lactation period.

Except for these findings, no interferences with the development and reproduction of the two generations were noted, and no teratogenic effects were observed up to the highest dose level of 3200 ppm (equal to about 238 mg/kg bw per day). A formal GLP compliance and QA statement was included in the report (laboratory certified by Eidgenössisches Departement des Innern, Bern, Switzerland) (Suter et al., 1989).

The herbicide bentazone was positively evaluated for inclusion in Annex I to Directive 91/414/EEC concerning the placing of plant protection products on the market based on the dossier submitted in 1995 by BASF AG. With regard to developmental end-points, bentazone has been evaluated in a two-generation reproductive toxicity study (Suter et al., 1989; see above). This study showed slightly reduced pup weight effects in the F_1 and F_2 generations at the middle dose (800 ppm) in the absence of obvious effects on parental weights, which were observed only at the high dose (3200 ppm). However, data from the long-term study of toxicity in rats (Takehara, 1984a) showed significant haematological and clinical chemistry alterations at 800 ppm.

Although the European Commission final review report on bentazone (European Commission, 2000) considered the effects demonstrated in the long-term study for the evaluation of parental toxicity, these effects were not considered in the most current evaluation of the same data by the USEPA (2010). Thus, the USEPA considers bentazone to affect offspring at non-parentally toxic doses.

In order to reassess these conflicting evaluations and to get a better picture about the origin of the pup weight effects, the two-generation reproductive toxicity study was re-evaluated. Due consideration was given to historical control data, and a focus on cofactors was included, including individual animal feed consumption and animal weight data as well as litter size distribution, in order to decide whether the pup weight impairment is a primary substance effect of bentazone or a secondary effect due to impairment of the dams.

Historical control data are valuable to differentiate between effects on a concurrent control group observed in a study and the inherent variability of biological parameters in studies conducted according to the same protocol in the same laboratory. The historical control data during the years 1985–1989 for this two-generation reproductive toxicity study with bentazone are compiled in a separate report (Gerspach, 2011) and are based on eight two-generation studies conducted in the same strain of rats.

The existence of an inverse relationship between pup body weight development and litter size, at least until culling at PND 4, due to competition for maternal milk is intensively described in Agnish & Keller (1997). This characteristic is also seen in the historical control data. Chahoud &

Paumgartten (2009) introduced an approach to standardize pup weight data for litter size effects by correction factors generated in a historical control cohort until the day of culling.

The reduced mean pup weight in the mid-dose F_1 generation was shown to be, to a considerable extent, due to the small litter size of the control group, as normalization of the mean pup weight to the litter size reduced this difference from a maximal 12% to 7%. In a next step, the remaining pup weight deviations were shown to be associated with litters from F_0 dams showing a significantly reduced feed intake and body weight gain in the relevant period of the lactation phase (PNDs 1–4). Although the group mean values of the middle dose do not vary significantly from the control group values, the individual analysis of the data showed that the litters with significantly reduced pup weights arise from dams showing relevant signs of toxicity, as demonstrated by a transient feed refusal within the early lactation phase and a severe maternal body weight loss or body weight gain reduction. Focusing only on those dams with clearly affected litters revealed reduced mean maternal feed consumption between PNDs 1 and 4 to 38% of the concurrent control and a mean body weight loss of 1.7 g between PNDs 1 and 4 in comparison with the control group, which gained about 16.7 g in this sensitive time frame. The respective pup body weight development is most likely impaired as a consequence of this nutritional deficit. A recovery was observed in dams and pups towards the end of the lactation phase, when the feed consumption levels were comparable with those of the control values. In the high-dose group, a maternal body weight reduction was apparent in all periods of the study, and the reduction in pup weight was shown to be attributable to a high litter size and/or a reduced maternal feed intake.

Similarly, the small mean litter size of the control animals in the F_2 generation led to higher mean pup weights in the control litters as compared with the treated groups. This led to an artificially high deviation of the treated animals from the control, but actually the F_2 generation mean pup weights are well within the naturally occurring variability of this parameter.

Therefore, this evaluation definitely supports the European conclusion that pup weight effects were observed only at maternally toxic levels. However, this re-evaluation showed the maternally toxic dose to lie at 800 ppm for the F_0 parental generation. The calculation of the effect levels in milligrams per kilogram body weight was done under consideration of the actual intake during the period where the toxicity was observed—namely, during the lactation period, PNDs 1–4.

The following relevant effect levels in parts per million and milligrams per kilogram body weight per day (according to the substance intake data) are considered appropriate for the two-generation study with bentazone. The NOAEL (maternal and developmental) is 200 ppm (equal to 22 mg/kg bw per day), based on the mean substance intake of F_0 dams between PNDs 1 and 4 during lactation, with a LOAEL (maternal and developmental) of 800 ppm (equal to 80 mg/kg bw per day). The resulting effect levels were derived on the basis of maternal toxicity evident for the F_0 females especially between PNDs 1 and 4 in the mid- and high-dose groups (800 and 3200 ppm) and in the F_1 females only in the high-dose group (3200 ppm). Reproductive/developmental toxicity is based on the slightly decreased pup body weights seen at PNDs 4 and 7 for the F_1 pups in the mid- and high-dose groups (800 and 3200 ppm) and for the F_2 pups in the high-dose group secondary to maternal toxicity (Chahoud & Paumgartten, 2009; Gerspach 2011; Kemény, 2011).

(b) Developmental toxicity

Rats

Bentazone (batch number and purity not given) was administered orally by gavage to groups of 20–36 pregnant Sprague-Dawley rats from day 6 to day 15 post-coitum. The doses administered in a 1% aqueous tylose solution were 0 (control), 22.2, 66.7 and 200 mg/kg bw per day. Two control groups were included. The rats were housed in pairs under controlled conditions and received standardized diet and water ad libitum. Clinical observations were recorded daily. Body weight was determined 3 times a week and on day 20 post-coitum. On this day, all animals were sacrificed and necropsied. The fetuses were dissected from the uterus, weighed, sexed and checked for any morphological abnormalities (external, gross pathological and skeletal examinations).

Following administration of the two lower doses, neither maternal toxicity nor embryo/fetal toxicity could be detected. In the highest dose group receiving 200 mg/kg bw per day, the resorption rate was drastically increased. In addition, the fetuses showed a decrease in body weight, an increase in the number of runts and an increase in the frequency of anasarca. The occurrence of anasarca was confined to this group. The total summary incidence of fetuses with anomalies of all types was also elevated. In contrast, maternal toxicity was not observed at this dose level.

In view of the above, the NOAEL was 200 mg/kg bw per day for maternal toxicity and 66.7 mg/kg bw per day for embryo/fetal toxicity, based on the high resorption rate and the fetal findings at the top dose level, which might suggest a fetotoxic or teratogenic potential of the test compound.

Although the study was conducted according to the "Guidelines for reproduction studies for safety evaluation of drugs from human use" (USFDA, 1966), it was not GLP compliant (Zeller & Peh, 1971).

The same study was repeated 6 years later. Bentazone (purity 92.5%) was tested for its prenatal toxicity in Sprague-Dawley rats. The test substance was administered at the same doses used in the previous study. Each group consisted of 26–29 rats. The animals were housed two per cage under controlled conditions and had free access to standardized diet and drinking-water. Clinical observations and mortality were checked daily. Body weight was determined 3 times a week, on day 0 of pregnancy and on days 6, 11, 15 and 20 post-coitum. On day 20, all animals were sacrificed and necropsied. The fetuses were dissected from the uterus, weighed, sexed and checked for any morphological abnormalities (external, gross pathological and skeletal examinations).

Bentazone was tolerated by all animals without any clinical symptoms and with no adverse effect on body weight or body weight gain. No animal died during the study period. No gross pathological changes were found. No differences between the control group and the substance-treated groups were noted with respect to conception rate, number of live or dead implantations or resorptions. Body weight of fetuses, their length and placenta weight remained unaffected. The examination of the fetuses did not reveal any abnormal findings.

Under the conditions of this study, no embryo/fetal toxicity or teratogenicity was noted. The NOAEL for both maternal and embryo/fetal toxicity was 200 mg/kg bw per day, the highest dose tested. The evidence of a fetotoxic or teratogenic potential of bentazone obtained in the previous study (see above) was not confirmed. Although the study was conducted according to the "Guidelines for reproduction studies for safety evaluation of drugs from human use" (USFDA, 1966), it was not GLP compliant (Hofmann & Merkle, 1978a).

In another study of embryotoxicity (including teratogenicity), bentazone (batch N 187, purity 97.8%) was administered daily to 25 presumably pregnant Wistar/HAN rats by stomach tube during GDs 6–15 at dose levels of 0, 40, 100 and 250 mg/kg bw per day. The age of the animals was at least 12 weeks, and the animals weighed around 185–225 g (post-coitum).

The treatment did not elicit any adverse effects at the low and middle doses. It did not produce any consistent signs of systemic maternal toxicity, such as clinical signs, mortality, changes in mean body weight or decreases in feed consumption. At the high dose, a slightly but significantly reduced maternal feed consumption was apparent (Table 15).

The maternal toxicity NOAEL was 250 mg/kg bw per day, the highest dose tested. Developmental toxicity, observed at 250 mg/kg bw per day, the highest dost tested, was characterized by an increase in post-implantation loss and a statistically significant increase in mean fetal resorptions per dam (but no increase in embryonic resorptions). This was accompanied by a statistically significant depression in the body weights (10.4%) of those fetuses surviving until day 21 sacrifice (Table 16).

Table 15. Feed consumption and body weight development in rats administered bentazone during days 6–15 of gestation

Parameter	0 mg/kg bw per day	40 mg/kg bw per day	100 mg/kg bw per day	250 mg/kg bw per day
Feed consumption (g/animal per day)				
Days 0–6	19.8	19.9	20.2	19.9
(% change relative to control)	—	(+0.5)	(+2.0)	(+0.5)
Days 6–11	21.3	21.1	20.6	20.1*
(% change relative to control)	—	(−0.9)	(−3.3)	(−5.6)
Days 11–16	22.5	21.9	22.1	21.8
(% change relative to control)	—	(−2.7)	(−1.8)	(−3.1)
Days 16–21	23.0	22.3	22.5	22.1
(% change relative to control)	—	(−3.0)	(−2.2)	(−3.9)
Body weight gain (g)				
Days 0–6	22	21	25	23
(% change relative to original weight at start of treatment)	(10.9)	(10.4)	(12.8)	(11.4)
Days 6–11	19	18	15	16
(% change relative to original weight at start of treatment)	(8.5)	(8.1)	(6.8)	(7.1)
Days 11–16	26	23	26	25
(% change relative to original weight at start of treatment)	(10.7)	(9.6)	(11.0)	(10.4)
Days 16–21	51	49	51	43
(% change relative to original weight at start of treatment)	(19.0)	(18.6)	(19.5)	(16.2)
Days 6–21	96	90	92	84
(% change relative to original weight at start of treatment)	(43.0)	(40.5)	(41.6)	(37.5)

From Becker et al. (1987a)
* $P < 0.05$

Bentazone also produced an effect upon the rate of growth, as evidenced by delayed or absence of ossification in the phalangeal nuclei of the extremities (digits of forelimb and hindlimb), sternebrae and cervical vertebrae. Examination of selected sites indicated that there was incomplete ossification of sternebra 5 (5 fetuses, 3 litters in controls versus 19 fetuses, 14 litters at the highest dose tested), absence of ossification in the metatarsals of toe 1 of the right hindlimb (18 fetuses, 8 litters in controls versus 53 fetuses, 19 litters at the highest dose tested) and absence of ossification in cervical vertebra 7 (1 fetus, 1 litter in control versus 9 fetuses, 5 litters at the highest dose tested) (Table 17).

The incidences of abnormal skeletal findings are shown in Table 18. The fetuses in group 4 (250 mg/kg bw per day) with incompletely ossified vertebrae and/or sternebrae are considered to mirror a delayed maturation, indicated by the reduced body weights of fetuses, and not a specific effect of the test article on skeletal development.

