IMAZAPYR

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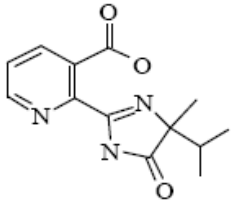
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Explanation

Imazapyr (Fig. 1) is the International Organization for Standardization–approved name of 2-[(*RS*)-4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl]nicotinic acid (International Union of Pure and Applied Chemistry), with Chemical Abstracts Service No. 81334-34-1. Imazapyr is a herbicide used for the control of grasses and broadleaf weeds in a variety of crops, including major uses in soya bean, sunflower, rice, maize, sugar cane, rape, wheat and non-crop areas such as vegetation management and forestry and minor uses in tobacco and oil palm. Imazapyr kills weeds by inhibiting the activity of the plant-specific enzyme acetohydroxyacid synthase, which catalyses the production of three branched-chain amino acids (valine, leucine and isoleucine) required for protein synthesis and cell growth. The rate of plant death is usually slow (several weeks) and is likely related to the amount of stored amino acids available to the plant.

Fig. 1. Chemical structure of imazapyr



Imazapyr has not previously been evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) and was reviewed by the present Meeting at the request of the Codex Committee on Pesticide Residues.

All critical studies contained statements of compliance with good laboratory practice (GLP).

Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution, metabolism and excretion

Rats

To determine the excretion pattern in rats after oral dosing with [^{14}C]imazapyr, a pilot study was undertaken. Each of four Crl:CD (SD) BR rats (two of each sex, 6–10 weeks old, weighing 150–250 g) was given a single dose of [^{14}C]imazapyr at a nominal rate of 10 mg/kg body weight (bw) by gavage. After dosing, the rats were individually housed in glass metabolism cages. Urine, faeces and expired air were collected daily for 3 days following dosing and analysed quantitatively for radioactive residues of [^{14}C]imazapyr. [^{14}C]Imazapyr used in this study had a specific activity of 0.39 MBq/mg and a radiochemical purity of 95.25%. The results showed that over a 3-day period, approximately 70–75% of the [^{14}C]imazapyr-related radioactivity was excreted in the urine and cage rinse, about 14–25% was excreted in the faeces and 0.05–0.15% was in the expired air. The excretion pattern was similar in both male and female rats. An average of about 90% of the dosed radioactivity was excreted within the first 24 hours. It was concluded that imazapyr was rapidly excreted from the body of the rat. The amount of radiocarbon found in the expired air was essentially "nil", leading to the conclusion that further metabolism studies of [^{14}C]imazapyr in rats need not be conducted in a closed system.

The study was GLP compliant, and a quality assurance (QA) statement was attached (Wu, 1992).

The definitive rat metabolism study was conducted to examine the absorption, distribution, metabolism and excretion of imazapyr, to aid the evaluation of test results from toxicological studies and extrapolation of data from experimental animals to humans. [^{14}C]Imazapyr labelled with ^{14}C at the 6-carbon position of the pyridine ring was used in this study. The position of ^{14}C label in imazapyr was considered to be metabolically stable, which allowed determination of the metabolic profile in rats. The ^{14}C tracer had a specific activity of 1.6 MBq/mg, with a radiochemical purity of 96.8% and a chemical purity of 93.4%. This material was diluted with non-radiolabelled imazapyr that had a chemical purity of 99.5%, to give final specific activities of 0.35 MBq/mg for the low dose and 0.01 MBq/mg for the high dose used in this study. The radiolabelled imazapyr was dissolved in corn oil for all oral dosing solutions, and the intravenous solution was prepared in normal saline. The study was conducted using both male and female Sprague-Dawley Crl:CD rats (53–66 days old; weight 150–250 g). The animals were divided into five groups. The sex and group distribution, route of administration and target doses are shown in Table 1.

Urine and faeces were collected from all treatment groups at specified time intervals, and selected samples were analysed for parent material and metabolites. Animals were sacrificed 7 days after administration of the test substance, and radiocarbon distributions were determined in select tissue samples. The collection of expired air in the definitive phase studies (Groups A, B, C and D) was not necessary, as the pilot study (Wu, 1992) had shown only trace amounts of radiocarbon in the expired air over a 3-day period.

The results revealed that imazapyr is quickly absorbed and excreted following administration to rats. The percentage of [^{14}C]imazapyr dose absorbed in the single oral dose group and multiple oral low-dose groups was calculated by comparing the percentage of the dose excreted in urine by these groups with the percentage of the dose excreted in urine by the intravenous low-dose group. The

Table 1. Details of the rat metabolism study

Group	Route of administration	Target dose (mg/kg bw)	Number of males	Number of females
A	Single oral low via gavage	9.5	5	5
B	Single oral high via gavage	924	5	5
C	Repeated oral low via gavage	14 days of unlabelled material (~10 mg/kg bw) followed by a single [^{14}C]imazapyr dose at 9.26 mg/kg bw	5	5
D	Single intravenous low	9.94	5	5
E (control)	Single oral via gavage (vehicle only)	Corn oil only	2	2

Source: Wu (1994)

Table 2. Route of excretion and total recovery of imazapyr in rats after 7 days

Group	Target dose (mg/kg bw)	Route of administration	Sex of animal	% of administered dose ± standard deviation			
				Urine[a]	Faeces	Carcass	Total
A	9.5	Single oral low	M	81.1 ± 5.9	26.4 ± 2.7	0.2 ± 0.2	107.7 ± 4.3
			F	78.5 ± 13.4	25.9 ± 9.2	0.1 ± 0.1	104.5 ± 6.3
B	924	Single oral high	M	78.8 ± 5.3	21.1 ± 5.3	< 0.1	99.9 ± 1.9
			F	76.7 ± 7.1	21.9 ± 6.2	0.1 ± 0.03	98.7 ± 2.6
C	9.26	Repeated oral low	M	75.0 ± 2.9	31.1 ± 3.3	0.1 ± 0.1	106.2 ± 4.5
			F	67.8 ± 4.6	33.2 ± 6.8	0.2 ± 0.07	101.2 ± 4.5
D	9.94	Intravenous	M	94.6 ± 7.7	6.6 ± 5.6	0.1 ± 0.02	101.3 ± 2.4
			F	86.5 ± 6.4	5.5 ± 4.1	0.1 ± 0.06	92.1 ± 3.8

F: female; M: male

[a] Includes cage wash and cage wipe.

Source: Wu (1994)

calculated absorption percentages were 80.6% and 80.0% for oral low-dose (Group A) males and females, respectively, and 75.6% and 71.4% for the repeated oral dose (Group C) males and females, respectively. For the oral high-dose group (Group B), the absorption percentages were 84.4% and 81.7% for males and females, respectively.

The majority of the administered dose was excreted through the urine and, to a lesser degree, through faeces. The test substance was mostly excreted as intact imazapyr, indicating a low level of biochemical alteration of the parent compound. Trace levels of polar and non-polar metabolites were formed and excreted in the urine and faeces. Only trace amounts of residues were detected in the liver and kidneys of the high-dose group.

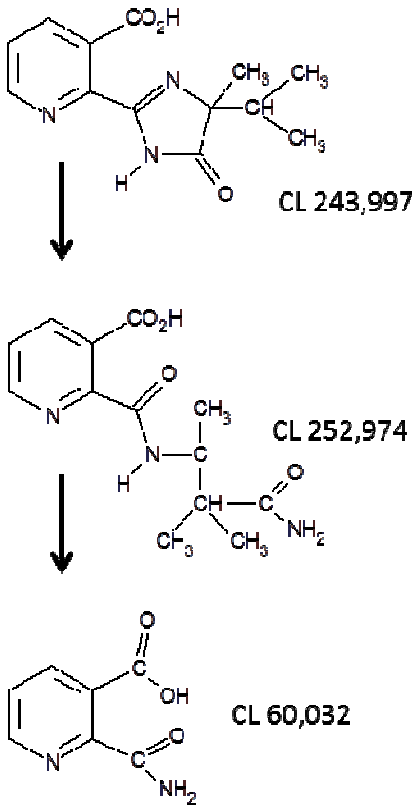
Urine was the major route of ^{14}C excretion (67.8–94.6%) (Table 2). Most of the elimination occurred within the first 24 hours after dosing (56.9–90.6%). The total level of ^{14}C radioactivity recovered in faeces ranged from 5.5% to 33.2% (Table 2), with a majority also eliminated within the first 24 hours (3.0–23.8%). Regardless of the treatment regimen, [^{14}C]imazapyr was rapidly eliminated by the rats, primarily through urine.

From the above, it can be concluded that regardless of the treatment regimen, imazapyr was eliminated rapidly by rats, and urine was the primary elimination route. The residue levels were below the limit of detection in tissues of rats dosed at 9.5 or 924 mg/kg bw, except for kidneys and liver from the high-dose group and ovaries from the multiple-dose group. There were no appreciable sex-related differences in absorption, elimination or distribution of radioactivity for rats dosed orally with

[^{14}C]imazapyr. The parent imazapyr was metabolized only to a limited degree and represented the major radiocarbon fraction in urine, faeces and examined tissues. Trace levels of polar and non-polar metabolites were formed and excreted in the urine and faeces.

The proposed metabolic pathway of imazapyr is shown in Fig. 2.

Fig. 2. Metabolic pathway of imazapyr



The study was GLP compliant, and a QA statement was attached (Wu, 1994).

The absorption, distribution, elimination and biotransformation of imazapyr were studied in male rats dosed orally with [^{14}C]imazapyr. Fifteen male Sprague-Dawley rats (age not specified), each weighing approximately 225 g, were housed in individual metabolism cages (Acme Metal Products, Inc., Chicago, Illinois) constructed to facilitate the separate collection of urine and faeces. Twelve rats were treated with [^{14}C]imazapyr at a dose of 4.4 mg/kg bw per day, and the remaining three rats were used as control animals. Three treated rats were sacrificed at each time interval (1, 2, 5 and 8 days) after dosing. One control rat was sacrificed on the 5th day, and two were sacrificed on the 8th day.

Approximately 87.2%, 93.3% and 94.9% of the total administered dose was rapidly excreted in the urine and faeces in 1, 2 and 4 days after treatment, respectively. Elimination of the administered dose was essentially completed by day 6 and accounted for 95.1% of the total dose. Overall recovery from the urine, faeces and cage rinse was 98.0% of the total administered dose by day 8 after treatment: 58.8% in urine, 36.3% in faeces and 2.9% in cage rinse. After 1 day, about 55.3% of the dose was excreted in the urine, and about 31.9% was excreted in the faeces. The half-life of imazapyr

in the rat was less than 1 day. The tissue residue level was less than 0.01 parts per million (ppm) on day 8.

The study was not GLP compliant, nor was any QA statement attached (Mallipudi, 1983).

2. Toxicological studies

2.1 Acute toxicity

The results of acute toxicity studies are summarized in Table 3. All these studies were conducted in compliance with GLP, and/or a QA statement was attached.

Table 3. Summary of acute toxicity studies with imazapyr

Species	Strain	Sex	Route	Batch no. and purity	LD_{50} (mg/kg bw) / LC_{50} (mg/L)	Reference
Rat	Sprague-Dawley Crl:CD®(SD)BR	M + F	Oral	AC 11574-27 98.8% w/w	> 5 000[a]	Lowe (1998)
Rabbit	New Zealand White	M + F	Oral	Not available	4 800 (probit analysis)	Kynoch (1983a)
Dog	Beagle	M + F	Oral	AC 4866-62 99.5%	> 5 000[b]	Fischer (1986)
Rat	Sprague-Dawley (HC/CFY)	M + F	Dermal	SCR 481/208 95.9%	> 2 000	Kynoch (1983b)
Rabbit	New Zealand White	M + F	Dermal	AC 4866-62 99.5%	> 2 000	Fischer (1990a)
Rat	Sprague-Dawley	M + F	Inhalation (4 h) MMAD ≤ 10 µm	AC 4361-97 93%	Actual gravimetric > 1.3 mg/L (nominal 5.1 mg/L)	Voss (1983)
Rabbit	New Zealand White	M	Skin Irritation	AC 4866-62 99.5%	Not irritating	Fischer (1990b)
Rabbit	New Zealand White	M	Eye irritation	AC 4866-62 99.5%	Irritating to eye	Fischer (1990c)
Guinea-pig	Hartley	M	Skin sensitization	AC4361-97 93%	Not sensitizing	Ledoux (1983)

F: female; LC_{50}, median lethal concentration; LD_{50}, median lethal dose; M: male; MMAD: mass median aerodynamic diameter; w/w: weight per weight

[a] The highest dose tested. The predominant clinical sign is salivation in male rats only.