Table 16. Caesarean section data

Parameter	0 mg/kg bw per day	40 mg/kg bw per day	100 mg/kg bw per day	250 mg/kg bw per day
Pregnancy status				
Mated (*n*)	25	25	25	25
Pregnant (*n*)	24	22	24	25
Conception rate (%)	96	88	96	100
Aborted (*n*)	0	0	0	0
Dams with viable fetuses (*n*)	24	22	24	25
Dams with all resorptions (*n*)	0	0	0	0
Mortality (*n*)	0	0	0	0
Pregnant at terminal sacrifice (*n*)	24	22	24	25
Caesarean section data				
Corpora lutea				
- mean (*n*)	13.5 ± 1.8[a]	13.5 ± 1.7	14.0 ± 1.7	13.7 ± 1.6
- total number (*n*)	324	297	335	343
Implantation sites				
- mean (*n*)	11.8 ± 2.2	11.5 ± 3.2	12.4 ± 2.2	12.2 ± 2.2
- total number (*n*)	282	253	298	305
Preimplantation loss (*n*)	42	44	37	38
Preimplantation loss (%)	13.0	14.8	11.0	11.1
Preimplantation loss mean (*n*)	1.8	2.0	1.5	1.5
Resorptions				
- mean/dam (*n*)	0.9	1.0	1.1	2.7
- total number (*n*)	21	21	26	67
- % of implantations	7.4	8.3	8.7	22.0
Early resorptions				
- mean/dam (*n*)	0.9	1.0	1.0	0.9
- total number (*n*)	21	21	25	23
- % of implantations	7.4	8.3	8.4	7.5
Late resorptions				
- mean/dam (*n*)	0.0	0.0	0.0	1.8*
- total number (*n*)	0	0	1	44*
- % of implantations	0	0	0.3	14.4*
Dead fetuses (*n*)	0	0	0	0
Live fetuses				
- mean/dam (*n*)	10.9 ± 2.1	10.5 ± 3.1	11.3 ± 2.1	9.5 ± 4.1
- total number (*n*)	261	232	272	238
- % of implantations	92.6	91.7	91.3	78.0
Total live female fetuses				
- mean/dam (*n*)	5.4 ± 2.0	5.8 ± 2.3	5.6 ± 1.8	5.3 ± 2.7
- total number (*n*)	129	127	134	133
- mean (%)	49.9	54.7	49.3	55.9
Total live male fetuses				

Parameter	0 mg/kg bw per day	40 mg/kg bw per day	100 mg/kg bw per day	250 mg/kg bw per day
- mean/dam (*n*)	5.5 ± 1.7	4.8 ± 1.9	5.8 ± 1.8	4.2 ± 2.0
- total number (*n*)	132	105	138	105
- mean (%)	50.6	45.3	50.7	44.1
Mean fetal weight (g)				
- males	4.9 ± 0.2	5.1 ± 0.3	5.0 ± 0.3	4.4 ± 0.4
- females	4.7 ± 0.3	4.7 ± 0.3	4.7 ± 0.3	4.1 ± 0.4
- males and females	4.8 ± 0.2	4.9 ± 0.3	4.9 ± 0.3	4.2 ± 0.4*

From Becker et al. (1987a)
* $P \leq 0.05$
[a] Standard deviation.

Table 17. Skeletal investigations of fetuses without abnormal findings

	Group 1 0 mg/kg bw per day	Group 2 40 mg/kg bw per day	Group 3 100 mg/kg bw per day	Group 4 250 mg/kg bw per day
No. of skeletons investigated	*132*	*115*	*143*	*120*
Sternebrae				
(B) sternebra 1	—	—	—	1 (0.8)
(B) sternebra 2	1 (0.8)[a]	—	—	1 (0.8)
(B) sternebra 3	—	—	—	1 (0.8)
(B) sternebra 4	—	—	—	1 (0.8)
(B) sternebra 5	5 (3.8)	3 (2.6)	10 (7.0)	18 (15.0)
Cervical vertebrae				
(A) vertebra 1	21 (15.9)	14 (12.2)	15 (10.5)	47 (39.2)
(C) vertebra 1	6 (4.5)	5 (4.3)	7 (4.9)	5 (4.2)
(A) vertebra 2	33 (25.0)	23 (20.0)	25 (17.5)	59 (49.2)
(A) vertebra 3	11 (8.3)	1 (0.9)	3 (2.1)	27 (22.5)
(A) vertebra 4	6 (4.5)	—	1 (0.7)	15 (12.5)
(A) vertebra 5	4 (3.0)	—	—	15 (12.5)
(A) vertebra 6	3 (2.3)	—	—	11 (9.2)
(A) vertebra 7	1 (0.8)	—	—	9 (7.5)
Ribs				
(D) rib no. 14 left side	1 (0.8)	1 (0.9)	3 (2.1)	5 (4.2)
(D) rib no. 14 right side	2 (1.5)	—	2 (1.4)	5 (4.2)
Phalangeal nuclei[b]				
Left-hand limb				
(A) toe 1 distal phalanx	25 (18.9)	19 (16.5)	20 (14.0)	27 (22.5)
(A) toe 1 metatarsalia	15 (11.4)	17 (14.8)	17 (11.9)	49 (40.8)
(A) toe 2 distal phalanx	—	—	—	3 (2.5)
(A) toe 2 proximal phalanx	89 (67.4)	73 (63.5)	88 (61.5)	110 (91.7)
(A) toe 3 distal phalanx	—	—	—	3 (2.5)
(A) toe 3 proximal phalanx	61 (46.2)	51 (44.3)	53 (37.1)	95 (79.2)
(A) toe 4 distal phalanx	—	—	—	3 (2.5)
(A) toe 4 proximal phalanx	58 (43.9)	45 (39.1)	48 (33.6)	93 (77.5)
(A) toe 5 distal phalanx	12 (9.1)	5 (4.3)	12 (8.4)	10 (8.3)
(A) toe 5 proximal phalanx	117 (88.6)	97 (84.3)	130 (90.9)	118 (98.3)

Table 17 (continued)

	Group 1 0 mg/kg bw per day	Group 2 40 mg/kg bw per day	Group 3 100 mg/kg bw per day	Group 4 250 mg/kg bw per day
Right-hand limb				
(A) toe 1 distal phalanx	18 (13.6)	11 (9.6)	15 (10.5)	19 (15.8)
(A) toe 1 metatarsalia	15 (11.4)	17 (14.8)	17 (11.9)	53 (44.2)
(A) toe 2 distal phalanx	—	—	—	3 (2.5)
(A) toe 2 proximal phalanx	89 (67.4)	78 (67.8)	92 (64.3)	114 (95.0)
(A) toe 3 distal phalanx	—	—	—	3 (2.5)
(A) toe 3 proximal phalanx	62 (47.0)	55 (47.8)	57 (39.9)	103 (85.8)
(A) toe 4 distal phalanx	—	—	—	3 (2.5)
(A) toe 4 proximal phalanx	58 (43.9)	53 (46.1)	52 (36.4)	99 (82.5)
(A) toe 5 distal phalanx	10 (7.6)	5 (4.3)	8 (5.6)	10 (8.3)
(A) toe 5 proximal phalanx	118 (89.4)	99 (86.1)	125 (87.4)	120 (100.0)
Calcaneum				
(A) left side	117 (88.6)	87 (75.7)	115 (80.4)	112 (93.3)
(A) right side	115 (87.1)	88 (76.5)	114 (79.7)	110 (91.7)

From Becker et al. (1987a)
A, ossification still absent; B, incompletely ossified; C, ossification centre dumbbell shape; D, supernumerary rib
[a] Percentage of skeletons showing the finding.
[b] Medical phalangeal nuclei of all fetuses still absent.

Table 18. Incidence of abnormal skeletal findings

Parameter	0 mg/kg bw per day	40 mg/kg bw per day	100 mg/kg bw per day	250 mg/kg bw per day
Litters evaluated (*n*)	25	22	24	25
Fetuses evaluated (*n*)	132	115	143	120
Live fetuses (*n*)	132	115	143	120
Dead fetuses (*n*)	0	0	0	0
Total abnormal skeletal findings				
Fetal incidence (%)	0 (0.0)	0 (0.0)	1 (0.7)	9 (7.5)
Litter incidence (%)	0 (0.0)	0 (0.0)	1 (4.2)	7 (28)
Individual abnormal skeletal findings, no. (corresponding mean fetal body weight in grams)				
Non-ossified sternebra				
- No. 2	0	0	1 (4.4)	0
- No. 6	0	0	0	4 (3.2)
- Nos 2, 5 and 6	0	0	0	1 (2.8)
Abnormally ossified and bipartite sternebra no. 4, abnormally ossified sternebra no. 5	0	0	0	1 (4.5)
Non-ossified vertebral body no. 1, non-ossified sternebrae nos 5 and 6	0	0	0	1 (2.6)
Non-ossified vertebral body no. 1	0	0	0	2 (3.4)

From Becker et al. (1987a)

The developmental NOAEL was 100 mg/kg bw per day, based on increased post-implantation loss, skeletal variations (incomplete or absent ossification in the phalangeal nuclei of the extremities, sternebrae and cervical vertebrae) and reduced body weights of fetuses surviving to day 21 at 250 mg/kg bw per day. There were no indications of teratogenic potential in this study up to the highest dose level tested. The study is GLP compliant (Becker et al., 1987a).

Bentazone (batch N 169, purity 93.9%) was administered daily to presumably pregnant Charles River CD rats via the diet during the entire gestation period (days 0–21) at a concentration of 0, 2000, 4000 or 8000 ppm (calculated to be equal to 0, 162, 324 and 631 mg/kg bw per day, respectively). The age of the animals was 7 weeks at the time of purchase. The animals were acclimatized for 11 days before administration of the test substance. The group mean weight was between 216 and 217 g.

No treatment-related effects were observed in the group dosed with 2000 ppm. The dietary level of 4000 ppm caused significant increases in water consumption and amniotic fluid weight in pregnant rats. Pregnant animals dosed with 8000 ppm displayed decreased feed intake, increased water consumption, suppression of body weight gain and various clinical signs suggesting haemorrhagic diathesis and signs of disturbance of fetal growth, characterized by an increased incidence of reduced fetal body weight and reduced ossification of cervical vertebrae. Additionally, haemorrhages were found in the liver of some high-dose pups. These effects are interpreted as secondary manifestations of toxic effects on the pregnant rats rather than being a direct influence of the test substance on the fetus. The study did not reveal any teratogenic potential.

The NOAEL for maternal effects was 2000 ppm (equal to 162 mg/kg bw per day), on the basis of increased water consumption at 4000 ppm (equal to 324 mg/kg bw per day), and the developmental toxicity NOAEL was 4000 ppm (equal to 324 mg/kg bw per day), on the basis of decreased fetal weight gain and reduced ossification of cervical vertebrae at 8000 ppm (equal to 531 mg/kg bw per day). Although the study was conducted prior to the implementation of GLP, it is stated that the study was run according to the principles of GLP. A formal GLP compliance and QA statement was included in the report (Itabashi et al., 1983).

A published literature study on the teratological effects of the pesticide Basagran on embryos of the albino rat was submitted. Groups of three pregnant albino rats (strain and source not specified) were orally administered single doses of 0, 25, 90 or 200 mg of the formulation Basagran (origin and purity not submitted) per kilogram body weight (corresponding to bentazone doses of 0, 12.0, 43.2 and 96 mg/kg bw) by gavage on GD 6, 8, 11, 14 or 16. On GD 20, all animals were sacrificed and necropsied. The fetuses were dissected from the uterus. Resorptions were counted, and the skeletons of fetuses were examined. The fetal findings observed consisted of an increased resorption rate, retardation of fetal development, incomplete ossification and absence of some bones. The increased resorption rates were noted at comparable incidences in all treated groups, irrespective of the dose administered. The incidence and severity of the findings decreased with the later times of administration. Thus, the findings were time dependent, but not dose dependent. The publication gave no details on maternal toxicity. The results of the gross pathological examination of the fetuses were only summarized in this study, and the frequency of the changes was not reported. In addition, the results of the examination of the control animals were not given.

Because of the inconsistency of the data reported, this investigation is considered unacceptable for evaluation purposes. However, it provides supplementary information, as the time dependence of fetal effects was investigated. The study is not GLP compliant (El-Mahdi & Lotfi, 1988).

Rabbits

In a study of developmental toxicity, groups of 15 Himalayan rabbits (ChBB:HM) were given bentazone (purity 92.5%) at a dose of 50, 100 or 150 mg/kg bw per day by gavage on GDs 6–18. Two

additional groups served as untreated controls or vehicle controls. At the time of procurement, the rabbits were between 22 and 83 weeks old with a mean weight of 2.449 kg. After a 10-day acclimatization period, the rabbits were fertilized by artificial insemination. The animals were observed daily for clinical signs and for mortality. Body weights, body weight gains and feed consumption were measured each day. At necropsy, the uterus was removed and the animals were examined for gross pathology. The number of corpora lutea, conception rate, number of implantations (live and dead implantations and early, intermediate and late resorptions) and number of dead fetuses were determined. The fetuses were removed from the uterus by caesarean section and examined. The weight and length of the fetuses were measured, and the placentas were weighed. The heads of the fetuses were fixed, and transverse sections were made and examined; skeletal assessment was undertaken by radiological examination.

One dam in the untreated control group aborted. One death was seen in each of the groups receiving bentazone at 100 or 150 mg/kg bw per day (these dams had severe vaginal haemorrhages), and a dam in the group at 100 mg/kg bw per day bore six fetuses prematurely on day 26 after conception. A dam at 100 mg/kg bw per day aborted on day 26 or 27 post-coitum. No other adverse clinical signs were noted, and there was no test material–related effect on maternal body weight gain. Feed consumption was lower in the dosed groups and in the vehicle control groups than in the group of untreated controls. No test material–related macroscopic abnormalities were seen in the animals that were killed at study termination. No intergroup differences were seen in conception rate or numbers of implantations and corpora lutea. Fetal body weights were increased at 100 and 150 mg/kg bw per day; however, this is not likely to be an adverse effect. Fetal length and placental weight were not affected by treatment. There were no differences between the groups in the frequency of anomalies, variations and retardations observed.