[b] Signs of toxicity limited to emesis at 1–1.5 hours after dosing.

2.2 Short-term studies of toxicity

(a) Oral administration

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

Rats

In a range-finding study, imazapyr (purity 93%; lot no. 4096-27A) was administered to Charles River CD strain albino rats (4 weeks old; five of each sex per dose) via the diet at a concentration of 0, 1000, 5000 or 10 000 ppm (equal to 0, 135, 675 and 1395 mg/kg bw per day, respectively, averaged over both sexes) for 28 consecutive days. Clinical observations were made twice a day. Body weight, feed consumption and water consumption were recorded once a week. Clinical, haematological, gross pathological and histopathological examinations were carried out at the end of the study.

At 10 000 ppm, body weight gain of male rats was significantly suppressed. No test substance–related overt toxic effects were observed at any treatment level during the course of the study. All rats survived the 28-day dosing period.

No test substance–related changes in feed intake, haematological parameters, biochemical parameters or organ weights were observed that could be attributed to ingestion of imazapyr. There were no gross or microscopic pathological changes that were attributable to ingestion of imazapyr.

It was concluded that the no-observed-adverse-effect level (NOAEL) for this study was 5000 ppm imazapyr in the diet (equal to 675 mg/kg bw per day), based on the suppression of body weight gain in males at 10 000 ppm (equal to 1395 mg/kg bw per day).

The study was conducted prior to the implementation of GLP (Fischer, 1982).

In a 90-day dietary study, imazapyr (purity 97.2%; lot no. AC 4468-114) was administered to Charles River CD strain albino rats (Sprague-Dawley derived) at a concentration of 0, 1000, 5000 or 10 000 ppm in the diet (equal to 0, 88.6, 438.6 and 879.1 mg/kg bw per day, respectively, averaged over both sexes). At the start of the trial, the rats were approximately 4 weeks old, and the average body weight was in the range of 86–98 g for males and 80–93 g for females. Each dose group had 30 animals of each sex. The animals were observed for toxic signs and morbidity twice daily and given a thorough examination at each weekly weighing. Feed consumption and body weights were recorded 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 at any treatment level during the course of the experiment. Some incidental findings occasionally seen among both control and treated rats were hair loss, body sores, malalignment of upper incisors, and reddish or black material on or around the eyes, nose and mouth. Feed intakes for both sexes at all dose levels were comparable. The body weight gains were somewhat decreased, although not significantly ($P < 0.05$), in both sexes at 5000 and 10 000 ppm. No treatment-related changes could be observed in haematological, biochemical or urine analysis parameters in test or control animals. Relative kidney weights were significantly ($P < 0.05$) increased in female rats at 10 000 ppm. Subsequently, histopathological examination failed to reveal any cause for this change. Therefore, the change was considered to be fortuitous. No other significant organ weight changes were observed in either sex at 10 000 ppm. Absolute and relative organ weights for both sexes at 1000 and 5000 ppm were unaffected by ingestion of imazapyr. No test substance–related gross or macroscopic changes were found at necropsy of the test animals.

In view of the above, the NOAEL was 10 000 ppm (equal to 879.1 mg/kg bw per day), the highest dose tested. The study was conducted as per GLP, and a QA statement was attached (Fischer, 1984).

Imazapyr (purity 99.3%; lot no. AC 4866-62) was administered to albino rats (Charles River CD rats, Sprague-Dawley derived) at a dietary concentration of 0, 15 000 or 20 000 ppm (equal to 0, 1336 and 1740 mg/kg bw per day, respectively, averaged over both sexes) in a 13-week oral toxicity study. The study comprised three groups, each containing 10 male and 10 female rats about 4.5 weeks

of age and weighing 100–130 g (males) or 102–120 g (females). The rats were observed daily for signs of overt toxicity, morbidity and mortality. Ophthalmological examinations were conducted prior to the study and at termination. Detailed clinical observations, individual body weights and individual feed consumption were recorded weekly. Haematological, clinical chemistry and urine analysis determinations were performed on all surviving animals at termination. At termination, all surviving animals were subjected to a gross necropsy, and selected organs were weighed. Samples of selected tissues were examined for histopathological evaluation from all test animals.

No overt clinical signs of toxicity or mortality were observed during the study period that could be attributed to administration of the test material. Feed consumption for both male and female rats at all dose levels was generally comparable to or in excess of those of the control group at most measurement intervals. Body weights were increased (not statistically significantly) during the 13-week study period for both sexes at all treatment levels in comparison with those of the control rats. Body weight gain for the treated groups was generally comparable to or in excess of those of the control groups, but total body weight gains for male and female rats that received imazapyr tended to be increased (2.5–6.5%) over those of the control group. Haematology, clinical chemistry and urine analysis parameters were unaffected by treatment with imazapyr. No changes were observed in absolute or relative (to body weight) organ weights that could be attributed to administration of the test material. There were no gross or microscopic pathological observations that were attributable to the treatment in any of the tissues evaluated.

The data for this study support a NOAEL in the rat of 20 000 ppm (equal to 1740 mg/kg bw per day), the highest dose tested.

The study was conducted in compliance with GLP, and a QA statement was attached (Fischer, 1992).

Dogs

In a 1-year study in Beagle dogs (aged 5–6 months and weighing around 6.2–8.4 kg for males and 6.3–8.4 kg for females), four groups of six males and six females were given diets containing imazapyr (purity 99.5%; lot no. AC 4866-62) at a concentration of 0 (control), 1000 (low dose), 5000 (middle dose) or 10 000 ppm (high dose). The study design and average imazapyr doses are shown in Table 4.

Table 4. Study design and doses of imazapyr ingested

Test group	Concentration in diet (ppm)	Dose per animal (study averages) (mg/kg bw per day)[a]		No. of animals assigned	
		Males	Females	Males	Females
1	0	0	0	6	6
2	1 000	30.4 ± 4.4	29.9 ± 3.9	6	6
3	5 000	141.2 ± 19.4	138.5 ± 21.2	6	6
4	10 000	282.1 ± 41.8	293.7 ± 27.8	6	6

[a] Calculated based on feed consumption and body weight data.
Source: Shellenberger, Nolen & Tegeris (1987)

Animals were examined daily for overt signs of toxicity. Body weights were determined prior to treatment, at the start of the study and weekly thereafter; feed consumption was measured daily. Blood biochemical and haematological parameters and urine analyses were determined prior to dosing, at 6 weeks and at 3, 6 and 12 months. Ophthalmological examinations were performed during the pretest period and at 6 and 12 months. All animals were necropsied for a complete gross pathological and histopathological evaluation and determination of absolute and relative organ weights.

No clinical signs were observed during the study that could be related to the test chemical. All clinical signs were seen in dogs of all dose groups and therefore were considered incidental. All dogs survived to terminal necropsy. Ophthalmological examinations revealed no ocular changes directly attributable to the test compound.

Mean weekly body weights of compound-treated males at all dose levels and of mid-dose (5000 ppm) females were similar to or exceeded those of the controls throughout the study. The only statistically significant differences were increased body weights, which were not toxicologically significant; mean body weights of low-dose (1000 ppm) and high-dose (10 000 ppm) females were slightly lower than those of the controls, but the differences were never statistically significant. Feed consumption of compound-treated males and females was essentially similar to or exceeded that of the controls, and the only statistically significant differences were increased consumption seen frequently in males. Blood biochemical and haematological parameters in males and females revealed no statistically significant differences that were considered to be compound related, as changes observed were not consistent. Occasional statistically significant differences between controls and compound-treated males and females were considered to be random occurrences, as mean values were generally within the normal expected range. Urine analysis parameters were considered to be similar between the control and compound-treated males and females throughout the study. There were no statistically significant differences in mean organ weights or in mean organ to body weight and organ to brain weight ratios between compound-treated and control males or females. The occasional distribution of gross lesions in males and females at termination indicated that these lesions were incidental to chemical treatment. Microscopic examination of all tissues revealed no changes attributable to the test chemical. Lesions observed at necropsy either occurred in near equal incidence in control and compound-treated animals or were found principally in control and mid-dose animals and were considered random occurrences.

In view of the above, the NOAEL in dogs in this 1-year study is 10 000 ppm (equal to 282.1 and 293.7 mg/kg bw per day for males and females, respectively), the highest dose tested.

A QA statement was attached (Shellenberger, Nolen & Tegeris, 1987).

(b) Dermal application

Rabbits

In a 21-day dermal toxicity study, imazapyr technical (purity 93%; lot no. AC 4361-97) was tested in New Zealand White rabbits (weight between 2.31 and 3 kg for males and 2.3 and 3.0 kg for females), with dermal doses of 0, 100, 200 and 400 mg/kg bw of the test substance applied to the back of each animal daily for a period of 6 hours.The area of test material application in both the treated and the control rabbits was covered with a gauze patch moistened with 5 mL of 0.9% saline and subsequently wrapped with an impervious plastic film, which was secured upon itself with an adhesive tape. After 6 hours of exposure, the rabbits were unwrapped, and all remaining test material was removed with soap and water. The rabbits were dried with a clean towel and then returned to their cages. This dosing procedure was repeated 5 days/week for 3 weeks.

No treatment-related clinical signs or mortalities were observed in this study. Two rabbits died with gross evidence of pneumonia, which was confirmed microscopically. There were no treatment-related effects on body weight or estimated feed consumption parameters measured in this study. Haematology, serum chemistry, clinical observation and histopathology revealed no consistent or distinct adverse effects associated with treatment.

In view of the above, there is no indication that imazapyr causes any systemic toxicity at levels of up to 400 mg/kg bw per day when applied topically for 5 days/week for 3 weeks to the back of the rabbit.

A QA statement was attached (Larson, 1983).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

Imazapyr (purity 99.5%; lot no. AC 4866-062) was administered to CD-1 mice (42 days old, weighing about 27 g [males, mean] and 21 g [females, mean] at the initiation of the study) at a dietary concentration of 0, 1000, 5000 or 10 000 ppm for a period of approximately 18 months. The study design and the doses of imazapyr administered are shown in Table 5.

Table 5. Study design and doses of imazapyr administered in a long-term study in mice

	Dietary concentration (ppm)	Dose per animal (study averages) (mg/kg bw per day)		No. of animals assigned	
		Males	Females	Males	Females
1	0	0	0	65	65
2	1 000	126–254	151–303	65	65
3	5 000	674–1 194	776–1 501	65	65
4	10 000	1 301–2 409	1 639–3 149	65	65

Source: Auletta (1988)

The detailed physical examinations for signs of local or systemic toxicity, tests for pharmacological effects and palpation of tissue masses were undertaken pretest and weekly after. Body weights were measured twice pretest, weekly through 14 weeks, biweekly in weeks 16 through 26, monthly thereafter and terminally (after fasting). Feed consumption was estimated pretest, weekly through 14 weeks, biweekly in weeks 16 through 26 and monthly thereafter. For laboratory studies, the experimental outlines are as shown in Table 6.

Table 6. Experimental outlines of laboratory studies in a long-term study in mice

Group	Dietary concentration (ppm)	Number of animals									
		Total		Haematology[a]		Necropsy				Histopathology[b]	
						12 months		18 months			
		M	F	M	F	M	F	M	F	M	F
I	0 (control)[c]	65	65	10	10	10	10	28	36	65	65
II	1 000	65	65	10	10	10	10	35	37	65	65
III	5 000	65	65	10[d]	10	10	10	34	28	65	65
IV	10 000	65	65	10	10	10	10	33	30	65	65

F: female; M: male

[a] Haematology was performed at month 12 and month 18.

[b] Microscopic evaluations were performed on selected tissues for all animals dying accidentally or spontaneously, killed in moribund condition or at the month 12 and month 18 sacrifices.

[c] Control animals received standard laboratory diet.

[d] At the 18-month interval, one animal died accidentally during terminal blood collection; therefore, haematology parameters could be measured only for nine Group III males.

Source: Auletta (1988)

Evaluations of mortality, physical observations, body weights, feed consumption, haematology values and organ weight data revealed no evidence of any effect of test material administration.

Evaluations of gross and microscopic pathology findings revealed a variety of abnormalities commonly seen in old mice. No dose-related differences in incidence or severity of these findings were seen, and no effect of test material administration was apparent. Neoplasms occurred in all groups (control and treated); no effect of test material was seen.

The above data supported a NOAEL of 10 000 ppm (equal to 1301 mg/kg bw per day for males and 1639 mg/kg bw per day for females), the highest dose tested.

The study was GLP compliant, and a QA statement was attached (Auletta, 1988).

Rats

In a chronic toxicity study, imazapyr (purity 99.5%; lot no. AC 4866-062) was administered to Sprague-Dawley CD rats (65 of each sex per group) via a diet containing 1000, 5000 or 10 000 ppm (equal to 49.9, 252.6 and 503.0 mg/kg bw per day for males and 64.2, 317.6 and 638.6 mg/kg bw per day for females, respectively) for 2 years. Control animals (65 of each sex per group) received standard laboratory diet only. At the initiation of the treatment, the animals were about 44 days old and weighed in the range of 158–221 g for males and 121–174 g for females. The animals were housed singly under controlled conditions and received standardized diet and water ad libitum. Clinical observations, body weight and feed consumption measurements were performed on all animals pretest and at selected intervals during the treatment period. Ophthalmoscopic examinations were performed on all animals at month 12 and at termination of the study. Haematology, clinical chemistry and urine analysis evaluations were performed on 10 animals of each sex per group at months 3, 6, 12 and 18 and at study termination. After approximately 12 months of treatment, 10 animals of each sex per group were sacrificed; all remaining survivors were sacrificed after 24 months of treatment. Selected organs were weighed and organ to body weight ratio and organ to brain weight ratio were calculated for all animals sacrificed after 12 months of treatment and for 10 animals of each sex per group after 24 months of treatment. Complete gross postmortem examination and histopathological evaluation of selected tissues were conducted on all animals. The experimental outlines for the laboratory studies are shown in Table 7.

Table 7. Experimental outlines for laboratory studies in a long-term study in rats

Group	Dietary concentration (ppm)	Number of animals							
		Total		Clinical laboratory studies (months 3, 6, 12, 18 and termination)		Necropsy and histopathology			
						12 months[a]		24 months[b]	
		M	F	M	F	M	F	M	F
I	0 (control)[c]	65	65	10	10	13	14	52	51
II	1 000	65	65	10	10	13	10	52	55
III	5 000	65	65	10	10	12	12	53	53
IV	10 000	65	65	10	10	13	10	52	55

F: female; M: male

[a] Includes unscheduled deaths prior to month 12.

[b] Includes unscheduled deaths between month 12 and study termination.

[c] Control animals received standard laboratory diet.

Source: Daly (1988)

There were no differences in the number of deaths of either sex among the control and treated groups. Physical observations were of the type commonly seen in laboratory rodents. There were no treatment-related ocular findings noted at the 12-month or terminal ophthalmological examination. Body weight data did not reveal any compound-related changes. Although there were slight increases (some were statistically significant) in mean feed consumption noted in all treated female groups,

generally during the 1st year, they were not considered of toxicological significance. There was no treatment-related alteration noted in the haematology, clinical chemistry or urine analysis. There were no treatment-related findings noted in the mean organ weights, organ to body weight ratios or organ to brain weight ratios of the treated male or female animals at both interim and terminal sacrifices. The results of the gross postmortem examination revealed a random distribution of gross lesions in the treated and control groups. The gross lesions were considered to be incidental changes, with no apparent relationship to the test material. Microscopically, the high-dose males (5/65, 7.69%) showed a higher incidence of C-cell carcinomas of the thyroid glands when compared with the control (1/65, 1.53%), low-dose (1/65, 1.53%) and mid-dose (1/63, 1.58%) males. Among females, one high-dose and one control rat exhibited C-cell carcinomas of the thyroid glands. The incidences of C-cell carcinoma in various groups are reproduced in Table 8. However, incidences of C-cell proliferative lesions in males showed a lack of a stepwise dose–response relationship and a lack of progression from C-cell hyperplasia to adenoma to carcinoma. None of the incidence data in the treated groups was statistically significantly different from those of the control group ($P > 0.05$). A summary of the incidences of proliferative lesions of thyroid glands in male rats is shown in Table 9. The earliest C-cell carcinomas among males were detected microscopically in a control animal at 88 weeks (spontaneous death) and in a high-dose animal at 92 weeks (spontaneous death). The latest were in a mid-dose and a high-dose animal at 106 weeks, both killed at the terminal sacrifice.

Table 8. Incidence of C-cell carcinomas

	Males				Females			
	Group I 0 ppm	Group II 1 000 ppm	Group III 5 000 ppm	Group IV 10 000 ppm	Group I 0 ppm	Group II 1 000 ppm	Group III 5 000 ppm	Group IV 10 000 ppm
No. of animal tissues examined	65	65	63	65	65	65	65	64
C-cell carcinoma number (%)	1 (1.53%)	1 (1.53%)	1 (1.58%)	5 (7.69%)	1 (1.53%)	0 (0%)	0 (0%)	1 (1.56%)

Source: Daly (1988)

Table 9. Summary of the incidence[a] of proliferative lesions of thyroid glands in male rats

	Group I 0 ppm	Group II 1 000 ppm	Group III 5 000 ppm	Group IV 10 000 ppm
No. of thyroid glands examined	65	65	63	65
C-cell hyperplasia	15 (23.10%)	8 (12.31%)	13 (20.63%)	6 (9.23%)
C-cell adenoma	2 (3.10%)	3 (4.62%)	9 (14.29%)	4 (6.15%)
C-cell carcinoma	1 (1.53%)	1 (1.53%)	1 (1.58%)	5 (7.69%)
C-cell adenoma and carcinoma combined	3 (4.62%)	4 (6.15%)	10 (15.87%)	9 (13.85%)
C-cell hyperplasia, adenoma and carcinoma combined	17 (26.15%)	12 (18.46%)	21 (33.33%)	15 (23.08%)

[a] The differences between the incidences of all groups are not statistically significant.
Source: Daly (1988)

A private consultant (Brown, 1988) who reviewed 260 male thyroid glands also confirmed that there were no significant biological differences in the incidences of proliferative C-cell lesions between the control and compound-treated male rats.

Further, the historical control data compiled during the period of 1979–1988 for male Charles River albino CD® rats at Bio/Dynamics Inc. revealed that the overall average incidence of C-cell carcinomas was 6.1%, with a range between studies of 0–22.7%; this range encompasses the incidence of carcinomas (7.69%) for the high-dose males in the 2-year study. Also, the concurrent control value for carcinomas (1/65, 1.5%) was significantly lower than the average historical control value of 6.1% for spontaneous carcinomas in male rats for the periods 1979–1988. The summary of historical control data is shown in Table 10.

Table 10. Historical control data for Charles River albino CD® (Sprague-Dawley) rats: studies terminated between 1979 and 1988 in Bio/Dynamics Inc. laboratories

Thyroid tissue findings	Incidence of finding	
	Males	Females
C-cell adenoma		
- Overall average	5.0% (134/2702)	4.9% (133/2730)
- Average range	0–20.9% (0/99–14/67)	0–15% (0/98–12/80)
C-cell carcinoma		
- Overall average	6.1% (166/2702)	5.3% (144/2730)
- Average range	0–22.7% (0/73–15/66)	0–16.4% (0/73–11/67)

Source: Bio/Dynamics Inc. Laboratories (1991)

From the literature, Suzuki, Mohr & Kemmerle (1979) reported a much higher incidence of C-cell carcinoma in Sprague-Dawley rats: 79% (33/42) in males and 49% (19/39) in females. Other studies also indicated an incidence of 16–40% C-cell carcinomas in other strains of rats, including Long-Evans, Sprague-Dawley, Wistar and wild rats (*Rattus norvegicus*) (VonSchilling, Frohberg & Oettel, 1967; Lindsay, Nichols & Chaikoff, 1968; Boorman, 1976).

In view of the above, it can be concluded that no proliferative lesions of C-cells in this study were deemed to be treatment related. None of the incident data in the treated groups was statistically significantly different from those of the control group. Further, the data do not show any stepwise dose–response relationship, progression from hyperplasia to adenoma to carcinoma or decreased latency in the development of C-cell carcinoma. Taking into account the historical control data and other factors as stated above, these lesions are not considered to be related to the test material.

There was an increased incidence of astrocytomas (a brain tumour) in high-dose male rats in comparison with the controls. No microscopic findings, neoplastic or non-neoplastic, were considered to be related to the test material.

The study was conducted in compliance with GLP, and a QA statement was attached (Daly, 1988).

The above study (Daly, 1988) was extended by a histopathological examination of the brain of male rats. A light microscopic pathological evaluation of the brains (original sections of the forebrain, midbrain and hindbrain and additional sections from the original paraffin blocks) and forebrains (additional sections from new paraffin blocks) of male rats from Groups I and IV was undertaken.

A higher incidence of primary brain tumours (astrocytomas) was observed in Group IV males receiving 10 000 ppm in the diet compared with control, low-dose or mid-dose males. The incidence in Group IV males (7.7%) was not statistically significantly increased ($P > 0.05$) compared with

control males (3.1%) and was close to or within the range of what might be expected in 2-year-old Sprague-Dawley rats, based on a present-day approach to brain tissue evaluation. However, it is noted that 14 brain sections per animal were examined histopathologically in the high-dose group and the control group, whereas 9 sections per animal were examined in the low- and mid-dose groups. There was no evidence of preneoplastic lesions, and all brain tumours tended to be well differentiated and non-expansive beyond the outer contours of the brain. There was no decreased time to the appearance of tumours in treated compared with control males. The incidences of primary brain tumours are presented in Table 11.

Table 11. Incidences of primary brain tumours in male rats

	Group I[a]	Group II[b]	Group III[b]	Group IV[a]
	0 ppm	1 000 ppm	5 000 ppm	10 000 ppm
No. of animals per group	65	65	65	65
Total no. of tumours per group	2 (3.1%)	1 (1.5%)	2 (3.1%)	5 (7.7%)
No. of astrocytomas per group	2 (3.1%)	0	1 (1.5%)	5* (7.7%)

*: $P < 0.05$ (Fischer's exact test)

[a] Fourteen brain sections per animal were examined histopathologically. Five of these sections represented two separate pieces of tissue from right and left forebrain.

[b] Nine brain sections per animal were examined histopathologically.