Accordingly, the NOAEL for maternal and fetal toxicity for bentazone was 150 mg/kg bw per day, the highest dose tested. Bentazone was not teratogenic. The study is not GLP compliant (Hofmann & Merkle, 1978b).

Bentazone (batch N 187, purity 97.8%) was administered daily to presumably pregnant Chinchilla rabbits by stomach tube during GDs 6–18 at a dose level of 0, 75, 150 or 375 mg/kg bw per day. The age of the animals at pairing was between 4 and 7 months, with weight (post-coitum) 2513–3539 g. The animals were examined for mortality, signs and symptoms twice daily. All animals were weighed daily from day 0 to day 28 post-coitum. The body weight change of the animals was calculated from these results during the treatment period beginning at day 6 (immediately prior to the first administration) and ended on day 19 post-coitum (approximately 24 hours after the last administration). In addition, the body weight gain corrected for uterine weight at necropsy was calculated.

On day 28 post-coitum, all females were killed by cervical dislocation and the fetuses removed by caesarean section. Postmortem examinations, including gross macroscopic examination of all internal organs, with emphasis upon the uterus, uterine contents, position of fetuses in the uterus and number of corpora lutea, were performed and the results recorded. The uteri (and contents) of all pregnant females were weighed on the scheduled day of necropsy and used to determine the corrected body weight gain. The uteri of all females that were found at necropsy to be not pregnant were placed in an aqueous solution of ammonium sulfide (Salewski, 1964) to accentuate possible haemorrhagic areas of implantation sites. All tissues and organs of the females were discarded. The fetuses were removed from the uterus, weighed, examined for gross external abnormalities and prepared for internal examinations. All fetuses were dissected carefully, the body cavities (thorax, abdomen, pelvis) and the organs investigated and any abnormal findings recorded. The sex of each fetus was noted and recorded. The heads of all fetuses were fixed in a solution of trichloroacetic acid and formaldehyde. The heads were cross-sectioned, and the cephalic viscera were examined (Wilson & Warkany, 1965). Descriptions of any abnormalities were recorded. After evaluation, the individual sections were preserved in a solution of ethyl alcohol and glycerine (one head per container). The crania of all fetuses were examined for ossification after the scalps were removed. The trunks of all

fetuses were placed in a solution of potassium hydroxide for clearing and stained with alizarin red (modified technique; Dawson, 1926). The skeletons were examined, and all abnormalities and variations were recorded. The specimens were preserved individually in plastic bags. The stained trunks of fetuses and the sections of the heads of fetuses were preserved.

No mortality was observed in this study. No signs or symptoms were observed in any female of the control, low-dose and mid-dose groups. Treatment-related effects were limited to the high dose (375 mg/kg bw per day) and consisted of signs of abortion in one dam, indicated by five aborted placentas found on day 22 post-coitum (in this dam, a total post-implantation loss was ascertained during necropsy on day 28 post-coitum), and a slight reduction of the mean feed consumption during the treatment period. No test article–related differences in comparison with the vehicle control group data were noted in the remaining parameters recorded and in all data of the low-dose group (75 mg/kg bw per day) and the mid-dose group (150 mg/kg bw per day).

Besides this, a single incidental finding (hydrocephalus internus) in one fetus of the mid-dose group (150 mg/kg bw per day) was noted during the gross pathological investigations. During the skeletal investigations, isolated abnormal findings were noted in all groups, including the controls. There were no signs of a test article relationship (Table 19).

There were no indications of a teratogenic potential at any dose. Based on these results, the NOAEL for maternal and prenatal developmental toxicity in rabbits is 150 mg/kg bw per day, based on signs of abortion and reduction of maternal feed consumption at 375 mg/kg bw per day. The study was GLP compliant, and a QA statement was attached (Becker et al., 1987b).

(c) Effects on spermatogenesis

A published literature study on the effects of a low dose of bentazone on spermatogenesis in mice exposed during fetal, postnatal and adult life, which was made available to the Committee, was reviewed. In this paper, the potential reproductive hazard to humans resulting from exposure to bentazone in drinking-water was studied in mice. Bentazone was administered in drinking-water at a concentration of 30 µg/l to (a) 10 adult male mice (3 months old) for 100 days, resulting in a dose of 21 µg/kg bw per day, and (b) 12 male mice exposed in utero, during lactation (from three dams, each was allowed to nurse four male offspring) and up to PND 100, resulting in a dose of 14 µg/kg bw per day.

With regard to male reproductive parameters, no histopathological changes were seen. Sperm number and morphology were not affected by the treatment. There were also no changes when using synaptonemal complex immunostaining or when using the micronucleus and comet assays (measures of chromosomal damage). The only statistically significant effect seen was an alteration of the frequency of some stages of sperm maturation in both experimental groups compared with the concurrent control groups, with no consistent pattern. According to a review article by Creasy (1997), quantifying stage frequency is not appropriate as an end-point itself. This parameter is used only to identify cell loss in the case of spermatid retention.

Therefore, this study is considered as supplementary information that confirms the absence of male reproductive toxicity in mice at doses below those chosen for risk assessment. This evaluation is supported by USEPA (2010). The study is not GLP compliant (Garagna et al., 2005).

2.6 Special studies

(a) Neurotoxicity

In a subchronic neurotoxicity study in Wistar CrlGlxBrlHan:Wi rats, bentazone (batch N 187; purity 96.9%) was administered to groups of 10 rats of each sex per dose at a dietary concentration of 0, 300, 1000 or 3500 ppm (equal to 0, 21.9, 73.6 and 258.1 mg/kg bw per day for males and 0, 27, 86.4 and 306.3 mg/kg bw per day for females, respectively) for at least 91 days. Each group was subdivided into two subsets (A and B) in order to balance the groups for functional observational battery and motor activity measurements. The animals were assigned to the treatment groups by

means of computer-generated randomization lists based on body weights. All the parameters, including functional observational battery, were examined as per approved guidelines.

Table 19. Caesarean section data in rabbits[a]

	0 mg/kg bw per day	75 mg/kg bw per day	150 mg/kg bw per day	375 mg/kg bw per day
Pregnancy status				
Mated (*n*)	16	16	16	16
Pregnant (*n*)	16	16	16	15
Conception rate (%)	100	100	100	94
Aborted/resorbed (*n*)	0	0	0	1
Dams with viable fetuses (*n*)	16	16	16	14
Mortality (%)	0	0	0	0
Pregnant terminal sacrifice (*n*)	16	16	16	14
Cesarean section data				
Corpora lutea				
- mean/dam	7.8 ± 1.6[b]	7.7 ± 1.5	8.6 ± 1.1	8.9 ± 1.5
- total number (*n*)	125	123	137	124
Implantation sites				
- mean/dam	7.7 ± 1.9	7.4 ± 1.5	8.4 ± 1.1	8.4 ± 1.9
- total number (*n*)	123	119	134	118
Preimplantation loss (%)	1.6	3.3	2.2	4.8
Post-implantation loss (%)	4.1	4.2	3.7	3.4 (8.8[c])
Resorptions				
- mean/dam	0.3	0.3	0.3	0.3
- total number (*n*)	5	5	5	4
- % of implantations	4.1	4.2	3.7	3.4
Early resorptions				
- mean/dam	0.3	0.1	0.3	0.1
- total number (*n*)	4	2	4	1
- % of implantations	3.3	1.7	3.0	0.8
Late resorptions				
- mean/dam	0.1	0.2	0.1	0.2
- total number (*n*)	1	3	1	3
- % of implantations	0.8	2.5	0.7	2.5
Dead fetuses (*n*)	0	0	0	0
Live fetuses				
- mean/dam	7.4 ± 2.2	7.1 ± 1.6	8.1 ± 1.5	8.1 ± 1.9
- total number (*n*)	118	114	129	114
Total live female fetuses				
- total number (*n*)	56	56	75	56
- mean (%)	47.5	49.1	58.1	49.1
Total live male fetuses				
- total number (*n*)	62	58	54	58

	0 mg/kg bw per day	75 mg/kg bw per day	150 mg/kg bw per day	375 mg/kg bw per day
- mean (%)	52.5	50.9	41.9	50.9
Mean fetal weight (g)				
- males[d]	37.9 ± 3.8	38.5 ± 4.6	37.0 ± 2.6	36.0 ± 3.5
- females[d]	37.1 ± 4.5	37.6 ± 5.2	35.7 ± 2.3	35.4 ± 2.9
- males and females[d]	37.7 ± 3.9	38.0 ± 4.7	36.4 ± 2.1	35.7 ± 2.8
- males and females	36.8 ± 5.0	37.3 ± 5.7	36.1 ± 3.8	35.3 ± 4.5

From Becker et al. (1987b)
[a] This table excludes dams #53 and #64 of the high-dose group.
[b] Standard deviation.
[c] Post-implantation loss under consideration of dam #64 (125 implantations and 11 losses).
[d] Unweighted mean of litter means and variation between litters.

No treatment-related clinical signs were observed throughout the study. Incidental observations included alopecia at various regions of the body in one mid-dose (1000 ppm) male and two high-dose (3500 ppm) females; piloerection in one high-dose female; and injury to the left ear in one control female.

No mortality or any treatment-related ophthalmoscopic findings were observed throughout the study. However, at termination, the only findings consisted of corneal stipplings in five control and three high-dose males and two control and five high-dose females. The incidence of this finding was within the expected range for rats of this age.

No treatment-related effects on body weight or body weight gain were observed. In the absence of statistical significance, the slightly lower terminal body weight and lower overall body weight gain of high-dose males were considered to be incidental. A statistically significant decrease in mean daily feed consumption was observed in high-dose males at days 63 and 77. The isolated occurrence was not indicative of a relationship to treatment. Except for a decrease in low-dose males at day 49, no statistically significant differences in feed efficiency were noted in any treated group. The isolated occurrence and the lack of a dose–response relationship indicated that the statistically significant difference in low-dose males was incidental.

No treatment-related functional observational findings were observed at any dose level. No statistically significant differences in defecation, number of rearings, forelimb and hindlimb grip strength or foot splay width were observed in any treated group. During home cage observation, deviations from (rank) "zero values" were obtained in several animals. However, all findings were equally distributed between treated groups and controls. Parameters investigated included posture, tremors, convulsions, abnormal movements and impairment of gait. During open-field observations and sensory motor tests/reflexes, deviations from "zero values" were obtained in several animals. However, as all findings were equally distributed between treated groups and controls, were without a dose–response relationship or occurred in single animals only, these observations were considered incidental. No treatment-related changes in motor activity were noted in treated groups.

There were some statistically significant differences between control and treated groups; however, these changes were neither dose related nor consistent over time. Therefore, these changes were considered incidental. These changes consisted of (in low-dose males) increased overall activity at day 50, decreased activity at interval 4 on day −7 and increased activity in low-dose males at intervals 8–10 and at day 50; (in mid-dose males) increased activity at interval 2 on day −7, decreased activity at interval 10 on day 22 and increased activity at intervals 9 and 10 on day 50; (in high-dose males) increased activity at interval 2 on day −7 and decreased activity at intervals 7 and 10 on day 22; (in low-dose females) increased activity at intervals 2 and 3 on day 22 and increased activity at interval 2 on day 50; (in mid-dose females) increased activity at intervals 2 and 3 on day 22; and (in high-dose females) increased activity at interval 2 on day 22.

Terminal body weights were comparable between all groups. Likewise, no statistically significant differences in absolute and relative brain weights were observed. No macroscopic lesions were observed at necropsy. With the exception of axonal degeneration of lumbar ganglia in one control male, no neurohistopathological lesions were noted in this study.

Dietary administration of bentazone to rats at dose levels of 0, 300, 1000 and 3500 ppm did not result in any indication of neurotoxicity. Under the conditions of the present study, the NOAEL for neurotoxicity was 3500 ppm (equal to 258.1 mg/kg bw per day in males and 306.3 mg/kg bw per day in females), the highest dose tested. The study was GLP compliant, and a QA statement was attached (Kaspers et al., 2004).

(b) Studies on metabolites

6-Hydroxybentazone and 8-hydroxybentazone are major plant metabolites of bentazone. Because crops of treated plants can be consumed by humans, farm animals or pets, an exposure to both of these compounds might be expected in principle. Although both metabolites have been demonstrated to be formed in mammals and therefore can be regarded as included in toxicological testing of the parent compound, specific toxicological studies were performed.

It has been shown that the 8-hydroxy and 6-hydroxy metabolites of bentazone are of comparable toxicity by the oral route of administration and are both less toxic than the parent compound. Additionally, both metabolites were negative in the Ames assay for the potential to induce point mutations in bacteria. As it is unlikely that a hydroxy group shift in the bentazone ring system dramatically changes the toxicity, it was decided to perform further investigations on 8-hydroxybentazone as a reference substance.

Therefore, 8-hydroxybentazone was investigated in a subchronic feeding study, in several mutagenicity studies and in a prenatal developmental study. These investigations revealed that the metabolites have no mutagenic or teratogenic potential and are less toxic than the parent substance.

Acute toxicity

The acute toxicity of 6-hydroxybentazone and 8-hydroxybentazone is summarized in Table 20.