Source: Broxup (1992)

The incidence of astrocytoma (7.7%) in males at 10 000 ppm was slightly above the upper bound of the historical control range of benign astrocytoma (0–1.4%) in CD rats, but within the range of malignant astrocytoma (0–8.8%). No malignant astrocytoma was observed in the study. Recently, glial tumours, including astrocytoma, encountered in carcinogenicity studies have been recognized as malignant due to the difficulty in appreciating their true future biological behaviour (Kaufmann et al., 2012). Therefore, the increase was accepted to be within the historical control range of combined astrocytomas and considered not to be treatment related. One secondary tumour (pituitary adenoma) was observed in each of the control, low-dose and high-dose groups, and two were observed in the mid-dose group. Miscellaneous non-neoplastic lesions were seen. These appeared to be incidental or due to other findings (e.g. compression of the ventral diencephalon by an extracerebral lesion). In view of the above, it is concluded that a higher incidence of primary brain tumours was observed in Group IV males receiving 10 000 ppm compared with control, low-dose or mid-dose males, and there is a strong possibility that this finding is incidental. The summary of historical control data for astrocytomas in Charles River albino CD® (Sprague-Dawley) rats (studies terminated between 1979 and 1988) in Bio/Dynamics Inc. laboratories is shown in Table 12.

Table 12. Historical control data for brain astrocytomas in Charles River albino CD® (Sprague-Dawley) rats: studies terminated between 1979 and 1988 in Bio/Dynamics Inc. laboratories

Tissues/finding	Males	Females
Malignant astrocytoma		
- Overall average	1.2% (34/2769)	1.1% (32/2790)
- Average range	0–8.8% (0/80–6/68)	0–4% (0/75–2/50)
Benign astrocytoma		
- Overall average	0.04% (1/2769)	–
- Average range	0–1.4% (0/80–1/69)	–

Source: Bio/Dynamics Inc. Laboratories (1991)

This histopathological evaluation was conducted according to GLP (Broxup, 1992).

The study by Daly (1988) was further extended by a statistical analysis of adrenal medullary tumours for female rats. Statistical analysis was performed on the histopathological data for adrenal medullary neoplasms, adenoma and carcinoma (Richter, 1992). Fisher's exact test was used to compare each treatment group with the control. The incidences in several treatment groups were too small for a valid Cochran-Armitage test. Analysis was done for adenoma, carcinoma and adenoma combined with carcinoma (total medullary tumours).

The incidence of tumours evaluated is shown in Table 13.

Table 13. Adrenal medullary tumours evaluated in female rats

	0 ppm	1 000 ppm	5 000 ppm	10 000 ppm
No. examined	65	65	65	65
Adenoma	2	2	0	4
Carcinoma	0	1	0	2
Carcinoma + adenoma	2	3	0	6

Source: Richter (1992)

There was no statistically significant increase in adenomas, carcinomas or adenomas plus carcinomas in any treatment group when compared with the control group (Richter, 1992).

The chronic dietary toxicity and carcinogenicity study by Daly (1988) was further extended by a study concerning the proliferative lesions in the adrenal medulla of female rats. The criteria for diagnosis of adrenal medullary tumours were reviewed. The original incidence of these lesions as reported by the study pathologist, Dr. Saulog, is tabulated in Table 14.

Table 14. Proliferative lesions in the adrenal medulla of female rats

	0 ppm	1 000 ppm	5 000 ppm	10 000 ppm
No. examined	65	65	65	65
Hyperplasia	6	5	8	7
Adenoma	1	2	0	6
Carcinoma	0	0	0	1
Total tumours	1	2	0	7

Source: Saulog & Richter (1991)

The slides were submitted to a consulting pathologist, Dr W.R. Brown, by the sponsor. Following his review, there were some differences in diagnosis between the two pathologists. The criteria for diagnosis of adrenal medullary tumours were reviewed and agreed upon. The pathologists reached a consensus on all neoplastic diagnoses. The revised incidence of adrenal medullary proliferative lesions is shown in Table 15.

No neoplastic alterations were observed in the adrenal medulla of 5000 ppm females. The incidences of the medullary adenomas and carcinomas and medullary hyperplasias occurred sporadically in the control and treated groups. The proliferative lesions in the adrenal medulla of females are not deemed to be treatment related.

The study was conducted according to GLP (Saulog & Richter, 1991).

Table 15. Agreed proliferative lesions in the adrenal medulla of female rats (after review)

	0 ppm	1 000 ppm	5 000 ppm	10 000 ppm
No. examined	65	65	65	65
Hyperplasia	5	5	8	8
Adenoma	2	2	0	4
Carcinoma	0	1	0	2
Total tumours	2	3	0	6

Source: Saulog & Richter (1991)

In this 2-year chronic toxicity and carcinogenicity study in rats, no signs of toxicity or carcinogenicity were observed at doses up to 10 000 ppm. No compound-related tumours were observed. Hence, the NOAEL for toxicity and carcinogenicity is 10 000 ppm (equal to 503 mg/kg bw per day), the highest dose tested (Daly, 1988).

2.4 Genotoxicity

Imazapyr was tested for genotoxicity in five in vitro and four in vivo studies. In all studies, imazapyr was found to be negative. All studies complied with GLP, and QA statements were attached. On the basis of these studies, it is concluded that imazapyr is unlikely to be genotoxic. A summary of the studies described is given in Table 16.

2.5 Reproductive and developmental toxicity

(a) Multigeneration studies

In a two-generation reproductive toxicity study, imazapyr technical (purity 99.5%; lot no. AC 4866-062) was administered to groups of Sprague-Dawley (CD-CRL: COBS CD(SD) BR) rats (25 of each sex per group; 43 days old and weighing 187–240 g [males] and 128–166 g [females]) at a dietary concentration of 0, 1000, 5000 or 10 000 ppm. After the acclimatization period, F_0 parental animals continuously received the test substance throughout the entire study. Achieved intakes are shown in Table 17. The parental generation animals received a 64-day premating treatment period. On day 0 postpartum, the pups were weighed by sex in each litter of four males and four females, where possible. The F_{1a} pups were weaned on day 21 postpartum, given an external examination and killed on day 21, 22 or 23 postpartum. Any pups with external abnormalities were given an internal examination. Following weaning of the F_{1a} litters, the dams were rested for a minimum of 21 days, and then the mating procedure was repeated, to produce the F_{1b} litters. Those F_{1b} generation rats selected for adult observations were treated from weaning (21 days postpartum) for at least 78 days before placement for mating. The males were then treated after the end of the second mating period until necropsy, whereas the females were treated throughout the gestation, parturition and lactation periods. The F_2 generation pups were killed following weaning.

There were no deaths, clinical signs or pathological findings that were considered related to treatment with imazapyr.

The body weights in the treated groups of males in the F_0 generation were not significantly different from those of the controls. However, occasional statistically significant intergroup differences in body weight gains were observed, which are not attributed to treatment. In treated groups of females of the F_0 generation, neither body weight nor body weight gain differed significantly from values of the control group for the premating period or the gestation and lactation periods of both reproductive phases.

The feed intake in both sexes was unaffected by the treatment. The conception rate for the first mating period of the F_0 generation at the 10 000 ppm level was significantly ($P < 0.05$) decreased. However, taking values for the second mating period into consideration in combination

Table 16. Summary of genotoxicity studies on imazapyr

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 uvrA	Imazapyr; 0, 50, 158, 500, 1 580, 5 000 µg/plate	93	Negative (±S9)	Allen (1983)
Bacterial reverse mutation assay (Ames test)	*S. typhimurium* TA98, TA100, TA1535, TA1537; *E. coli* WP2 uvrA	Imazapyr; 0, 3.3, 10, 33, 100, 333, 1 000, 2 500, 5 000 µg/plate	99.6	Negative (±S9)	Woitkowiak (2012)
Chromosomal aberration assay in mammalian cells	CHO cells	Imazapyr; 50, 170, 500, 1 700, 5 000 µg/mL	93	Negative (±S9)	Farrow & Cortina (1984)
Unscheduled DNA synthesis	Rat hepatocytes	50, 100, 500, 1 000, 5 000 µg/mL	93	Negative	Sernau & Farrow (1984)
Forward mutation assay in mammalian cells (HPRT test)	CHO cells	Imazapyr technical; 6, 9, 12 mg/mL	93	Negative (±S9)	Johnson & Allen (1984)
In vivo					
Chromosome analysis (micronucleus test)	NMRI mouse	Imazapyr technical; 0, 500, 1 000, 2 000 mg/kg bw (two administrations)	100	Negative	Schwind & Landsiedel (2006)
Chromosome analysis (micronucleus test)	NMRI mouse	Imazapyr technical; 0, 500, 1 000, 2 000 mg/kg bw (single administration)	99.1	Negative	Honarvar (2006)
Dominant lethal assay for male fertility	Rat, Charles River CD	Imazapyr technical; 0, 125, 250, 300, 1 000, 2 000 mg/kg bw per day (administration on days 1 through 5)	93	Negative	Salamon & Enloe (1983a)
Dominant lethal assay	Rat (male), Charles River	Imazapyr technical; 0, 250, 500, 1 000 mg/kg bw per day (administration on days 1 through 5)	93	Negative	Salamon et al. (1984)

CHO: Chinese hamster ovary; DNA: deoxyribonucleic acid; HPRT: hypoxanthine–guanine phosphoribosyltransferase; S9: 9000 × *g* supernatant fraction from rat liver homogenate

with values of the first mating period indicates this not to be of toxicological significance. There was no significant difference in fertility indices, day of mating or other parameters of parental performance (Tables 18–21).

Although there were marked intergroup variations in the incidence of dead pups at birth, which on occasion attained statistical significance, there was no consistent trend indicative of a relationship with treatment. Other parameters of maternal performance, including gestational index, length of gestation, numbers of live pups at birth and sex ratio, were similar to control values.

As far as the F_{1a}, F_{1b}, F_{2a} and F_{2b} pups are concerned, the viability, survival and lactation indices were unaffected, as was the clinical condition of the pups in treated groups. On all but one

Table 17. Study design and range of weekly achieved intakes of imazapyr

Test group	Dietary concentration (ppm)	Dose per animal (mg/kg bw per day)		No. of animals assigned	
		Males	Females	Males	Females
F_0 generation					
1	0	0	0	25	25
2	1 000	43.1–117.0	67.6–131.5	25	25
3	5 000	202.1–586.2	331.4–646.9	25	25
4	10 000	379.8–1 150.6	637.0–1 284.3	25	25
F_{1b} generation					
1	0	0	0	25	25
2	1 000	48.3–142.8	80.2–149.9	25	25
3	5 000	252.8–720.8	404.7–736.1	25	25
4	10 000	483.4–1 471.8	761.3–1 537.1	25	25

Source: Robinson et al. (1987)

Table 18. Group parental performance, F_0 generation, first mating phase

	No. of males	No. of females	No. of females failing to mate	Mean no. of days to mating (SD)	No. of pregnant females	Mating index (%)	Fertility index (%)	Conception rate (%)
Group 1	25	25	2	2.70	22	92	88	95.7
0 ppm				(2.120)				
Group 2	25	25	0	3.40	21	100	84	84
1 000 ppm				(2.739)				
Group 3	25	25	0	3.61	21	100	84	84
5 000 ppm				(2.904)				
Group 4	25	25	0	2.44	18	100	72	72*
10 000 ppm				(1.044)				

SD: standard deviation; *: $P < 0.05$ (Fisher's)
Source: Robinson et al. (1987)

Table 19. Group parental performance, F_0 generation, second mating phase

	No. of males	No. of females	No. of females failing to mate	Mean no. of days to mating (SD)	No. of pregnant females	Mating index (%)	Fertility index (%)	Conception rate (%)
Group 1	25	25	2	2.86	17	92	68	73.9
0 ppm				(2.587)				
Group 2	25	25	2	2.78	21	92	84	91.3
1 000 ppm				(1.704)				
Group 3	25	25	1	2.04	18	96	72	75
5 000 ppm				(0.955)				
Group 4	25	25	1	2.54	21	96	84	87.5
10 000 ppm				(1.141)				

SD: standard deviation
Source: Robinson et al. (1987)

Table 20. Group parental performance, F_0 generation, first and second mating phases

	No. of males/females	No. of males producing at least one pregnancy	No. of females pregnant at least once	No. of males producing two pregnancies	No. of females pregnant twice
Group 1 0 ppm	25	22	22	16	16
Group 2 1 000 ppm	25	23	23	19	19
Group 3 5 000 ppm	25	22	22	16	17
Group 4 10 000 ppm	25	22	22	16	16

Source: Robinson et al. (1987)

Table 21. Group parental performance, F_{1b} generation, first and second mating phases

	No. of males/females	No. of males producing at least one pregnancy	No. of females pregnant at least once	No. of males producing two pregnancies	No. of females pregnant twice
Group 1 0 ppm	25	23	23	19	19
Group 2 1 000 ppm	25	21	22	18	18
Group 3 5 000 ppm	25	23	23	21	21
Group 4 10 000 ppm	25	22	22	17	17

Source: Robinson et al. (1987)

occasion, the pup body weights in the treated groups were not significantly different from control values. There were no pathological findings related to the treatment.

In view of the above, it can be concluded that treatment of groups of male and female rats of both the F_0 and F_{1b} generations with imazapyr at a dose of 1000, 5000 or 10 000 ppm in the diet did not cause any significant effects upon mortality, clinical condition, body weight, feed consumption or pathological status. There were also no significant adverse effects upon reproductive performance or development of the pups in the F_{1a}, F_{1b}, F_{2a} or F_{2b} generation.

Based on the results of this two-generation reproductive toxicity study with imazapyr technical in Wistar rats, the NOAEL for parental, reproductive and offspring toxicity was considered to be 10 000 ppm (equal to about 1471.8 mg/kg bw per day), the highest dose tested.

A formal GLP compliance and QA statement was included in the report (Robinson et al., 1987).

(b) Developmental toxicity

Rats

Imazapyr (purity 93%; lot no. AC 4361-97) was administered orally by gavage to groups of 25 pregnant Sprague-Dawley rats from day 6 through day 15 postcoitum. Dose levels of 100, 300 and

1000 mg/kg bw per day were administered as a test article/0.1% Tween 80 deionized water mixture at a dosing volume of 10 mL/kg bw. Dosing volumes were calculated based on each animal's body weight on gestation day 6. Dosage materials were given employing a syringe equipped with a 16 gauge ball-tipped gavage needle. Vehicle control group animals were treated in a similar manner with a 0.1% Tween 80 deionized water solution. Polygamous cohabitation among rats was initiated following completion of the quarantine period. On the following day, the females were examined for signs of copulation (determined by the presence of a copulatory plug in the vagina or sperm-positive results in the vaginal smear). Those females for which copulation was confirmed were assigned to a treatment group, and that day was taken as day 0.

The study design and pregnancy outcomes are shown in Table 22.

Table 22. Design of a developmental toxicity study in rats

Test group	Dose for females (mg/kg bw per day)	Number of bred females	Number of resulting pregnancies[a]
1	0	25	22
2	100	25	24
3	300	25	23
4	1 000	25	22

[a] Only data from gravid females were used for analysis.
Source: Salamon & Enloe (1983b)

All animals were observed at least twice daily for mortality, morbidity and overt signs of toxicity. Body weights were recorded for all females assigned to this study on gestation days 0, 6, 9, 12, 15 and 20 (final sacrifice). Feed consumption was monitored by visual observation on a daily basis. All females were sacrificed on gestation day 20. The gravid uterus was excised, weighed and examined to determine the numbers of implantation sites, resorption sites and fetuses. The number of corpora lutea was also recorded. The thoracic and abdominal organs of each female were examined for gross morphological change. Maternal tissues were retained for possible later histopathological examination.

Each fetus was removed from the chorion and examined for external developmental anomalies. Approximately two thirds of the fetuses from each litter were retained in ethyl alcohol for subsequent examination of their skeletal development. The remaining fetuses were retained in Bouin's fixative and subsequently examined for visceral development.

All statistical analyses of maternal and litter data were performed with the levels of significance at $P < 0.05$ and $P < 0.01$ using analysis of variance (ANOVA), analysis of covariance (ANCOVA) as well as the chi-squared test, Kruskal-Wallis test and Dunnett's multiple comparison test.

All females assigned to this study survived to final sacrifice. Maternal body weights and body weight gains obtained for the treated groups' dams were similar to those in the control group. Salivation, which occurred during the dosing period, was observed in 6/22 gravid females in the 1000 mg/kg bw per day group, as shown in Table 23.

This effect was observed only at variable gestation days and appeared to be a treatment-related effect. However, because the observation was transient in nature and not found in other studies as well as other doses, it was not considered to be an adverse effect. It might be a result of the high dose and the irritating effect of the compound.

No other noteworthy observations were noted for the treated dams. No gross pathological changes were noted that were considered to be a result of the maternal exposure to imazapyr. Reproductive data obtained for the treated dams were similar to those of the controls (Table 24).

Table 23 Maternal observation of salivation

Serial no.	Animal no.	Gestation day on which salivation was observed
1	AE 3673	8
2	AE 3666	9
3	AE 3752	11, 13, 15
4	AE 3653	10
5	AE 3740	13, 14
6	AE 3765	14

Source: Salamon & Enloe (1983b)

Table 24. Reproduction data in a developmental toxicity study in rats[a]

Test group dose (mg/kg bw per day)	No. of pregnant animals		No. of corpora lutea	No. of implantation sites	No. of resorptions		No. of viable fetuses	No. of dead fetuses
					Early	Late		
0	22	Mean	16.3	14.7	0.4	0.0	14.3	0.0
		SD	2.00	2.41	0.50	0.21	2.32	
100	24	Mean	17.4	15.0	0.7	0.0	14.3	0.0
		SD	2.89	3.76	0.92	0.20	3.71	
300	23	Mean	15.9	14.3	0.4	0.0	13.9	0.0
		SD	2.70	2.62	0.66	0.21	2.71	
1 000	22	Mean	14.8	13.8	0.9	0.0	12.9	0.0
		SD	3.12	3.41	1.48		3.60	

SD: standard deviation

[a] No statistically significant intergroup differences were noted.

Source: Salamon & Enloe (1983b)

Mean fetal body weight and crown–rump length data obtained for the imazapyr group fetuses were comparable to those for the control group fetuses. Examinations of the fetal external, skeletal and visceral development revealed no aberrant structural changes that appeared to be the result of maternal exposure to the test article.

Imazapyr was not found to be teratogenic with this test system/study design under the ambient conditions at this facility.

In view of the above, the NOAEL for maternal toxicity was 300 mg/kg bw per day, based on salivation observed in 6/22 gravid females at 1000 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 1000 mg/kg bw per day, the highest dose tested.

The study was GLP compliant (Salamon & Enloe, 1983b).

Rabbits

A teratology pilot study was conducted in New Zealand White albino rabbits to establish the exposure levels of imazapyr to be employed in the conduct of a teratology study using animals of the same species. Young female rabbits weighing 3.84–4.26 kg (group mean) (five per group) were treated daily by oral gavage with imazapyr (purity 93%; lot no. AC 4361-97) at 0, 250, 500, 1000 or 2000 mg/kg bw on days 6 through 18 of gestation. The test article was administered as a test article/0.1% Tween 80 deionized water mixture. Immediately after copulation, the bred doe was given

an intravenous injection of luteinizing hormone (approximately 1 mg/kg bw) in the marginal ear vein. The study design was as shown in Table 25.

Table 25. Design of a teratology pilot study in rabbits

Test group	Dose for females (mg/kg bw per day)	Number of bred females	Number of resulting pregnancies
1 (vehicle control)	0	5	4
2 (Test I)	250	5	5
3 (Test II)	500	5	3
4 (Test III)	1 000	5	5
5 (Test IV)	2 000	5	5

Source: Salamon & Enloe (1983c)

The animals were examined for mortality, signs and symptoms twice daily. Body weights were recorded on defined gestation days. Feed consumption was monitored by visual observation on a daily basis. On day 28 postcoitum, 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 were recorded. The uteri (and contents) of all pregnant females were examined to determine the numbers of implantation sites, resorption sites and fetuses. Tissues were retained in 10% neutral buffered formalin as needed to confirm gross findings.

All statistical analyses of maternal data were performed with the levels of significance at $P < 0.05$ and $P < 0.01$ using ANOVA, Kruskal-Wallis test and Dunnett's multiple comparison test.

Two of the five animals in the 250 mg/kg bw per day dose group, four of five animals in the 1000 mg/kg bw per day dose group and all five animals in the 2000 mg/kg bw per day dose group died prior to final sacrifice. Necropsy examinations of these animals revealed stomach lesions (discolorations/depressions) in four animals in the 1000 mg/kg bw per day dose group, two of which were classified by the pathologist as erosive lesions and two as gastric ulcers. Gastric mucosal changes classified by the pathologist as erosive lesions were noted for four of the five animals in the 2000 mg/kg bw per day dose group. The remaining 2000 mg/kg bw per day dose group female that died exhibited gastric and pyloric mucosal discolorations. One of the 250 mg/kg bw per day dose group females that died exhibited fluid in the trachea, and the pathologist noted chronic non-suppurative pneumonia involving 50% of the lung; the other 250 mg/kg bw per day dose group mortality exhibited pulmonary exudate and discoloration, gastric mucosal depressions and ulcers, as noted by the pathologist. Body weight data and observations noted for the treated group does that survived to final sacrifice (gestation day 28) were similar to those for the control group. The mean numbers of corpora lutea, implantation sites, resorption sites and viable fetuses obtained for the sacrificed does revealed no intergroup differences that appeared to be related to the exposure to imazapyr. Gross necropsy examinations performed upon the does that survived to final sacrifice revealed no alterations that could be correlated with the exposure to the test article.

The imazapyr exposure levels of 1000 and 2000 mg/kg bw per day resulted in maternal death. This toxicity appears to have been due to local effects of the compound on the gastrointestinal tract. There were no indications of any exaggerated pharmacological or embryocidal effects at the imazapyr exposure level of 250 or 500 mg/kg bw per day. In view of the above, the maternal NOAEL in this pilot study was 500 mg/kg bw per day, based on the maternal deaths at 1000 mg/kg bw per day and above.

The study was GLP compliant, and a QA statement was attached (Salamon & Enloe, 1983c).

In a study of developmental toxicity, groups of 18 female New Zealand White albino rabbits were given imazapyr (purity 93%; lot no. 4361-97) at a dose of 0, 25, 100 or 400 mg/kg bw per day by gavage on gestation days 6–18. The test article was administered as a test article/0.1% Tween 80 deionized water mixture orally via gavage. At the time of procurement, the rabbits were young, with a group mean weight of 3.92–4.07 kg. Each female was placed in the male's cage (1 : 1 pair) and allowed to remain until successful copulation was observed or until the female's rejection of the male was apparent. Immediately following copulation, the bred doe was given an intravenous injection of luteinizing hormone (approximately 1 mg/kg bw) in the marginal ear vein. The study design was as shown in Table 26.

Table 26. Design of a developmental toxicity study in rabbits

Test group	Dose for females (mg/kg bw per day)	Number of bred females	Number of resulting pregnancies
1 (vehicle control)	0	18	17
2 (TI)	25	18	18
3 (TII)	100	18	16
4 (TIII)	400	18	17

Source: Salamon, Stephen & Enloe (1983)

The animals were observed twice daily for mortality, morbidity and overt signs of toxicity. Body weights were recorded on defined gestation days. Feed consumption was monitored by visual observation on a daily basis. All females were killed on gestation day 28. The gravid uterus was excised, weighed and examined to determine the numbers of implantation sites, resorption sites and fetuses. The number of corpora lutea was also recorded. The thoracic and abdominal organs of each female were examined for gross morphological change. Maternal tissues were retained for possible later histopathological examination.