Table 20. Acute toxicity of bentazone metabolites

Species	Strain	Sex	Route	Batch no. / purity	LD_{50} (mg/kg bw)	Reference
6-Hydroxybentazone						
Rat	Wistar	Male Female	Oral	E-106251 / > 98%	> 5000	Kieczka & Kirsch (1987b)
Mouse	NMRI	Male Female	Oral	E-106251 / > 98%	> 5000	Kieczka & Kirsch (1987c)
8-Hydroxybentazone						
Rat	Wistar	Male Female	Oral	Batch not given / > 98.5%	> 5000	Kieczka & Kirsch (1987a)
Mouse	NMRI	Male Female	Oral	Batch not given / > 98.5%	> 5000	Kirsch & Hildebrand (1987)

Genotoxicity

The genotoxicity of 6-hydroxybentazone and 8-hydroxybentazone is summarized in Table 22.

Table 22. Genotoxicity of bentazone metabolites

Study	Test system	Concentration/ dose	Purity (%)	Results	Reference
6-Hydroxybentazone					
Bacterial reverse mutation assay (Ames test)	*S. typhimurium* TA98, TA100, TA1535, TA1537	20, 100, 500, 2500, 5000 µg/plate	> 98	Negative (±S9)	Engelhardt & Gelbke (1987b)
8-Hydroxybentazone					
Bacterial reverse mutation assay (Ames test)	*S. typhimurium* TA98, TA100, TA1535, TA1537, plate incorporation and preincubation assay; with/without S9 mix	20, 100, 500, 2500, 5000 µg/plate	99.9	Negative (±S9)	Engelhardt & Gelbke (1987a)
In vitro forward mutation assay in mammalian cells (HPRT test)	V79 cells; with/without S9 mix	Among others, 300, 1000, 2000, 3000; and 500, 1000, 2500, 5000 µg/ml	99.9	Negative (±S9)	Muellerschoen (1992)
In vivo mouse micronucleus test	NMRI mouse, male and female (single oral gavage; vehicle: 0.5% aqueous carboxymethylcellulose)	625, 1250, 2500 mg/kg bw	99.9	Negative	Engelhardt & Hoffmann (1993)

S9, 9000 × g supernatant fraction of rat liver homogenate

Subchronic oral toxicity (8-hydroxybentazone)

To study and determine the toxicity profile and NOAEL, 8-hydroxybentazone (batch no. L 47-213; purity 99.9%) was administered to groups of 10 rats (Chbb:Thom(SPF)) of each sex per dose group (42 days old and weighing around 186 [176–201] g [males] and 143 [134–150] g [females]) at a dietary concentration of 0, 400 (low dose), 1200 (intermediate dose) or 3600 ppm (top dose) (equal to 0, 28, 85 and 259 mg/kg bw per day for males and 0, 34, 101 and 304 mg/kg bw per day for females, respectively) for at least 90 days. The mean substance intakes for both sexes were 0, 31, 93 and 282 mg/kg bw per day, respectively. The animals were examined for all parameters as per guidelines.

The administration of 8-hydroxybentazone did not result in any substance-related effects. One low-dose male died on study day 27, and one mid-dose male died on study day 63. The cause of death could not be determined. These mortalities are not considered treatment related. No treatment-related ophthalmological findings were noted. The only findings observed at the end of the treatment period were mainly remainders of the pupillary membrane and occasionally corneal stippling. The body weight development, body weight changes and feed consumption of the treated male and female animals did not differ substantially from those of the control animals.

In terms of haematological parameters, the only statistically significant change seen was a slight increase in reticulocyte count in females at the end of the study, a finding not considered biologically relevant. No substance-related effects were noted in the clotting analyses. A few spurious statistically significant clinical chemistry findings were observed.

A few spurious statistically significant decreased concentrations of inorganic phosphorus at 1200 and 3600 ppm, minimally decreased concentrations of calcium at 1200 ppm and decreased concentrations of total bilirubin, total protein and globulins at 400 ppm were seen only in males and only at the end of the study. In the absence of a dose–effect relationship or associated adverse findings, these findings are not considered to be of toxicological relevance.

No treatment-related changes in urinary parameters were observed.

The only finding of statistical significance was a decrease in the absolute weight of the adrenal glands in males of the 400 ppm group compared with controls. There was no dose–response relationship, and there were no significant differences in the absolute weights of the adrenal glands at higher doses. Furthermore, the relative adrenal weights did not show significant differences when compared with the controls. Therefore, a substance-related effect can be ruled out. No other statistically significant changes in absolute or relative organ weights were observed.

All gross lesions and histopathological findings were biologically equally distributed over the control and treatment groups and are considered as having developed spontaneously.

Dietary administration of 8-hydroxybentazone to rats at dose levels up to 3600 ppm did not result in any compound-related effects. The NOAEL under the conditions of the present comparative study was 3600 ppm (equal to about 259 mg/kg bw per day in males and 304 mg/kg bw per day in females, or 282 mg/kg bw per day for both sexes combined), the highest dose tested. Therefore, the metabolite 8-hydroxybentazone proved to be less toxic than the parent compound bentazone. The study was GLP compliant, and a QA statement was attached (Mellert & Hildebrand, 1993).

Developmental toxicity of 8-hydroxybentazone

8-Hydroxybentazone was tested for its toxicity in Wistar (Chbb:THOM(SPF)) rats (77–79 days old, weighing 232.5 g [mean]). The test substance (batch no. 108 746, purity 99.9%) was administered as an aqueous suspension to 20–25 pregnant female rats per group by stomach tube at a dose of 40, 100 or 250 mg/kg bw on day 6 through day 15 post-coitum. A standard dose volume of 10 ml/kg bw was used. The control group was dosed with the vehicle only (0.5% aqueous carboxymethylcellulose solution). On day 20 post-coitum, all females were sacrificed and assessed by gross pathology. The fetuses were dissected from the uterus, sexed, weighed and further investigated for any external and/or skeletal findings.

There were no substantial substance-related effects on the dams concerning feed consumption, body weight, body weight change, uterine weights, corrected body weight change, or clinical and necropsy observations up to and including the dose of 250 mg/kg bw per day. There were no differences of biological relevance between the control and the substance-treated groups (40, 100 and 250 mg/kg bw per day) in conception rate, mean numbers of corpora lutea, total implantations, resorptions and live fetuses, fetal sex ratio or preimplantation and post-implantation losses.

No dose- or substance-related differences were recorded for placental and fetal body weights. The external, soft tissue and skeletal examinations of the fetuses revealed no differences between the control and the substance-treated groups that might be related to the test substance administration. Number and type of the fetal external, soft tissue and skeletal findings, which were classified as malformations, variations and/or retardations, recorded for the 40, 100 and 250 mg/kg bw per day fetuses were substantially similar to actual and/or historical control values.

Thus, under the conditions of this study, 8-hydroxybentazone caused no signs of maternal toxicity and no signs of embryo/fetal toxicity up to and including a dose of 250 mg/kg bw per day. There were no indications of teratogenic effects that could be causally related to the test substance administration.

Bentazone technical at a dose of 250 mg/kg bw per day induced some signs of maternal (reduced feed consumption, impaired body weight gains) and developmental (reduced fetal body weights and delayed maturation of the fetal skeletons) toxicity, but no teratogenic effects were seen (Becker et al., 1987a).

For this prenatal toxicity study with 8-hydroxybentazone in rats, the NOAEL for maternal and fetal toxicity was 250 mg/kg bw per day, whereas the NOAEL was 100 mg/kg bw day in the preceding toxicity study (Becker et al., 1987a) with bentazone technical, the parent compound.

The study was GLP compliant, and a QA statement was attached (Hellwig & Hildebrand, 1993).

(c) Dermal penetration

Rats in vivo

The absorption and elimination of bentazone (purity 97%) have been studied in Sprague-Dawley rats after dermal application of [^{14}C]bentazone sodium salt in an aqueous solution at a concentration of 0.002, 0.02, 0.2 or 2 mg/cm^2 as the free acid, which correspond to dermally applied doses of 0.12, 1.2, 12 and 120 mg/kg bw, respectively.

At each dose level, groups of four animals were sacrificed at 0.5, 1, 2, 4 and 10 hours after dosing. The remaining four rats per group were transferred to metabolism cages after coverings had been removed, and their backs were washed with methanol at 10 hours after dose application. From these animals, samples of urine and faeces were collected during intervals of 10–24, 24–48 and 48–72 hours. At 72 hours after dosing, rats were killed, and samples of blood and tissues were taken for analysis. In addition, concentrations of radioactivity in liver and plasma and excretion of radioactivity in urine and faeces after oral administration of bentazone sodium salt at a dose level equal to 4 mg/kg bw as the free acid to male rats were determined. The orally dosed animals were allocated to groups of four per dose level and sacrificed at the same times after dosing as indicated for the dermal application groups. Rats scheduled for termination after 72 hours were housed in metabolism cages. Urine and faeces were collected separately during intervals of 0–6, 6–12, 12–24, 24–48 and 48–72 hours. The animals received standardized diet and water ad libitum.

Only small proportions of the dose (1.23%, 1.90%, 1.46% and 0.79%, respectively) were absorbed at dermally applied concentrations of 0.002, 0.02, 0.2 and 2 mg/cm^2. This dermally absorbed part was rapidly eliminated, with urinary excretion being the main route. Less than 0.1% of the dose was excreted via the faeces within 10 hours. After a 4 mg/kg bw oral dose of [^{14}C]bentazone sodium salt, a mean of 90% of the total dose was excreted in the urine during 72 hours. A major part (85%) was excreted during the first 12 hours. These results clearly indicated that the oral dose was extensively and rapidly absorbed and eliminated. The time course of radioactive plasma levels at the different times confirms the limited extent of percutaneous absorption.

The proportion of the dose remaining in skin and fur was much greater at lower dose levels. Means of 61%, 28%, 13% and 2%, respectively, remained, after washing with cotton wool swabs moistened with water, on the treated skin of animals sacrificed at 72 hours after application of the 0.002, 0.02, 0.2 and 2 mg/cm^2 doses, respectively. This finding might suggest that a certain amount of test compound could not be easily removed from the skin or fur.

Mean concentrations of radioactivity in tissues after dermal application of 0.12, 1.2 and 12 mg/kg bw were generally low and mostly below the level of quantification. The concentrations in the eye, brain and testes after dermal application were low and frequently below the level of quantification.

Mean concentrations of radioactivity in plasma after the 4 mg/kg bw oral dose were 7.07, 6.29, 3.46, 1.84, 0.166 and 0.0041 µg/ml. The concentrations of radioactivity in liver were 2.46, 1.94, 1.25, 0.789, 0.117 and < 0.0091 µg/g, respectively.

In view of the above, it can be concluded that only about 1–2% of the amount applied dermally was absorbed. In contrast, 90% of the test substance administered orally was excreted with the urine within 72 hours, confirming the high degree of absorption following oral administration. Seventy-two hours after dermal application of even the highest dose, only traces of radioactivity were present in the animal body. Elimination of the dermally absorbed amount was rapid and effective, with the urine being the main route. It has been stated that although GLP was not compulsory when the study was performed, the study was run according to the principles of GLP (Hawkins et al., 1986b).

Human skin in vitro

The dermal penetration of [^{14}C]bentazone sodium salt (batch no. 210-2201, radiochemical purity 97.3%) through human skin was assessed by a single topical application of about 4933, 49.3 or

8.22 μg/cm^2 of active ingredient formulated in BAS 351 32 H to split thickness skin membranes mounted on Franz-type diffusion cells. The doses represent the formulation concentrate or two representative spray dilutions (1:100 and 1:600) for field use, respectively. The study was performed using five diffusion cells per dose.

Diffusion cells were operated in the static mode with tap water as the receptor fluid. The openings of the donor compartments were covered with Fixomull® stretch adhesive fleece (semi-occlusive conditions) after application. After a 6-hour exposure period, the surface of the skin membranes was washed. The adhesive fleece cover of the donor compartment was reconstituted, and the study continued up to 24 hours. During the study period, amounts of the receptor fluid were collected from each cell at several time points in order to determine kinetic parameters (lag phase, absorption rate and permeability constant). At the end of the sampling period, the test substance was recovered from all compartments of each diffusion cell. The recovery results are summarized as non-absorbed dose (skin washing and tape stripping), amount associated with the skin membrane and absorbed dose (receptor fluid, receptor chamber washing and receptor samples including washout). The mean recovery rates and absorption kinetic parameters are presented in Table 22.

Table 22. Recovery results in a human skin dermal penetration study

Parameter		Group 1	Group 2	Group 3
Target applied dose of test preparation	(mg or μl/cm^2)	10	10	10
Target applied dose of test substance	(μg/cm^2)	4933	49.3	8.22
Mean nominal applied dose of test substance	(μg/cm^2)	5020	51.2	8.47
Recovery				
Mean total recovery rate	(% of applied dose)	101.7	98.5	96.2
Mean non-absorbed dose	(% of applied dose)	101.6	97.7	95.3
Mean amount associated with skin	(% of applied dose)	0.04	0.50	0.57
Mean absorbed dose	(% of applied dose)	0.00	0.30	0.30
Absorption kinetics				
K_p	(×10^{-5} cm/h)	—[a]	0.236[b]	1.01[b]
Absorption rate	(μg/(cm^2·h))	—[a]	0.012[b]	0.008[b]
Lag time	(h)	—[a]	1.8[b]	2.5[b]

From Gamer & Landsiedel (2009)
K_p, permeability coefficient
[a] The absorbed dose was too low for meaningful calculation of kinetic parameters.
[b] Kinetic parameters are doubtful due to low absorbed dose.