Each fetus was removed from the chorion and examined for external developmental anomalies. Each fetus was examined for internal development, sexed and eviscerated. The heads were removed from approximately one third of the fetuses per litter, retained in Bouin's fixative and subsequently examined. The carcasses of these fetuses as well as all remaining fetuses per litter were preserved in ethyl alcohol and subsequently examined for skeletal structure development.

All statistical analyses of maternal and litter data were performed with the levels of significance at $P < 0.05$ and $P < 0.01$ using ANOVA and ANCOVA as well as the chi-squared test, Kruskal-Wallis test and Dunnett's multiple comparison test.

Maternal body weight data obtained (0, 6, 12, 15, 18, 24 and 28 days of gestation) for the treated groups' does were comparable with those of the control does. No untoward antemortem observations were noted that could be correlated with the exposure to imazapyr. Two animals from the control group and two from the 400 mg/kg bw per day group died during the dosing period; gross pathological findings for these does were limited to pulmonary changes. At study termination, the surviving test animals were found to have a slightly higher incidence of common and expected pulmonary and hepatic changes (Table 27). This increased incidence was not judged to be test article related.

Maternal reproductive data and fetal weight and crown–rump length data obtained for the imazapyr-treated groups were comparable with those for the controls. The examinations of fetal external development revealed that one fetus in the 25 mg/kg bw per day group and four fetuses in the 400 mg/kg bw per day group exhibited anomalies. The 25 mg/kg bw per day group fetus exhibited a short tail. The anomalies noted for the 400 mg/kg bw per day group were one fetus with a kink at the tip of its tail, two fetuses from the same litter with talipes and one anurous fetus with talipes and spina bifida. The incidences of spina bifida in this study were 1/144 fetuses (0.7%) and 1/16 litters (6.3%).

This study was conducted in New Zealand White albino rabbits in 1983. Unfortunately, corresponding historical control data are not available for the laboratory where the study was conducted. However, in two sets of recent historical control data from studies conducted from 2000 to 2003, the spina bifida incidences were in the range of 0–0.5% per fetus and 0–4.2% per litter.

Table 27. Gross pathological findings of lungs and liver of rabbits that died during the dosing period and at study termination

Group/ dose (mg/kg bw per day)	Gross pathological findings of lungs[a]			Gross pathological findings of liver[a]		
	No. of dead showing lesion	No. of sacrificed showing lesion/total no. sacrificed	Findings	No. of dead showing lesion	No. of sacrified showing lesion/ total no. sacrificed	Findings
0	2	2/4	Discoloration, diffuse, red, ~50–75% involved, multiple focal	0	1/4	Discoloration, white surface (multiple focal)
TI: 25	0	4/7	Discoloration, diffuse, red, ~50–75% involved, multiple focal, consolidation	0	2/7	Discoloration, white surface (multiple focal) Exaggerated lobular pattern, diffuse, pale surface
TII: 100	0	4/6	Discoloration, diffuse, red, ~50–75% involved, multiple focal, consolidation	0	1/6	Depression, solitary, white surface, firm, ~0.2 cm
TIII: 400	2	4/7	Discoloration, diffuse, red, ~50–75% involved, multiple focal, consolidation	0	2/7	Discoloration, multiple focal / diffuse, pale, ~75–100%

[a] "Dead" refers to rabbits that died during the dosing period; "sacrificed" refers to rabbits that died at study termination.

Source: Salamon, Stephen & Enloe (1983)

In addition, the left eye of a fetus in the 25 mg/kg bw per day group appeared larger than normal; however, this eye appeared normal in size during the internal examination of the head. The remaining fetuses obtained were judged to be free of grossly apparent external anomalies. There were no consistent, deleterious findings noted during the evaluations of the fetal internal, skeletal or internal head development that were considered to be a result of exposure to imazapyr. Statistical evaluations of the fetal anomaly data revealed no statistically significant differences in the numbers of treated groups' litters with fetuses exhibiting external, internal, skeletal or internal head anomalies when compared with the controls (Table 28).

In view of the above, imazapyr was not found to be teratogenic with this test system/study design under ambient conditions. Accordingly, the NOAEL for maternal and embryo/fetal toxicity for imazapyr was 400 mg/kg bw per day, the highest dose tested.

The study was GLP compliant (Salamon, Stephen & Enloe, 1983).

Table 28. Fetal developmental findings in rabbits[a]

Findings	0 mg/kg bw per day		25 mg/kg bw per day		100 mg/kg bw per day		400 mg/kg bw per day	
	No. of fetuses	No. of litters	No. of fetuses	No. of litters	No. of fetuses	No. of litters	No. of fetuses	No. of litters
Runt[b]	1/108 (0.9%)	1/13 (7.7%)	6/152 (3.9%)	5/17 (29.4%)	3/147 (2%)	3/16 (18.8%)	3/144 (2.1%)	3/16 (18.8%)
Haematoma[b]	0/108	0/13	0/152	0/17	0/147	0/16	2/144 (1.4%)	1/16 (6.3%)
Left eye larger than normal	0/108	0/13	1/152[c] (0.7%)	1/17 (5.9%)	0/147	0/16	0/144	0/16
Short tail	0/108	0/13	1/152 (0.7%)	1/17 (5.9%)	0/147	0/16	0/144	0/16
Anurous	0/108	0/13	0/152	0/17	0/147	0/16	1/144 (0.7%)	1/16 (6.3%)
Slight kink, tip of tail	0/108	0/13	0/152	0/17	0/147	0/16	1/144 (0.7%)	1/16 (6.3%)
Spina bifida	0/108	0/13	0/152	0/17	0/147	0/16	1/144 (0.7%)	1/16 (6.3%)
Talipes								
- Both posterior appendages	0/108	0/13	0/152	0/17	0/147	0/16	1/144 (0.7%)	1/16 (6.3%)
- Both anterior appendages	0/108	0/13	0/152	0/17	0/147	0/16	2/144 (1.4%)	1/16 (6.3%)
13th rib bilateral[b]	37/108 (34.3%)	8/13 (61.5%)	51/152 (33.6%)	14/17 (82.4%)	45/147 (30.6%)	12/16 (75%)	38/144 (26.4%	15/16 (93%)
13th rib unilateral[b]	10/108 (9.3%)	6/13 (46.2%)	23/152 (33.6%)	14/17 (82.4%)	14/147 (9.5%)	8/16 (50%)	13/144 (9%)	9/16 (56.3%)
Absent cauda vertebra	0/108	0/13	0/152	0/17	0/147	0/16	1/144 (0.7%)	1/16 (6.3%)
Assymetrical ossification (sternum)	0/108	0/13	0/152	0/17	1/147 (0.7%)	1/16 (6.3%)	0/144	0/16
Dual ossification centre								
- Sternebrae	0/108	0/13	2/152 (1.3%)	2/17 (11.0%)	2/147 (1.4%)	2/16 (12.5%)	1/144 (0.7%)	1/16 (6.3%)
- Vertebrae	0/108	0/13	0/152	0/17	1/147 (0.7%)	1/16 (6.3%)	0/144	0/16
Irregular ossification pattern								
- Frontal	0/76	0/13	3/108 (2.8%)	1/17 (5.9%)	1/147 (0.7%)	1/16 (6.3%)	0/102	0/16
- Parietal	2/76 (2.6%)	2/13 (15.4%)	5/108 (4.6%)	3/17 (17.6%)	5/105 (4.8%)	4/16 (25%)	1/102 (1%)	1/16 (6.3%)

Findings	0 mg/kg bw per day		25 mg/kg bw per day		100 mg/kg bw per day		400 mg/kg bw per day	
	No. of fetuses	No. of litters	No. of fetuses	No. of litters	No. of fetuses	No. of litters	No. of fetuses	No. of litters
Misshapen vertebra	0/108	0/13	0/152	0/17	0/147	0/16	1/144 (0.7%)	1/16 (6.3%)
Misshapen sternum	0/108	0/13	0/152	0/17	1/147 (0.7%)	1/16 (6.3%)	0/144	0/16
Misshapen underdeveloped sternebra	0/108	0/13	2/152 (1.3%)	2/17 (11.8%)	2/147 (1.4%)	2/16 (12.5%)	1/144 (0.7%)	1/16 (6.3%)
Misaligned caudal vertebra	0/108	0/13	0/152	0/17	1/147 (0.7%)	1/16 (6.3%)	0/144	0/16
Misaligned compressed sacral vertebra	0/108	0/13	0/152	0/17	0/147	0/16	1/144 (0.7%)	1/16 (6.3%)
Extra thoracic vertebral body	0/108	0/13	0/152	0/17	0/147	0/16	1/144 (0.7%)	1/16 (6.3%)
Fused ribs	0/108	0/13	0/152	0/17	0/147	0/16	1/144 (0.7%)	1/16 (6.3%)
Spiny process from lumbar vertebra	0/108	0/13	0/152	0/17	1/147 (0.7%)	1/16 (6.3%)	0/144	0/16
Incompletely ossified								
- Frontal[b]	4/76 (5.3%)	1/13 (7.7%)	6/108 (5.6%)	4/17 (23.5%)	7/105 (6.7%)	4/16 (25%)	7/102 (6.9%)	5/16 (31.3%)
- Frontal and parietal[b]	0/76	0/13	2/108 (1.9%)	2/17 (11%)	4/105 (3.8%)	4/16 (25%)	3/102 (2.9%)	2/16 (12.5%)
- Interparietal[b]	0/76	0/13	2/108 (1.9%)	2/17 (11%)	1/105 (1%)	1/16 (6.3%)	0/102	0/16
- Parietal[b]	1/76 (1.3%)	1/13 (7.7%)	1/108 (0.9%)	1/17 (5.9%)	1/105 (1%)	1/16 (6.3%)	1/102 (1%)	1/16 (6.3%)
- Sternebrae[b]	108/108 (100%)	13/13 (100%)	151/152 (99.3%)	17/17 (100%)	145/147 (98.6%)	16/16 (100%)	143/144 (99.3%)	16/16 (100%)
- Vertebrae[b]	1/108 (0.9%)	1/13 (7.7%)	1/152 (0.7%)	1/17 (5.9%)	0/147	0/16	1/144 (0.7%)	1/16 (6.3%)
Non-ossified								
- Sternebrae[b]	52/108 (48.1%)	9/13 (69.2%)	62/152 (40%)	13/17 (76.5%)	52/147 (35.4%)	14/16 (87.5%)	56/144 (38.9%)	13/16 (81.3%)
- Vertebrae[b]	0/108	0/13	1/152 (0.7%)	1/17 (5.9%)	0/147	0/16	1/144 (0.7%)	1/16 (6.3%)

[a] Nine out of the 100 mg/kg bw per day group fetuses were improperly fixed, and only the head and ribs were examined for developmental alteration. Therefore, those nine fetuses were omitted from those incidences/percentages of skeletal findings that do not include the head or ribs.
[b] Considered to be incidental findings.
[c] Appeared normal during internal examination.
Source: Salamon, Stephen & Enloe (1983)

2.6 Special studies

(a) Neurotoxicity

To ascertain the possibility of acute neurotoxic effects of imazapyr, groups of 10 male and 10 female Wistar (Crl:WI(Han)) rats (aged about 49 days and weighing between 195.3 and 238.5 g for males and between 45.0 and 187.3 g for females at dosing) were exposed to imazapyr (purity 99.4%; batch no. 9129H01HA) in a single oral administration by gavage at a dose level of 0 (drinking-water containing 1% carboxymethyl cellulose served as vehicle control; test group 0), 200 (test group 1), 600 (test group 2) or 2000 mg/kg bw (test group 3). The administration volume was 10 mL/kg bw. The animals were observed up to 2 weeks after dosing. The general state of health of the rats was examined daily. Body weight determinations, functional observational batteries and motor activity measurements were carried out 7 days prior to and on the day of the administration as well as 7 and 14 days after administration. At the end of the study, five animals of each sex per test group were fixed by in situ perfusion under deep anaesthesia with isoflurane and subjected to neuropathological examinations. The remaining animals were killed using carbon dioxide without further examination.

For clinical examinations, Dunnett's test (two-sided) was used for comparison of each dose group with the control group for body weight and body weight change, and non-parametric one-way analysis using the Kruskal-Wallis test (two-sided) was used for comparison of each dose group with the control group for faeces, rearing, gripping intensity of front extremities, gripping intensity of hind extremities, foot splay test and motor activity. For neuropathology, non-parametric one-way analysis using the Kruskal-Wallis test (two-sided) was used for comparison of each dose group with the control group for weight parameters.

Clinical examinations, functional observational battery as well as motor activity measurements revealed no test substance–related effects in male and female Wistar rats on the day of test substance administration or on study days 7 and 14 at any dose level. Regarding neuropathology, brain weight determination, necropsy and neuropathology examinations by light microscopy did not reveal any treatment-related neuropathological findings.

From the above findings, it can be concluded that imazapyr when administered as a single oral gavage dose caused no test substance–related adverse effects. Therefore, under the conditions of the present study, the NOAEL was 2000 mg/kg bw, the highest dose tested, for male and female Wistar rats.

The study was GLP compliant, and a QA statement was attached (Buesen et al., 2011a).

In a subchronic neurotoxicity study in Wistar (Crl:WI(Han)) rats, imazapyr (purity 99.4%; batch N 9129HO1HA) was administered to groups of 10 rats (approximately 49 days old) of each sex per dose at a dietary concentration of 0, 100, 300 or 1000 ppm (equal to 0, 89, 272 and 924 mg/kg bw per day for males and 0, 92, 283 and 933 mg/kg bw per day for females, respectively) for at least 91 days (13 weeks). Each group was subdivided into two subsets (A and B) in order to balance the groups for functional observational battery and motor activity measurements. For functional observational battery and motor activity measurements, the animals were tested in randomized order. It was ensured that all animals were examined within the same day after start of dosing so that the time of testing for all animals remained identical. The examinations of all subsets were performed at the same time of day to avoid potential diurnal effects.

Functional observational battery and motor activity measurements were carried out prior to the test substance administration on days −7, 1, 22, 50 and 85. At the end of the study, five animals of each sex per dose group were fixed by in situ perfusion under deep anaesthesia with isoflurane and subjected to neuropathological examinations. The remaining animals were killed using carbon dioxide without further examination.

Clinical examinations, functional observational battery and motor activity measurements revealed no test substance–related neurobehavioural effects at any dose level on study days 1, 22, 50

and 85. Regarding neuropathology, brain weight determination, necropsy and neuropathology examinations by light microscopy did not reveal any treatment-related neuropathological findings.

In view of the above, imazapyr administered at dose-adjusted concentrations over a period of 3 months revealed no adverse neurobehavioural effects in Wistar rats. No test substance–related findings were observed in the neurohistopathology investigation at any dose level.

Under the conditions of this study, the NOAEL for neurotoxicity was 924 mg/kg bw per day for Wistar rats, the highest dose tested.

The study was GLP compliant, and a QA statement was attached (Buesen et al., 2011b).

(b) Immunotoxicity

To determine the immunotoxic potential of imazapyr, imazapyr (purity 99.4%; batch no. 9129H01HA) was administered to female C57BL/6 J Rj mice (49 ± 1 days old) at a dose level of 0 (control; test group 0), 500 (test group 1), 1500 (test group 2) or 5000 ppm (test group 3), equal to 0, 155, 525 and 1668 mg/kg bw per day (mean intake), respectively, via the diet over a period of 4 weeks. At the same time, a positive control group (test group 4) including eight female C57BL/6 J Rj mice received cyclophosphamide monohydrate (purity 100%; batch no. 1362353) by gavage at 10 mg/kg bw per day. All animals were immunized 6 days before blood sampling and necropsy using 0.5 mL sheep red blood cells (4×10^8 sRBC/mL) administered intraperitoneally.

Feed consumption and body weight were determined weekly. The animals were examined for signs of toxicity or mortality at least once a day. Detailed clinical examinations in an open field were conducted prior to the start of the administration period and weekly thereafter. Measurement of the sRBC immunoglobulin M (IgM) antibody titres was performed at the end of the study. Additionally, all animals were assessed by gross pathology, and the organ weights of spleen and thymus were measured.

During the clinical examinations, imazapyr did not cause signs of general systemic toxicity in any test group. No animal died prematurely in the present study. The administration of cyclophosphamide monohydrate as the positive control substance (10 mg/kg bw per day) led to expected effects on feed consumption and body weight. With regard to clinical pathology, the sRBC IgM antibody titres in female mice dosed with the test substance were not affected by treatment. In contrast, the mice dosed with the positive control compound had significantly reduced sRBC IgM antibody titres, which is indicative of immunotoxicity.

Regarding pathology, the test substance led to no pathological findings in female mice. The administration of the positive control substance (10 mg/kg bw per day) led to effects indicative of immunotoxicity (i.e. reduced absolute and relative spleen and thymus weights).

The oral administration of the positive control substance cyclophosphamide monohydrate (10 mg/kg bw per day) led to findings indicative of immunotoxicity. This was represented by lower sRBC IgM antibody titres as well as reduced spleen and thymus weights. Thus, assay sensitivity was verified in the present immunotoxicity study performed in female mice.

Under the conditions of the study, imazapyr did not reveal any signs of immunotoxicity when administered via the diet over a period of 4 weeks to female mice.

Thus, the NOAEL for general systemic toxicity as well as immunotoxicity was identified to be 5000 ppm (equal to 1668 mg/kg bw per day).

The study was GLP compliant, and a QA statement was attached (Buesen et al., 2011c).

(c) Studies on metabolites

Plant metabolism studies with imazapyr were conducted in six different crops: soya bean, maize, sugar cane, oil palm, clover and Bermuda grass. In each crop, it was found that parent imazapyr was the only major compound found. Minor metabolites, including 2,3-pyridine

dicarboxylic acid (PDC), were identified only in clover and Bermuda grass, which are consumed only by livestock. It has been shown that PDC is of comparable acute toxicity (LD_{50}) to imazapyr by the oral and dermal routes of administration. However, a greater number of clinical signs were observed post-dosing for the metabolite than for the parent. As regards skin and mucous membrane irritation, there was not much difference between parent and metabolite. Additionally, PDC is also negative in the in vivo mouse micronucleus test.

Acute toxicity

The acute toxicity of PDC is summarized in Table 29.

Table 29. Acute toxicity of imazapyr metabolite PDC

Species	Strain	Sex	Route	Batch no. / purity	LD_{50} (mg/kg bw)	Reference
Rat	Crl:CD(SD)BR	Male + female	Oral	AC 5561-131 99.1%	> 5 000[a]	Lowe (1990)
Rat	Crl:CD(SD)BR	Male + female	Oral	AC 5561-131 9.1%	> 5 000[a]	Lowe (1997)
Rabbit	New Zealand White	Male + female	Dermal	AC 5561-131 99.1%	> 2 000	Fischer (1990d)
Rabbit	New Zealand White	Male	Skin irritation	AC 5561-131 99.1%	Slightly irritating to rabbit skin	Fischer (1990e)
Rabbit	New Zealand White	Male	Eye irritation	AC 5561-131 99.1%	Irreversibly irritating to rabbit eye	Lowe (1991)

[a] Toxic signs such as decreased activity, epistaxis, diuresis, diarrhoea, urine staining, brown staining of anogenital area, anorexia, chromodacryorrhoea, bloating and piloerection were observed. There was complete recovery by day 6.

Genotoxicity

The genotoxicity of PDC is summarized in Table 30.

Table 30. Genotoxicity of imazapyr metabolite PDC

Study	Test system	Dose (mg/kg bw)	Purity (%)	Results	Reference
In vivo mouse micronucleus test	Crl:NMRI mouse, male (treated twice through oral gavage at a 24 h interval, and samples of bone marrow were taken 24 h after the last treatment; vehicle: corn oil)	500, 1 000 and 2 000	99.7	Negative ±S9	Schulz & Landsiedel (2008)

S9: 9000 × *g* supernatant fraction of rat liver homogenate

Combined repeated-dose toxicity study

To study and detect possible effects of PDC on the integrity and performance of the reproductive system of both sexes of rats, including gonadal function, mating behaviour, conception, gestation and parturition, and also to obtain information about the general toxicological profile,

including target organs and the NOAEL after repeated oral administration, a combined repeated-dose toxicity study of PDC with the reproductive/developmental toxicity screening test in Wistar rats was conducted through oral administration (gavage).

PDC (purity 99.7%; batch no. 080319) was administered orally by gavage to groups of 10 male and 10 female Wistar rats (Crl:WI(Han)), aged about 8–12 weeks (F_0 animals), at a dose level of 0 mg/kg bw per day (drinking-water containing 1% carboxymethyl cellulose served as vehicle control; test group 0), 50 mg/kg bw per day (test group 1), 250 mg/kg bw per day (test group 2) or 1000 mg/kg bw per day (test group 3) (Table 31).

Table 31. Design of a combined repeated-dose toxicity study with reproductive/developmental toxicity screening test on PDC in rats

Test group	Dose (mg/kg bw per day)	Concentration (mg/100 mL)	Dosing volume (mL/kg bw)	Number of animals	
				Males	Females
0	0	0	10	10	10
1	50	500	10	10	10
2	250	2 500	10	10	10
3	1 000	10 000	10	10	10

Source: Buesen et al. (2010)

On clinical examination, signs of general systemic toxicity were observed only in male parental animals at a dose level of 1000 mg/kg bw per day during the premating period, as there were significantly lower body weights accompanied by reduced feed consumption, but not by reduced general condition. Male animals of the 50 and 250 mg/kg bw per day test groups did not show any findings. No test substance–related findings were observed for female animals at any dose level during the entire study period. When compared with the control group, the terminal body weights of F_0 males were significantly decreased to 99%, 98% and 94% ($P < 0.01$) of the control group values at 30, 250 and 1000 mg/kg bw per day, respectively.

One female animal (No. 135) in test group 3 (1000 mg/kg bw per day) was found dead in the 2nd week of the study. This finding was considered to be incidental and not treatment related, as no pathological findings could explain the premature death. In addition, all other animals of test group 3 (1000 mg/kg bw per day) did not show any severe clinical findings.

Concerning clinical pathology, no treatment-related adverse effects were found.

During histopathological examination, male and female animals of test group 3 (1000 mg/kg bw per day) revealed a minimal to moderate increase in incidence of apoptosis in the epithelium of the caecum. In addition, male animals of test groups 2 and 3 (250 and 1000 mg/kg bw per day) showed minimal to mild desquamation of the caecal epithelial cells (Table 32). These findings were not regarded to be treatment related.

The reduction in body weight was possibly related to these caecum findings. Here, males revealed desquamation of the caecal epithelial cells. This finding could have led to disturbances in digestion and/or water retention, which, in turn, may have led to weight loss or reduced weight gain. Furthermore, the caecum of males and females of test group 3 (1000 mg/kg bw per day) revealed an increased incidence of apoptosis. This cell loss (desquamation) and programmed cell death (apoptosis) may not be regarded to be induced by the test substance and adverse in nature. Female animals exclusively showed apoptosis and no shedding/desquamation of the epithelial cells. In the thymus of some test substance–treated animals, an increase of starry sky cells (uptake of apoptotic cells/debris by macrophages) was observed. As there was no clear dose–response relationship (males: 0/2/0/3, females: 0/3/1/2), a treatment-related effect is regarded to be very unlikely.