The mean total recovery rates fulfil the quality criteria put forward in the test guidelines.

No meaningful absorbed doses were measured in diffusion cells treated with the high dose. The mean absorbed dose was very low for the middle and low doses also (0.30% each). Also, the amount of test substance associated with the skin membranes was very low (0.04%, 0.50% and 0.57% for the high, middle and low doses, respectively).

Summing up the absorbed dose with that associated with the skin membrane, 0.04%, 0.80% and 0.87% of the applied dose were recovered from diffusion cells for the high, middle and low doses, respectively. For the high dose, no cumulative absorbed dose curves could be generated. Consequently, no absorption rate, permeability coefficient or lag time could be calculated for this dose.

The receptor samples start to show quantifiable amounts of radioactivity (test substance) 2–4 hours after application in the mid-dose group and 2–6 hours after application in the low-dose group.

Therefore, the mean cumulative absorbed dose curves of these test groups show the steepest slopes between 1 and 2 hours after application, and the curves show an unsteady and decreasing slope thereafter. Although for the middle and low doses cumulative absorbed dose curves could be generated, the kinetic parameters calculated from these curves are doubtful due to the very low absorbed doses obtained.

Within the variability of the method, the absorption rates for the middle and low doses are comparable and do not reflect the dilution factor between these doses.

Consequently, the permeability coefficient of the low-dose group is higher than that for the mid-dose group. The absorption lag times calculated in the mid- and low-dose groups were 1.8 and 2.5 hours, respectively, and show the presence of a functional barrier in the skin samples used. According to the categorization schemes suggested by Marzulli, Brown & Maibach (1969) and Barber, Hill & Schum (1995), the permeability coefficients derived from the steepest parts of the penetration–time curves show a very slow to slow diffusion of [^{14}C]bentazone sodium salt from the spray dilutions through human skin membranes. For the formulation concentrate (high dose), virtually no diffusion of [^{14}C]BAS 351 H (bentazone sodium salt) through human skin membranes was present under the test conditions used.

In view of the above, it can be concluded that in vitro dermal penetration of bentazone formulated as an aqueous soluble (liquid) concentrate formulation of bentazone sodium through human skin is appropriately calculated as per cent absorbed dose. Considering the amount of radiolabelled substance associated with the skin (remaining skin and tape strips 3–6) after washing as absorbable and combining this with the absorbed amount detected in the receptor, the extent of dermal penetration through human epidermis is about 0.06% for the concentrate, 1.31% for the 1:100 spray strength dilution and 1.23% for the 1:600 dilution.

The study was GLP compliant, and a QA statement was attached (Gamer & Landsiedel, 2009).

3. Observations in humans

3.1 Medical surveillance of manufacturing plant personnel

All persons handling crop protection products are surveyed by regular medical examinations. There are no specific parameters available for effect monitoring of bentazone. Thus, the medical monitoring programme is designed as a general health checkup, with special interest in the primary target organs presumed to be relevant by analogy from animal experiments. The surveillance programme includes a general physical examination, including neurological status, red and white blood cell counts and liver enzymes. Adverse health effects suspected to be related to bentazone exposure have not been observed. Some cases of irritation of the eyes and the skin have been registered in the BASF internal clinical incident log in persons exposed to bentazone. No other adverse health effects due to bentazone have been documented in the BASF internal medical files.

At BASF sites in Germany and the USA, studies have been performed among employees who had been assigned to bentazone production facilities in the 1970s and 1980s. However, these studies do not address potential effects of the final product, but those of a specific multi-step batch process that included a large number of process starting materials and chemical intermediates and that is now obsolete (Nasterlack et al., 2007).

3.2 Direct observation

A literature search (24 October 2011) retrieved several case reports of suicide attempts with bentazone, some of which resulted in deaths:

- After ingestion of approximately 36 g bentazone for a suicide attempt, a 41-year-old man developed rhabdomyolysis with acute renal failure, vomiting, palpitation, fever and somnolence. Fifteen hours after intake, urine output decreased, and blood, urine nitrogen,

creatinine and creatinine kinase levels were increased. On the 5th day of hospitalization, laboratory findings had returned to normal levels (Emre et al., 2011).

- A 23-year-old healthy male farmer attempted to commit suicide by consumption of approximately 80 ml of bentazone (35.3 g, 569 mg/kg bw). He developed nausea, vomiting, cough, abdominal pain and nasogastric irritation and received a gastric lavage. The patient recovered and was discharged 5 days after admission. Another 31-year-old man, suffering from alcohol abuse and schizophrenia, ingested approximately 200 ml bentazone (88.2 g; 1.764 mg/kg bw). Icteric sclera and multiple reddish ulcers in the oral cavity, tongue base and posterior wall of the oropharynx were found. Over the next few days, the patient developed acute renal failure, fluid overloading and high anion gap metabolic acidosis; he died 5 days after admission (Wu et al., 2008).
- A 59-year-old woman who intentionally ingested 100–200 ml Basagran (about 50–100 g bentazone) was taken to the hospital with cardiac arrest 2 days after she had consumed the herbicide. During this period, she suffered vomiting, urination and diarrhoea, and she was drowsy with a muddled speech. Biological samples obtained at the autopsy were analysed, and the presence of bentazone, alcohol and an active metabolite of citalopram was detected. Blood concentrations of bentazone, alcohol and desmethyl-citalopram were 625 mg/kg, 0.62 g/l and 0.03 mg/kg, respectively (Müller et al., 2003).
- A case of fatal suicidal bentazone poisoning was presented along with a description of the different analytical methods involved. A 56-year-old farmer was examined by the family doctor 1 hour after voluntarily ingesting 500 ml of FIGHTER (about 250 g bentazone). He presented a Glasgow score of 15, polypnoea, diarrhoea and vomiting. During transport by ambulance to the hospital, he tossed, sweated and suddenly presented breathing difficulty followed by heart failure. The patient died within 2 hours post-ingestion. Blood and urine samples were taken just before death. Bentazone plasma and urine levels were 1500 and 1000 mg/l, respectively (Turcant et al., 2003).
- A 27-year-old robust man, without any medical or surgical history, attempted to commit suicide by consuming 300 ml Basagran (about 130 g bentazone). This poisoning resulted in vomiting, fever, sweating, pipe-like muscle rigidity, sinus tachycardia, drowsiness, leukocytosis, rhabdomyolysis and hepatorenal damage. Empirical treatment with bromocriptine was temporally associated with resolution of the above signs and symptoms. His clinical presentations and the effect of bromocriptine may be indicative that Basagran poisoning mimicks neuroleptic malignant syndrome (Lin et al., 1999).

There have been several unpublished reports of deaths after ingestion of bentazone. The lowest reported dose associated with a death was 20 g; however, this information is related to a case that occurred in China and could not be verified.

Comments

Biochemical aspects

Toxicokinetic studies performed on mice, rats and rabbits indicate that bentazone is rapidly and almost completely absorbed via the oral route (> 99%), and maximum blood concentrations of radioactivity are achieved in approximately 15 minutes at low doses (4 mg/kg bw) and by 1 hour at high doses (200 mg/kg bw). Administration of bentazone either as the sodium salt or as the free acid did not result in any significant differences in absorption. There was no evidence of penetration into the central nervous system or spinal cord, and elimination from other tissues was rapid, with no indication of bioaccumulation.

Elimination was almost exclusively via the urine (approximately 91% within 24 hours); 5 days after dosing, less than 2% was found in faeces and less than 0.02% in expired air. Biliary

excretion of radioactivity was minimal. No significant differences were found in absorption and elimination among the different species investigated (rat, rabbit, mouse).

Bentazone is minimally metabolized in vivo, with the parent compound being the predominant excretion product. Only small amounts of 6-hydroxybentazone (up to approximately 6% of the dose) and minimal amounts of 8-hydroxybentazone (less than approximately 0.2% of the dose) were detected in urine.

Toxicological data

Bentazone has moderate acute toxicity when administered orally to rats, guinea-pigs and rabbits and low toxicity when administered dermally or by inhalation to rats. In rats, the LD_{50} was greater than or equal to 850 mg/kg bw. The dermal LD_{50} in rats was greater than 5000 mg/kg bw. The inhalation LC_{50} was greater than 5.1 mg/l of air (4-hour exposure; nose only). Bentazone was moderately irritating to the eye but not irritating to the skin in rabbits. It was a dermal sensitizer in the Magnusson and Kligman maximization test and the Buehler test in guinea-pigs.

Repeated-dose toxicity studies (subchronic and chronic) in mice, rats and dogs indicate that effects on haematology and blood coagulation (e.g. prolongation of prothrombin time and partial thromboplastin time) were consistently observed.

Three short-term oral rat studies demonstrated an overall NOAEL of 400 ppm (equal to 25.3 mg/kg bw per day), with a LOAEL of 800 ppm (equal to 40 mg/kg bw per day) for decreased body weight gain, decreased feed consumption, increased serum total cholesterol levels, increased urine output and prolonged prothrombin time and partial thromboplastin time.

In 90-day and 1-year dog studies, clinical signs, anaemia and effects on blood coagulation were noted. In the 90-day study, the NOAEL was 300 ppm (equal to 12.0 mg/kg bw per day), on the basis of sedation and ulceration and alopecia on the leg of one dog at 1000 ppm (equal to 39.6 mg/kg bw per day). The NOAEL for the 1-year study was 400 ppm (equal to 13.1 mg/kg bw per day), on the basis of anaemia, altered blood coagulation parameters, clinical signs and weight loss at the highest dietary concentration of 1600 ppm (equal to 52.3 mg/kg bw per day).

In a 2-year dietary toxicity and carcinogenicity study in mice, the NOAEL was 100 ppm (equal to 12 mg/kg bw per day), based on prolongation of prothrombin time and an increased incidence of calcification of the testicular tunica albuginea and deferent canals in the males at 400 ppm (equal to 47 mg/kg bw per day). No carcinogenic effects were observed in this study.

In a 2-year combined toxicity and carcinogenicity study in rats, the NOAEL was 200 ppm (equal to 9 mg/kg bw per day), based on clinical chemistry changes indicative of effects on liver and kidney and effects on blood coagulation parameters at 800 ppm (equal to 35 mg/kg bw per day). No carcinogenic effects were observed in this study.

The Meeting concluded that bentazone was not carcinogenic in rats or mice.

Bentazone was tested for genotoxicity in an adequate range of assays, both in vitro and in vivo. It showed no evidence of genotoxicity.

The Meeting concluded that bentazone is unlikely to be genotoxic.

In view of the lack of genotoxicity and the absence of carcinogenicity in rats and mice, the Meeting concluded that bentazone is unlikely to pose a carcinogenic risk to humans.

In a two-generation dietary reproduction study in rats, the NOAEL for parental and offspring toxicity was 200 ppm (equal to 14 mg/kg bw per day), on the basis of reduced parental feed consumption and body weight gain and reduced pup body weight resulting from parental toxicity at 800 ppm (equal to 59 mg/kg bw per day). There were no effects on reproduction at 3200 ppm (240 mg/kg bw per day), the highest dose tested.

In two studies of developmental toxicity in rats treated by gavage, the overall NOAEL for maternal toxicity was 250 mg/kg bw per day, the highest dose tested. The overall developmental NOAEL was 200 mg/kg bw per day, on the basis of increased post-implantation loss, reduced weight

of fetuses surviving to day 21 and skeletal anomalies at the next higher dose of 250 mg/kg bw per day.

In a third study of developmental toxicity, in which rats were given diets containing bentazone from day 0 to day 21, the NOAEL for maternal toxicity was 2000 ppm (equal to 162 mg/kg bw per day), on the basis of increased water consumption at 4000 ppm (equal to 324 mg/kg bw per day). The developmental NOAEL was 4000 ppm (equal to 324 mg/kg bw per day), on the basis of decreased fetal weight gain and reduced ossification of cervical vertebrae at 8000 ppm (equal to 631 mg/kg bw per day).

In two gavage studies of developmental toxicity in rabbits, the overall NOAEL for maternal and developmental toxicity was 150 mg/kg bw per day, on the basis of a reduction in maternal feed consumption and increased post-implantation losses at 375 mg/kg bw per day.

The Meeting concluded that bentazone was not teratogenic in rats or rabbits.

In a subchronic neurotoxicity study, there was no indication of neurotoxicity at doses up to 3500 ppm (equal to 258 mg/kg bw per day), the highest dose tested.