Table 32. Histopathological findings in caecum in F_0 parental animals administered PDC

	Males				Females			
	0 mg/kg bw per day	50 mg/kg bw per day	250 mg/kg bw per day	1 000 mg/kg bw per day	0 mg/kg bw per day	50 mg/kg bw per day	250 mg/kg bw per day	1 000 mg/kg bw per day
No. of organs examined	10	10	10	10	10	10	10	10
Desquamation of epithelium	0	0	4	7	0	0	0	0
- Grade 1	–	–	4	4	–	–	–	–
- Grade 2	–	–	–	3	–	–	–	–
Apoptosis increased	0	1	0	5	0	0	0	7
- Grade 1	–	–	–	1	–	–	–	1
- Grade 2	–	1	–	3	–	–	–	5
- Grade 3	–	–	–	1	–	–	–	1

Source: Buesen et al. (2010)

The animal that was found dead (animal no. 135) revealed the most severe increase in starry sky cells in the thymus. The appearance of the starry sky cells is known to occur during involution of the thymus, starving or stress. In this animal, the increase in starry sky cells is regarded to have been due to the stress during agony.

Fertility indices for male and female animals were not impaired by test substance administration, even at a dose level of 1000 mg/kg bw per day. In addition, live birth and viability indices of pups in all test groups were not influenced.

Under the conditions of this combined repeated-dose toxicity study with the reproductive/ developmental toxicity screening test in Wistar rats, the NOAEL for systemic toxicity, reproductive performance and fertility in male and female Wistar rats was 1000 mg/kg bw per day, the highest PDC dose tested. The NOAEL for embryo and fetal toxicity was 1000 mg/kg bw per day, the highest PDC dose tested.

The study was GLP compliant, and a QA statement was attached (Buesen et al., 2010).

3. Observations in humans

3.1 Medical surveillance on manufacturing plant personnel

No information was provided.

3.2 Direct observation

No data on exposure of the general public or epidemiological studies are available from the manufacturer, nor have any epidemiological studies performed by third parties been identified.

Comments

Biochemical aspects

Imazapyr is quickly and extensively absorbed following oral administration to rats. There were no substantial sex differences in the absorption, elimination or distribution of radioactivity in rats receiving an oral dose of radiolabelled imazapyr. The majority of the administered dose was

excreted in urine (68–95%) and, to a lesser extent, in faeces (5.5–33%). Most of the elimination occurred within the first 24 hours after dosing (57–91% in urine; 3–24% in faeces). The half-life of imazapyr in the rat was less than 1 day. Imazapyr was excreted mostly unchanged. Trace levels of polar and non-polar metabolites were formed and excreted in urine and faeces. Only trace amounts of tissue residues were detected in the liver and kidneys of the high-dose group, indicating no bioaccumulation.

Toxicological data

The oral LD_{50}s were greater than or equal to 5000 mg/kg bw in rats, rabbits and dogs, but some clinical signs were noted in dogs immediately after dosing. The dermal LD_{50}s in rats and rabbits were greater than 2000 mg/kg bw. Imazapyr appears to be of low acute inhalation toxicity, but characterization by this route was limited due to the high median particle sizes. Imazapyr was irritating to the eye but not irritating to the skin in rabbits. It was not a dermal sensitizer in guinea-pigs.

Repeated-dose toxicity studies in rats and dogs indicate no effects except for reduced body weight gain in a 28-day oral toxicity study in rats, in which there was decreased body weight gain in males at the highest dose tested, 10 000 ppm (equal to 1395 mg/kg bw per day). However, in two 13-week studies in rats, no treatment-related effects were observed up to 20 000 ppm (equal to 1740 mg/kg bw per day), and in a 1-year study in dogs, up to 10 000 ppm (equal to 282.1 mg/kg bw per day).

In two long-term oral toxicity and carcinogenicity studies in mice and rats (18 months in mice, 2 years in rats), no substance-related effects were observed. In the mouse study, no treatment-related effects occurred up to the highest dose tested (10 000 ppm, equal to 1301 mg/kg bw per day). Imazapyr did not show any carcinogenic potential. In rats, the NOAEL was the highest dose tested, 10 000 ppm (equal to 503 mg/kg bw per day). No compound-related tumours were observed. Three supplementary evaluations (two histopathological and one statistical) supported the results of the 2-year rat study.

The Meeting concluded that imazapyr is not carcinogenic in mice or rats.

Imazapyr 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 imazapyr is unlikely to be genotoxic.

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

In a two-generation dietary reproductive toxicity study in rats, the NOAEL for parental and offspring toxicity was 10 000 ppm (equal to about 1471.8 mg/kg bw per day), the highest dose tested. There was also no effect on reproduction at the highest dose tested.

In a study of developmental toxicity in rats treated by gavage, salivation observed in 6/22 gravid females at 1000 mg/kg bw per day was considered to be due to the high dose and the irritating effect of the compound. The NOAEL for embryo and fetal toxicity was 1000 mg/kg bw per day, the highest dose tested.

In a pilot study for developmental toxicity in rabbits, treatment-related maternal toxicity, including death, was observed at 1000 mg/kg bw per day and above. This toxicity appears to have been due to local effects of the compound on the gastrointestinal tract. In the main study, the NOAEL for maternal and embryo/fetal toxicity was 400 mg/kg bw per day, the highest dose tested.

The Meeting concluded that imazapyr is not teratogenic in rats or rabbits.

In acute and subchronic neurotoxicity studies with imazapyr, there was no indication of neurotoxicity (including functional observational battery and motor activity measurements), with NOAELs of 2000 mg/kg bw and 924 mg/kg bw per day, respectively. Both doses were the highest doses tested.

The Meeting concluded that imazapyr is not neurotoxic.

In a 4-week dietary study in mice, no immunotoxic effects were seen up to the highest dose tested, 1668 mg/kg bw per day.

Toxicological data on metabolites and/or degradates

PDC, a minor plant metabolite of imazapyr, is of low acute toxicity by the oral (LD_{50} > 5000 mg/kg bw) and dermal (LD_{50} > 2000 mg/kg bw) routes of administration. However, a greater number of clinical signs were observed post-dosing for the metabolite than for the parent. PDC is negative in the in vivo mouse micronucleus test. In a combined repeated-dose toxicity/reproductive and developmental toxicity study, in which rats were treated by gavage from 2 weeks premating through to 1 week post-mating in males and up to day 4 of lactation in females, the NOAEL for systemic and embryo and fetal toxicity was 1000 mg/kg bw per day, the highest dose tested.

Human data

No information is available on adverse health effects or poisoning in manufacturing plant personnel or in operators and workers exposed to imazapyr.

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

Toxicological evaluation

The Meeting established an acceptable daily intake (ADI) of 0–3 mg/kg bw, derived from a NOAEL of 282 mg/kg bw per day, the highest dose tested, from the 1-year study of oral toxicity in dogs. A safety factor of 100 was applied. Although imazapyr is generally of low toxicity, the Meeting concluded that an ADI was necessary because effects were observed at high doses in the 28-day oral toxicity study in rats.

The Meeting concluded that it was not necessary to establish an acute reference dose (ARfD) for imazapyr in view of its low acute toxicity and the absence of developmental toxicity and any other toxicological effects that would be likely to be elicited by a single dose.

Levels relevant to risk assessment of imazapyr

Species	Study	Effect	NOAEL	LOAEL
Mouse	Two-year study of toxicity and carcinogenicity[a]	Toxicity	10 000 ppm, equal to 1 301 mg/kg bw per day[b]	–
		Carcinogenicity	10 000 ppm, equal to 1 301 mg/kg bw per day[b]	–
Rat	Short-term studies of toxicity[a,c]	Toxicity	5 000 ppm, equal to 675 mg/kg bw per day	10 000 ppm, equal to 1 395 mg/kg bw per day
	Two-year studies of toxicity and carcinogenicity[a,c]	Toxicity	10 000 ppm, equal to 503 mg/kg bw per day[b]	–
		Carcinogenicity	10 000 ppm, equal to 503 mg/kg bw per day[b]	–

Species	Study	Effect	NOAEL	LOAEL
	Two-generation study of reproductive toxicity[a]	Reproductive toxicity	10 000 ppm, equal to 1 471.8 mg/kg bw per day[b]	–
		Parental toxicity	10 000 ppm, equal to 1 471.8 mg/kg bw per day[b]	–
		Offspring toxicity	10 000 ppm, equal to 1 471.8 mg/kg bw per day[b]	–
	Developmental toxicity study[d]	Maternal toxicity	1 000 mg/kg bw per day[b]	–
		Embryo and fetal toxicity	1 000 mg/kg bw per day[b]	–
Rabbit	Developmental toxicity study[d]	Maternal toxicity	400 mg/kg bw per day[b,e]	–
		Embryo and fetal toxicity	400 mg/kg bw per day[b]	–
Dog	One-year study of toxicity[a]	Toxicity	10 000 ppm, equal to 282.1 mg/kg bw per day[b]	–

LOAEL: lowest-observed-adverse-effect level; NOAEL: no-observed-adverse-effect level
[a] Dietary administration.
[b] Highest dose tested.
[c] Two or more studies combined.
[d] Gavage administration.
[e] Maternal deaths occurred at higher doses in the pilot study, likely due to local effects.

Estimate of acceptable daily intake

0–3 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 imazapyr

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid; absorption is ~80%
Dermal absorption	No data
Distribution	Widely distributed
Potential for accumulation	None
Rate and extent of excretion	Rapid, 68–90% within 24 h, mainly via urine
Metabolism in animals	Minimal
Toxicologically significant compounds in animals, plants and the environment	Imazapyr

Acute toxicity	
Rat, LD_{50}, oral	> 5 000 mg/kg bw
Rat, LD_{50}, dermal	> 2 000 mg/kg bw
Rat, LC_{50}, inhalation	No reliable data
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Irritating
Dermal sensitization	Non-sensitizing (Magnusson and Kligman test)
Short-term studies of toxicity	
Target/critical effect	No effect at highest dose tested
Lowest relevant oral NOAEL	282 mg/kg bw per day, the highest dose tested (dog)
Lowest relevant dermal NOAEL	400 mg/kg bw per day, the highest dose tested (rabbit)
Lowest relevant inhalation NOAEC	No data
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	No effects at highest dose tested
Lowest relevant NOAEL	503 mg/kg bw per day, the highest dose tested (rat)
Carcinogenicity	Not carcinogenic
Genotoxicity	
	Not genotoxic
Reproductive toxicity	
Reproduction target/critical effect	No reproductive effects
Lowest relevant parental NOAEL	1 471.8 mg/kg bw per day, the highest dose tested (rat)
Lowest relevant offspring NOAEL	1 471.8 mg/kg bw per day, the highest dose tested (rat)
Lowest relevant reproductive NOAEL	1 471.8 mg/kg bw per day, the highest dose tested (rat)
Developmental toxicity	
Developmental target/critical effect	None (but maternal deaths observed in pilot study, likely due to local effects)
Lowest relevant maternal NOAEL	400 mg/kg bw per day, the highest dose tested (rabbit)
Lowest relevant embryo/fetal NOAEL	400 mg/kg bw per day, the highest dose tested (rabbit)
Neurotoxicity	
	Not neurotoxic
Other toxicological studies	
Immunotoxicity	Not immunotoxic
Medical data	
	No information on health effects in manufacturing personnel

LC_{50}: median lethal concentration; LD_{50}: median lethal dose; NOAEC: no-observed-adverse-effect concentration; NOAEL: no-observed-adverse-effect level

Summary

	Value	Study	Safety factor
ADI	0–3 mg/kg bw	One-year oral toxicity study (dogs)	100
ARfD	Unnecessary	—	—

ADI: acceptable daily intake; ARfD: acute reference dose

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