6-Hydroxybentazone and 8-hydroxybentazone are major plant metabolites of bentazone. Both were less acutely toxic than the parent compound. Neither of the metabolites induced mutations in bacterial tests, and 8-hydroxybentazone was also not genotoxic in an in vitro mammalian forward mutation test and an in vivo mouse micronucleus test. In a subchronic dietary toxicity study and a developmental toxicity study in rats with 8-hydroxybentazone, the NOAEL was approximately 250 mg/kg bw per day, the highest dose tested.

No adverse health effects or poisoning in manufacturing plant personnel or in operators and workers exposed to bentazone have been reported.

Several case reports of suicide attempts due to ingestion of bentazone formulations have been reported in the literature, including four cases resulting in death. The range of doses ingested that resulted in death was 35–250 g of bentazone. The poisoning symptoms and signs included nausea, vomiting, abdominal pain, rhabdomyolysis, hepatorenal damage and cardiac failure.

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

Toxicological evaluation

The Meeting established an ADI of 0–0.09 mg/kg bw derived from a NOAEL of 9 mg/kg bw per day from the 2-year study of toxicity and carcinogenicity in rats, on the basis of prolonged blood coagulation and clinical chemistry changes indicative of effects on liver and kidney at 35 mg/kg bw per day. A safety factor of 100 was applied. This ADI was supported by the NOAEL of 13.1 mg/kg bw per day observed in the 1-year study in dogs for anaemia, altered blood coagulation parameters, clinical signs and weight loss seen at the highest dose of 52.3 mg/kg bw per day; by the NOAEL of 14 mg/kg bw per day in the two-generation study in rats, on the basis of reduced parental feed consumption and body weight gain and reduced pup body weight resulting from parental toxicity at 59 mg/kg bw per day; and by the NOAEL of 12 mg/kg bw per day in a 2-year toxicity and carcinogenicity study in mice, based on prolongation of prothrombin time and an increased incidence of testicular calcification at 47 mg/kg bw per day.

The Meeting reaffirmed its previous conclusion that no ARfD is necessary. It considered that the post-implantation loss seen in the rat developmental study was not caused by a single dose and that no other effects were observed in repeated-dose studies that could be due to a single dose.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Two-year study of toxicity and carcinogenicity[a]	Toxicity	100 ppm, equal to 12 mg/kg bw per day	400 ppm, equal to 47 mg/kg bw per day
		Carcinogenicity	2000 ppm, equal to 242 mg/kg bw per day[b]	—
Rat	Short-term studies of toxicity[c]	Toxicity	400 ppm, equal to 25.3 mg/kg bw per day	800 ppm, equal to 40 mg/kg bw per day
	Two-year studies of toxicity and carcinogenicity[a,c]	Toxicity	200 ppm, equal to 9 mg/kg bw per day	800 ppm, equal to 35 mg/kg bw per day
		Carcinogenicity	4000 ppm, equal to 274 mg/kg bw per day[b]	—
	Two-generation study of reproductive toxicity[a]	Reproductive toxicity	3200 ppm, equal to 240 mg/kg bw per day[b]	—
		Parental toxicity	200 ppm, equal to 14 mg/kg bw per day	800 ppm, equal to 59 mg/kg bw per day
		Offspring toxicity	200 ppm, equal to 14 mg/kg bw per day	800 ppm, equal to 59 mg/kg bw per day
	Developmental toxicity studies[c,d]	Maternal toxicity	250 mg/kg bw per day[b]	—
		Embryo and fetal toxicity	200 mg/kg bw per day	250 mg/kg bw per day
Rabbit	Developmental toxicity study[d]	Maternal toxicity	150 mg/kg bw per day	375 mg/kg bw per day
		Embryo and fetal toxicity	150 mg/kg bw per day	375 mg/kg bw per day
Dog	Ninety-day and 1-year studies of toxicity[a,c]	Toxicity	400 ppm, equal to 13.1 mg/kg bw per day	1000 ppm, equal to 39.6 mg/kg bw per day

[a] Dietary administration.
[b] Highest dose tested.
[c] Two or more studies combined.
[d] Gavage administration.

Estimate of acceptable daily intake for humans

0–0.09 mg/kg bw

Estimate of acute reference dose

Unnecessary

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

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

Critical end-points for setting guidance values for exposure to bentazone

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapidly and almost completely absorbed (> 90%)
Dermal absorption	Poorly absorbed (1–2%)
Distribution	Widely distributed
Potential for accumulation	None
Rate and extent of excretion	Rapid, more than 90% within 24 h, mainly via urine
Metabolism in animals	Minimal
Toxicologically significant compounds in animals, plants and the environment	Parent compound
Acute toxicity	
Rat, LD_{50}, oral	≥ 850 mg/kg bw
Rat, LD_{50}, dermal	> 5000 mg/kg bw
Rat, LC_{50}, inhalation	> 5.1 mg/l of air
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Irritating
Dermal sensitization	Sensitizer (Magnusson & Kligman test)
Short-term studies of toxicity	
Target/critical effect	Blood coagulation
Lowest relevant oral NOAEL	12 mg/kg bw per day (dog)
Lowest relevant dermal NOAEL	1000 mg/kg bw per day (highest dose tested) (rabbit)
Lowest relevant inhalation NOAEC	No data
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Blood coagulation, liver and kidney effects
Lowest relevant NOAEL	9 mg/kg bw per day (rat)
Carcinogenicity	Not carcinogenic in rats or mice
Genotoxicity	
	Not genotoxic
Reproductive toxicity	
Reproduction target/critical effect	No reproductive effects
Lowest relevant parental NOAEL	14 mg/kg bw per day (rat)
Lowest relevant offspring NOAEL	14 mg/kg bw per day (rat)
Lowest relevant reproductive NOAEL	240 mg/kg bw per day (highest dose tested) (rat)
Developmental toxicity	
Developmental target/critical effect	Post-implantation loss, reduced fetal weight and skeletal anomalies
Lowest relevant maternal NOAEL	150 mg/kg bw per day (rabbit)
Lowest relevant embryo/fetal NOAEL	150 mg/kg bw per day (rabbit)
Neurotoxicity	
Subchronic neurotoxicity	No effect up to 258 mg/kg bw per day (highest dose tested)

Medical data	
	No significant health effects in manufacturing personnel. Six cases of intentional poisoning have been reported with various critical symptoms.

Summary

	Value	Study	Safety factor
ADI	0–0.09 mg/kg bw	2-year chronic toxicity and carcinogenicity study (rat)	100
ARfD	Unnecessary	—	—

References

Agnish ND, Keller KA (1997). The rationale for culling of rodent litters. *Fundamental and Applied Toxicology*, 38(1):2–6.

Allen TR (1989). 1st amendment to report: 52-week oral toxicity (feeding) study with bentazon technical (ZST No.: 86/48) in the dog. Unpublished report no. 1989/0153. RCC – Research & Consulting Co. AG, Itingen, Switzerland. Submitted to WHO by BASF.

Allen TR et al. (1989). 52-week oral toxicity (feeding) study with bentazon technical (ZST No.: 86/48) in the dog. Unpublished report no. 1989/0049. RCC – Research & Consulting Co. AG, Itingen, Switzerland. Submitted to WHO by BASF.

Anonymous (1981). Thirty-day oral toxicity study of bentazon in mice. Unpublished report no. 1981/10239. Nippon Institute for Biological Science, Tokyo, Japan. Submitted to WHO by BASF.

Barber ED, Hill T, Schum DB (1995). The percutaneous absorption of hydroquinone (HQ) through rat and human skin in vitro. *Toxicology Letters*, 80:167–172.

Becker H et al. (1987a). Embryotoxicity (including teratogenicity) study with bentazon technical in the rat. Unpublished report no. 1986/421. RCC – Research & Consulting Co. AG, Itingen, Switzerland. Submitted to WHO by BASF.

Becker H et al. (1987b). Embryotoxicity (including teratogenicity) study with bentazon technical in the rabbit. Unpublished report no. 1987/058. RCC – Research & Consulting Co. AG, Itingen, Switzerland. Submitted to WHO by BASF.

Booth GM (1974). Metabolism of bentazon in the mouse (*Mus musculus*). Unpublished report no. 1974/5117. Department of Zoology, Brigham Young University. Submitted to WHO by BASF.

Butler WH (1985). Review of the studies on the 24 months oral chronic toxicity and potential carcinogenicity of bentazon in rats. Unpublished report no. 1985/440. Nippon Institute for Biological Science, Tokyo, Japan. Submitted to WHO by BASF.

Butler WH (1986). Supplemental report of the studies on the 24-month oral chronic toxicity and potential carcinogenicity of bentazon in rats. Unpublished report no. 1986/0438. Nippon Institute for Biological Science, Tokyo, Japan. Submitted to WHO by BASF.

Butler WH (1988). Two-year chronic/oncogenic feeding studies of bentazon in rat and mouse. Unpublished report no. 1988/0155. Bibra Toxicology International, Carshalton, Surrey, England. Submitted to WHO by BASF.

Cannon GE et al. (1974). Two year chronic oral toxicity study of BAS 351-H in rats. Unpublished report no. 1974/004. Cannon Laboratories Inc., Reading, PA, USA. Submitted to WHO by BASF.

Carlton WW et al. (1987). Review of hepatic and pulmonary tissues of 24-month chronic oral toxicity study of bentazon Reg. No. 51 929 in mice. Unpublished report no. 1987/0139. Nippon Institute for Biological Science, Tokyo, Japan. Submitted to WHO by BASF.

Chahoud I, Paumgartten FJR (2009). Influence of litter size on the postnatal growth of rat pups: is there a rationale for litter-size standardization in toxicity studies? *Environmental Research*, 109:1021–1027.

Chasseaud LF et al. (1979). A comparison of the bioavailability and metabolic fate of (^{14}C)-bentazon free acid and (^{14}C)-bentazon sodium salt in the rat, after oral administration. Unpublished report no. 1979/077. Huntingdon Research Centre Ltd, Huntingdon, Cambridgeshire, England. Submitted to WHO by BASF.

Cifone MA (1985a). Evaluation of bentazon 84/140 in the in vivo mouse hepatocyte unscheduled DNA synthesis assay. Unpublished report no. 1985/159. Litton Bionetics Inc., Kensington, MD, USA. Submitted to WHO by BASF.

Cifone MA (1985b). Evaluation of bentazon in the in vitro mouse primary hepatocyte unscheduled DNA synthesis assay. Unpublished report no. 1985/067. Litton Bionetics Inc., Kensington, MD, USA. Submitted to WHO by BASF.

Creasy DM (1997). Evaluation of testicular toxicity in safety evaluation studies: the appropriate use of spermatogenic staging. *Toxicologic Pathology*, 25(2):119–131.

Dawson AB (1926). A note on the staining of the skeleton of cleared specimens with alizarin red S. *Stain Technology*, 1:123–124.

den Boer WC (1985). Mutagenicity evaluation of bentazon techn. 84/140 in the CHO HGPRT forward mutation assay. Unpublished report no. 1985/403. Litton Bionetics, Veenendaal, the Netherlands. Submitted to WHO by BASF.

El-Mahdi MM, Lotfi MM (1988). Teratological effects of pesticide (Basagran) on embryo of albino rat. *Archiv für Experimentelle Veterinärmedizin*, 42(2):261–266.

Emre H et al. (2011). Rhabdomyolysis and acute renal failure as a result of bentazone intoxication. *Eastern Journal of Medicine*, 16(1):59–61.

Engelhardt G, Gelbke H-P (1983). Report on the study of Reg. No. 51 929 (bentazone) (ZNT test substance No.: 83/3) in the Ames test (standard plate test with *Salmonella typhimurium*). Unpublished report no. 1983/222. BASF AG, Ludwigshafen/Rhein, Federal Republic of Germany. Submitted to WHO by BASF.

Engelhardt G, Gelbke H-P (1985a). Report on the study of bentazone (ZNT test substance No.: 84/140) in the Ames *Salmonella*/microsome plate assay and reverse mutation assay—*E. coli* WP2 uvrA (standard plate test). Unpublished report no. 85/108. BASF AG, Ludwigshafen/Rhein, Federal Republic of Germany. Submitted to WHO by BASF.

Engelhardt G, Gelbke H-P (1985b). Report on the study of bentazone Na (pure active ingredient) ZNT No.: 84/298 and bentazon Na (technical grade) ZNT No.: 84/299 in the Ames test (standard plate test with *Salmonella typhimurium*). Unpublished report no. 1985/081. BASF AG, Ludwigshafen/Rhein, Federal Republic of Germany. Submitted to WHO by BASF.

Engelhardt G, Gelbke H-P (1985c). Cytogenetic investigations in NMRI mice after a single oral administration of bentazone—Micronucleus test. Unpublished report no. 1985/036. BASF AG, Ludwigshafen/Rhein, Federal Republic of Germany. Submitted to WHO by BASF.

Engelhardt G, Gelbke H-P (1987a). Report on the study of 8-hydroxy-bentazon (ZNT test substance No.: 86/391) in the Ames test (standard plate test and preincubation test with *Salmonella typhimurium*). Unpublished report no. 1987/0168. BASF AG, Ludwigshafen/Rhein, Federal Republic of Germany. Submitted to WHO by BASF.

Engelhardt G, Gelbke H-P (1987b). Report on the study of 6-hydroxy-bentazon (ZNT test substance No.: 86/244) in the Ames test (standard plate test and preincubation test with *Salmonella typhimurium*). Unpublished report no. 1987/023. BASF AG, Ludwigshafen/Rhein, Federal Republic of Germany. Submitted to WHO by BASF.

Engelhardt G, Hoffmann HD (1993). Cytogenetic study in vivo of 8-OH-bentazon in mice—Micronucleus test—Single oral administration. Unpublished report no. 1993/10424. BASF AG, Ludwigshafen/Rhein, Germany. Submitted to WHO by BASF.

European Commission (2000). *Review report for the active substance bentazone*. EC 7585/VI/97-final, 30 November 2000 (http://ec.europa.eu/food/plant/protection/evaluation/existactive/list1-14_en.pdf).

Gamer AO, Landsiedel R (2009). ^{14}C-BAS 351 H (bentazone Na-salt) in BAS 351 32 H—Study of penetration through human skin in vitro. Unpublished report no. 2009/1041499. BASF SE, Ludwigshafen/Rhein, Germany. Submitted to WHO by BASF.

Garagna S et al. (2005). Effects of a low dose of bentazon on spermatogenesis of mice exposed during foetal, postnatal and adult life. *Toxicology*, 212:165–174.

Gerspach R (2011). 8 two-generation studies 1985–1990 in the Han Wistar rat (KFM:WIST) performed at RCC Ltd. Unpublished report no. 2011/1145234. Harlan Laboratories Ltd, Füllinsdorf, Switzerland. Submitted to WHO by BASF.

Haseman JK, Huff J, Boorman GA (1984). Use of historical control data in carcinogenicity studies in rodents. *Toxicologic Pathology*, 12(2):126–135.

Hathway DE et al. (1971). The metabolism of bentazon in rats. Unpublished report no. 1971/0069. Huntingdon Research Centre Ltd, Huntingdon, Cambridgeshire, England. Submitted to WHO by BASF.

Hawkins DR et al. (1986a). Investigation of urinary metabolites of bentazon in the rat. Unpublished report no. 1986/090. Huntingdon Research Centre Ltd, Huntingdon, Cambridgeshire, England. Submitted to WHO by BASF.

Hawkins DR et al. (1986b). Dermal absorption of ^{14}C-bentazon in rats. Unpublished report no. 1985/299. Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, England. Submitted to WHO by BASF.

Hawkins DR et al. (1987). The biokinetics and metabolism of ^{14}C-bentazon in rats. Unpublished report no. 1987/0429. Huntingdon Research Centre Ltd, Huntingdon, Cambridgeshire, England. Submitted to WHO by BASF.

Hellwig J, Hildebrand B (1993). Study of the prenatal toxicity of 8-OH-bentazon in rats after oral administration (gavage). Unpublished report no. 1993/10572. BASF AG, Ludwigshafen/Rhein, Germany. Submitted to WHO by BASF.

Hofmann HT (1972a). Bericht ueber die Pruefung der akuten oralen Toxizitaet - Bentazon techn. an der Ratte Unpublished report no. 1972/051. BASF AG, Ludwigshafen/Rhein, Federal Republic of Germany. Submitted to WHO by BASF.

Hofmann HT (1972b). Bericht ueber die Pruefung der akuten intraperitonealen Toxizitaet - Bentazon techn. an der Ratte. Unpublished report no. 1972/10130. BASF AG, Ludwigshafen/Rhein, Federal Republic of Germany. Submitted to WHO by BASF.

Hofmann HT (1973a). Acute oral toxicity of 3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one-2,2-dioxide to the rat. Unpublished report no. 1973/022. BASF AG, Ludwigshafen/Rhein, Federal Republic of Germany. Submitted to WHO by BASF.

Hofmann HT (1973b). Acute oral toxicity of the sodium salt of 3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one-2,2-dioxide to the rat. Unpublished report no. 1973/023. BASF AG, Ludwigshafen/Rhein, Federal Republic of Germany. Submitted to WHO by BASF.

Hofmann HT (1974). Acute oral toxicity of the sodium salt of 3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one-2,2-dioxide (bentazon) to the guinea pig. Unpublished report no. 1974/035. BASF AG, Ludwigshafen/Rhein, Federal Republic of Germany. Submitted to WHO by BASF.

Hofmann HT (1991). Report on the study of the acute oral toxicity of 3-isopropyl-2,1,3-benzo-thiadiazinon-(4)-2,2-dioxide (= bentazon) in guinea pigs. Unpublished report no. 1991/10147. BASF AG, Ludwigshafen/Rhein, Germany. Submitted to WHO by BASF.

Hofmann HT, Merkle J (1978a). Investigation to determine the prenatal toxicity of 3-isopropyl-2,1,3-benzothiadiazin-4-one-2,2-dioxide on rats. Unpublished report no. 1978/039. BASF AG, Ludwigshafen/Rhein, Federal Republic of Germany. Submitted to WHO by BASF.

Hofmann HT, Merkle J (1978b). Study to determine the prenatal toxicity of 3-(1-methylethyl)-1H-2,1,3-benzothiadiazin-4(3H)-one-2,2-dioxide in rabbits. Unpublished report no. 1984/048. BASF AG, Ludwigshafen/Rhein, Federal Republic of Germany. Submitted to WHO by BASF.

Hofmann HT, Peh J (1973). Report on the testing of 3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one-2,2-dioxide for mutagenicity after intraperitoneal administration to the male mouse. Unpublished report no. 1973/025. BASF AG, Ludwigshafen/Rhein, Federal Republic of Germany. Submitted to WHO by BASF.

Hofmann HT, Zeller H (1969a). Acute inhalation toxicity (inhalation danger) of 3-isopropyl-2,1,3-benzothiadiazinone-(4)-2,2-dioxide on rats. Unpublished report no. 1969/003. BASF AG, Ludwigshafen/Rhein, Federal Republic of Germany. Submitted to WHO by BASF.

Hofmann HT, Zeller H (1969b). Acute intraperitoneal toxicity of 3-isopropyl-2,1,3-benzo-thiadiazinone-(4)-2,2-dioxide in the mouse. Unpublished report no. 1969/018. BASF AG, Ludwigshafen/Rhein, Federal Republic of Germany. Submitted to WHO by BASF.

Hunter B et al. (1978). Tumorigenicity of bentazone acid to mice in long term dietary administration. Unpublished report no. 1978/034. Huntingdon Research Centre Ltd, Huntingdon, Cambridgeshire, England. Submitted to WHO by BASF.

IARC (1979). *Pathology of tumours in laboratory animals. Vol. II. Tumours of the mouse.* Lyon, France, International Agency for Research on Cancer (IARC Scientific Publications No. 23).

Ioannou YM (1989). Bentazon—review of additional data concerning the two-year chronic toxicity/oncogenicity studies in rats and mice. Unpublished report no. 1989/10485. United States Environmental Protection Agency, Washington, DC, USA. Submitted to WHO by BASF.

Itabashi M et al. (1981). One month toxicity tests for bentazon in rats (tests to determine the dosage levels for 24-month toxicity tests). Unpublished report no. 1981/10240. Nippon Institute for Biological Science, Tokyo, Japan. Submitted to WHO by BASF.

Itabashi M et al. (1983). Teratogenicity study of bentazon, Reg. No. 51 929 (ZNT No. 81/273) in rats by dietary administration. Unpublished report no. 1984/066. Nippon Institute for Biological Science, Tokyo, Japan. Submitted to WHO by BASF.

Jaeckh R, Gelbke H-P (1985). Report on a point mutation test carried out on CHO cells (HGPRT locus) with the test substance bentazon (substance No. 84/140). Unpublished report no. 1985/396. BASF AG, Ludwigshafen/Rhein, Federal Republic of Germany. Submitted to WHO by BASF.

Jeang C-L, Li G-C (1978). Screening of pesticides for mutagenicity in the microbial systems. Unpublished report no. 1978/10236. Plant Protection Center. Submitted to WHO by BASF.

Kaspers U et al. (2004). BAS 351 H (bentazone) subchronic neurotoxicity study in Wistar rats—Administration in the diet for 3 months. Unpublished report no. 2004/1013171. BASF AG, Ludwigshafen/Rhein, Germany. Submitted to WHO by BASF.

Kemény M (2011). Bentazone (BAS 351 H): reassessment of the maternal and developmental toxicity in the 2-generation study. Unpublished report no. 2011/1248852. BASF SE, Limburgerhof, Germany. Submitted to WHO by BASF.

Kieczka H (1986). Amendment to the report on the open epicutaneous test (OET) for the sensitizing potential of bentazon-Na 600 g/L in the guinea pig. Unpublished report no. 1986/347. BASF AG, Ludwigshafen/Rhein, Federal Republic of Germany. Submitted to WHO by BASF.

Kieczka H, Hildebrand B (1986). Report on the open epicutaneous test (OET) for the sensitizing potential of bentazon-Na 600 g/L in the guinea pig. Unpublished report no. 1986/221. BASF AG, Ludwigshafen/Rhein, Federal Republic of Germany. Submitted to WHO by BASF.

Kieczka H, Kirsch P (1986). Report on the maximization test for the sensitizing potential of Reg. No. 51 929 – bentazon in guinea pigs. Unpublished report no. 1986/195. BASF AG, Ludwigshafen/Rhein, Federal Republic of Germany. Submitted to WHO by BASF.

Kieczka H, Kirsch P (1987a). Report on the study of acute oral toxicity on the rat based on OECD and EPA (FIFRA) of 8-hydroxy-bentazon. Unpublished report no. 1987/030. BASF AG, Ludwigshafen/Rhein, Federal Republic of Germany. Submitted to WHO by BASF.

Kieczka H, Kirsch P (1987b). Report on the study of acute oral toxicity on the rat based on OECD and EPA (FIFRA) of 6-hydroxy-bentazon. Unpublished report no. 1987/002. BASF AG, Ludwigshafen/Rhein, Federal Republic of Germany. Submitted to WHO by BASF.

Kieczka H, Kirsch P (1987c). Report on the study of acute oral toxicity on the mouse based on OECD and EPA (FIFRA) of 6-hydroxy-bentazon. Unpublished report no. 1987/003. BASF AG, Ludwigshafen/Rhein, Federal Republic of Germany. Submitted to WHO by BASF.

Kirsch P, Hildebrand B (1983a). Report on the study of the acute oral toxicity in rats of Reg. No. 51 929 – bentazon. Unpublished report no. 1983/114. BASF AG, Ludwigshafen/Rhein, Federal Republic of Germany. Submitted to WHO by BASF.

Kirsch P, Hildebrand B (1983b). Report on the study of acute oral toxicity in the rat of Reg. No. 51 929. Unpublished report no. 1983/113. BASF AG, Ludwigshafen/Rhein, Federal Republic of Germany. Submitted to WHO by BASF.

Kirsch P, Hildebrand B (1983c). Report on the study of the irritation to the intact and abraded dorsal skin of the white rabbit based on Draize of Reg. No. 51 929 – bentazon. Unpublished report no. 1983/081. BASF AG, Ludwigshafen/Rhein, Federal Republic of Germany. Submitted to WHO by BASF.

Kirsch P, Hildebrand B (1983d). Report on the study of the irritation to the eye of the white rabbit based on Draize of Reg. No. 51 929 – bentazon. Unpublished report no. 1983/083. BASF AG, Ludwigshafen/Rhein, Federal Republic of Germany. Submitted to WHO by BASF.

Kirsch P, Hildebrand B (1983e). Report on the study of the acute intraperitoneal toxicity in rats of Reg. No. 51 929 – bentazon. Unpublished report no. 1983/161. BASF AG, Ludwigshafen/Rhein, Federal Republic of Germany. Submitted to WHO by BASF.

Kirsch P, Hildebrand B (1987). Report on the study of acute oral toxicity on the mouse based on OECD and EPA (FIFRA) of 8-hydroxy-bentazon. Unpublished report no. 1987/0322. BASF AG, Ludwigshafen/Rhein, Federal Republic of Germany. Submitted to WHO by BASF.

Kirsch P, Hildebrand B (1993). Study of the dermal toxicity of Reg. No. 51 929 in white rabbits—Application to the intact skin over 3 weeks. Unpublished report no. 1993/10760. BASF AG, Ludwigshafen/Rhein, Germany. Submitted to WHO by BASF.

Klecak G (1977). Identification of contact allergens: predictive tests in animals. In: Marzulli FN, Maibach HI, eds. *Advances in modern toxicology: dermatotoxicology and pharmacology*. *Vol. 4*. Washington, DC, USA, Hemisphere Publishing Corporation, pp. 305–339.

Klimisch H-J (1986). Acute inhalation toxicity LC 50 4 hours (rat)—Dust aerosol study of Reg. No. 51 929 / bentazon. Unpublished report no. 1986/220. BASF AG, Ludwigshafen/Rhein, Federal Republic of Germany. Submitted to WHO by BASF.

Leuschner F (1971). To assess the effect of oral administration of bentazon on the fertility of male Sprague-Dawley rats (with particular reference to dominant lethal factors). Unpublished report no. 1971/018. Laboratorium für Pharmakologie und Toxikologie, Hamburg, Federal Republic of Germany. Submitted to WHO by BASF.

Leuschner F, Otto H (1972). 13 weeks oral toxicity study in Beagle dogs with 3-isopropyl-2,1,3-benzothiadiazinon-(4)-2,2-dioxide (here called bentazon). Unpublished report no. 1972/0061. Laboratorium für Pharmakologie und Toxikologie, Hamburg, Federal Republic of Germany. Submitted to WHO by BASF.

Leuschner F, Otto H (1973). Supplement: 13 weeks oral toxicity study in Beagle dogs with 3-isopropyl-2,1,3-benzothiadiazinon-(4)-2,2-dioxide (here called bentazon). Unpublished report no. 1973/005. Laboratorium für Pharmakologie und Toxikologie, Hamburg, Federal Republic of Germany. Submitted to WHO by BASF.

Leuschner F et al. (1970). 13-week toxicity of 3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one-2,2-dioxide (hereafter referred to as XIX/410) to Beagles when administered with the food. Unpublished report no. 1970/009. Laboratorium für Pharmakologie und Toxikologie, Hamburg, Federal Republic of Germany. Submitted to WHO by BASF.

Leuschner F et al. (1971). 21-day toxicity of 3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one-2,2-dioxide to NZW rabbits on local application. Unpublished report no. 1971/005. Laboratorium für Pharmakologie und Toxikologie, Hamburg, Federal Republic of Germany. Submitted to WHO by BASF.

Leuschner F et al. (1973). Chronic oral toxicity of bentazon in a reproduction study covering three generations of Sprague Dawley rats. Unpublished report no. 1973/010. Laboratorium für Pharmakologie und Toxikologie, Hamburg, Federal Republic of Germany. Submitted to WHO by BASF.

Lin TJ et al. (1999). Acute Basagran poisoning mimicking neuroleptic malignant syndrome. *Human & Experimental Toxicology*, 18(8):493–494.

Marzulli FN, Brown DWC, Maibach HI (1969). Techniques for studying skin penetration. *Toxicology and Applied Pharmacology*, Supplement 3:76–83.

Mellert W, Hildebrand B (1993). Subchronic oral toxicity study with 8-OH-bentazon in Wistar rats—Administration in the diet for 3 months. Unpublished report no. 1993/11011. BASF AG, Ludwigshafen/Rhein, Germany. Submitted to WHO by BASF.

Millar WF (1987). The Birth Defect Prevention Act (SB 950)—Request for toxicology study evaluation worksheets for bentazon. Unpublished report no. 1987/10417. California Department of Food and Agriculture, Sacramento, CA, USA. Submitted to WHO by BASF.

Moriya M (1984). Bentazon: Microbial mutagenicity study—Addendum. Unpublished report no. 1984/10285. Institute of Environmental Toxicology, Tokyo, Japan. Submitted to WHO by BASF.

Moriya M et al. (1983). Further mutagenicity studies on pesticides in bacterial reversion assay systems. *Mutation Research*, 116:185–216.

Muellerschoen H (1991). Gene mutation assay in Chinese hamster ovary CHO cells in vitro with bentazon technical. Unpublished report no. 1991/11108. Cytotest Cell Research GmbH & Co. KG, Rossdorf, Germany. Submitted to WHO by BASF.

Muellerschoen H (1992). Gene mutation assay in Chinese hamster V79 cells in vitro with 8-OH-bentazon Reg. No. 108 746. Unpublished report no. 1992/11329. Cytotest Cell Research GmbH & Co. KG, Rossdorf, Germany. Submitted to WHO by BASF.

Müller IB et al. (2003). Fatal overdose of the herbicide bentazone. *Forensic Science International*, 135(3):235–236.

Nasterlack M et al. (2007). Epidemiological and clinical investigations among employees in a former herbicide production process. *International Archives of Occupational and Environmental Health*, 80(3):234–238.

Neuschl J, Kacmar P (1993). [Acute oral toxicity of bentazone, a herbicide developed in Czechoslovakia, in pheasants and rabbits and the clinical symptoms of poisoning.] *Veterinarni Medicina (Praha)*, 38(2):115–121 (in Slovak).

Oesch F (1977). Ames test for bentazone. Unpublished report no. 1977/028. Universität Mainz, Mainz, Federal Republic of Germany. Submitted to WHO by BASF.

Otto S (1974). Investigations of rabbit urine and feces after oral administration of ^{14}C-bentazon. Unpublished report no. 1974/9000. BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Federal Republic of Germany. Submitted to WHO by BASF.

Postica F et al. (1982). Potential mutagenic evaluation of the bentazone. Unpublished report no. 82/10236. Institute of Hygiene and Public Health, Iasi, Romania (Prof. V. Rugina). Submitted to WHO by BASF.

Salewski E (1964). Staining method for a macroscopic test for implantation points in the uterus of the rat. *Naunyn-Schmiedebergs Archiv für Experimentelle Pathologie und Pharmakologie*, 247:367–368.

Schilling K, Hildebrand B (1988). Study on the dermal toxicity of Reg. No. 51 929 / bentazon in rabbits—Application to the intact skin for 3 weeks (21 applications). Unpublished report no. 1988/0350. BASF AG, Ludwigshafen/Rhein, Federal Republic of Germany. Submitted to WHO by BASF.

Shirasu Y et al. (1976). Mutagenicity testing on bentazon in microbial systems. Unpublished report no. 1976/009. Institute of Environmental Toxicology, Tokyo, Japan. Submitted to WHO by BASF.

Shirasu Y et al. (1982). Mutagenicity screening studies on pesticides. In: *Proceedings of the 3rd Environmental Mutagens and Carcinogens International Conference, 21–27 September 1981, Tokyo, Japan*. pp. 331–335.

Siebert D, Lemperle E (1974). Genetic effects of herbicides: induction of mitotic gene conversion in *Saccharomyces cerevisiae*. *Mutation Research*, 22:111–120.

Suter P et al. (1989). Two-generation reproduction study with bentazon technical (ZST-No. 86/48) in the rat. Unpublished report no. 1989/0068. RCC – Research & Consulting Co. AG, Itingen, Switzerland. Submitted to WHO by BASF.

Taalman RDFM (1987). Clastogenic evaluation of bentazon (ZNT No. 86/48) in an in vitro cytogenetic assay measuring chromosome aberration frequencies in Chinese hamster ovary (CHO) cells. Unpublished report no. 1987/0169. Hazleton Biotechnologies, Veenendaal, the Netherlands. Submitted to WHO by BASF.

Takehara K (1984a). Studies on the 24-month chronic toxicity of bentazon in rats. Unpublished report no. 1985/433. Nippon Soda Co. Ltd, Tokyo, Japan. Submitted to WHO by BASF.

Takehara K (1984b). Studies on the 24-month chronic toxicity of bentazon Reg. No. 51 929 (ZNT No. 81/273) in mice. Unpublished report no. 1985/432. Nippon Institute for Biological Science, Tokyo, Japan. Submitted to WHO by BASF.

Takehara K (1985). Supplemental report of the studies on the 24-month chronic toxicity of bentazon Reg. No. 51 929 (ZNT No. 81/273) in mice. Unpublished report no. 1985/431. Nippon Institute for Biological Science, Tokyo, Japan. Submitted to WHO by BASF.

Takehara K (1986). Supplemental report of the studies on the 24-month oral chronic toxicity and potential carcinogenicity of bentazon in rats, confidential report to Dr. W.H. Butler. Unpublished report no. 1986/0438. Nippon Institute for Biological Science, Tokyo, Japan. Submitted to WHO by BASF.

Takehara K, Tajima M (1982). Thirty-day dose range finding study for chronic toxicity studies in mice. Unpublished report no. 1982/1000611. Nippon Institute for Biological Science, Tokyo, Japan. Submitted to WHO by BASF.

Tarone RE, Chu KC, Ward JM (1981). Variability in the rates of some common naturally occurring tumors in Fischer 344 rats and (C57BL/6N×C3H1HeN)F1(B6C3F1) mice. *Journal of the National Cancer Institute*, 63:849–854.

Tennekes H et al. (1987). 13-week oral toxicity (feeding) study with bentazon technical (ZNT No. 86/48) in the rat. Unpublished report no. 1987/017. RCC – Research & Consulting Co. AG, Itingen, Switzerland. Submitted to WHO by BASF.

Toyoshima S (1978). Acute dermal toxicity studies of bentazon-acid in the rat. Unpublished report no. 1978/055. Keio University, Tokyo, Japan. Submitted to WHO by BASF.

Toyoshima S et al. (1978a). Acute oral, subcutaneous and intraperitoneal toxicity studies of bentazon-acid in the rat. Unpublished report no. 1978/053. Keio University, Tokyo, Japan. Submitted to WHO by BASF.

Toyoshima S et al. (1978b). Acute subcutaneous and intraperitoneal toxicity studies of bentazon-acid in the mouse. Unpublished report no. 1978/054. Keio University, Tokyo, Japan. Submitted to WHO by BASF.

Turcant A et al. (2003). Fatal acute poisoning by bentazone. *Journal of Analytical Toxicology*, 27(2):113–117.

USEPA (2010). Bentazone registration review: revised human-health assessment scoping document. Washington, DC, USA, United States Environmental Protection Agency, March.

USFDA (1966). *Guidelines for reproduction studies for safety evaluation of drugs from human use*. Rockville, MD, USA, United States Department of Health and Human Services, Food and Drug Administration.

Vesselinovitch SD, Mihailovich N, Rao KUN (1978). Morphology and metastatic nature of induced hepatic nodular lesions in C57BL × C3HF1 mice. *Cancer Research*, 38:2003–2010.

Ward JM et al. (1978). Neoplastic and nonneoplastic lesions in aging (C57BL/6N×C3H1HeN)F1(B6C3F1) mice. *Journal of the National Cancer Institute*, 63:849–854.

Welsh J et al. (1974). 18 month chronic oral toxicity study of BAS 351-H in mice. Unpublished report no. 1974/041. Cannon Laboratories Inc., Reading, PA, USA. Submitted to WHO by BASF.

Wilson JG, Warkany J, eds (1965). *Teratology, principles and techniques*. Chicago, IL, USA, University of Chicago Press, pp. 265–277.

Wu IW et al. (2008). Acute renal failure induced by bentazone: 2 case reports and a comprehensive review. *Journal of Nephrology*, 21(2):256–260.

Xu HH, Schurr KM (1990). Genotoxicity of 22 pesticides in microtitration SOS chromotest. *International Journal*, 5:1–14.

Yamate J (1988). Additional histopathological investigation into the salivary and mammary glands taken from the long term feeding study in mice with bentazon. Unpublished report no. 1988/0483. Nippon Institute for Biological Science, Tokyo, Japan. Submitted to WHO by BASF.

Zeller H (1969). Acute dermal toxicity of 3-isopropyl-2,1,3-benzo-thiadiazinone-(4)-2,2-dioxide on rats. Unpublished report no. 1969/002. BASF AG, Ludwigshafen/Rhein, Federal Republic of Germany. Submitted to WHO by BASF.

Zeller H, Birnstiel H (1969). Acute oral toxicity of 3-isopropyl-2,1,3-benzo-thiadiazinone-(4)-2,2-dioxide (technical grade) on rabbits. Unpublished report no. 1969/005. BASF AG, Ludwigshafen/Rhein, Federal Republic of Germany. Submitted to WHO by BASF.

Zeller H, Hofmann HT (1969). Acute oral toxicity of thianon in rats. Unpublished report no. 1969/0013. BASF AG, Ludwigshafen/Rhein, Federal Republic of Germany. Submitted to WHO by BASF.

Zeller H, Kirsch P (1970). 90-day feeding trial on rats with 3-isopropyl-2,1,3-benzothiadiazinone-(4)-2,2-dioxide. Unpublished report no. 1970/008. BASF AG, Ludwigshafen/Rhein, Federal Republic of Germany. Submitted to WHO by BASF.

Zeller H, Magoley J (1970a). Acute oral toxicity of 3-isopropyl-2,1,3-benzo-thiadiazinone-(4)-2,2-dioxide to cats. Unpublished report no. 1970/016. BASF AG, Ludwigshafen/Rhein, Federal Republic of Germany. Submitted to WHO by BASF.

Zeller H, Magoley J (1970b). Acute oral toxicity of 3-isopropyl-2,1,3-benzo-thiadiazinone-(4)-2,2-dioxide in dogs. Unpublished report no. 1970/017. BASF AG, Ludwigshafen/Rhein, Federal Republic of Germany. Submitted to WHO by BASF.

Zeller H, Peh J (1971). Bericht ueber die Pruefung von 3-Isopropyl-2,1,3-benzo-thiadiazinon-(4)-2,2-dioxid (= Bentazon) auf etwaige teratogene Wirkung an der Ratte bei peroraler Applikation. Unpublished report no. 1971/0041. BASF AG, Ludwigshafen/Rhein, Federal Republic of Germany. Submitted to WHO by BASF.

Zimmermann FK et al. (1984). Testing of chemicals for genetic activity with *Saccharomyces cerevisiae*: a report of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutation Research*, 133:199–244.