CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

Substance Name: Imidazole

EC Number: **206-019-2**

CAS Number: **288-32-4**

Index Number: **--**

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	***Imidazole***
EC number:	***206-019-2***
CAS number:	***288-32-4***
Annex VI Index number:	***NA***
Degree of purity:	***≥ 99.5 - ≤ 99.9 % (w/w)***
Impurities:	***Impurities are not considered relevant for the classification and labelling of the substance.***

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation	**Directive 67/548/EEC (Dangerous Substances Directive; DSD)**
Current entry in Annex VI, CLP Regulation	No classification	No classification
Current proposal for consideration by RAC	Classification Acute Tox. 4, H302 Skin Corr. 1C, H314 Eye damage Cat.1, H318 Developm. Repr. Cat. 1B, H360D Labelling GHS05, GHS07, GHS08 H302, H314, H360D, Dgr	Classification Repr. Cat 2; R61 Xn; R22 C; R34 Labelling T R: 61-22-34

Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Classification Acute Tox. 4, H302 Skin Corr. 1C, H314 Eye damage Cat.1, H318 Developm. Repr. Cat. 1B, H360D Labelling GHS05, GHS07, GHS08 H302, H314, H360D, Dgr	Classification Repr.Cat 2; R61 Xn; R22 C; R34 Labelling T R: 61-22-34

1.3 Proposed harmonised classification and labelling based on CLP Regulation and/or DSD criteria

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives				Reason for no classification: conclusive but not sufficient for classification
2.2.	Flammable gases				Reason for no classification: conclusive but not sufficient for classification
2.3.	Flammable aerosols				Reason for no classification: conclusive but not sufficient for classification
2.4.	Oxidising gases				Reason for no classification: conclusive but not sufficient for classification
2.5.	Gases under pressure				Reason for no classification: conclusive but not sufficient for classification
2.6.	Flammable liquids				Reason for no classification: conclusive but not sufficient for classification
2.7.	Flammable solids				Reason for no classification: conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures				Reason for no classification: conclusive but not sufficient for classification
2.9.	Pyrophoric liquids				Reason for no classification: conclusive but not sufficient for classification
2.10.	Pyrophoric solids				Reason for no classification: conclusive but not sufficient for classification
2.11.	Self-heating substances and mixtures				Reason for no classification: conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases				Reason for no classification: conclusive but not sufficient for classification
2.13.	Oxidising liquids				Reason for no classification: conclusive but not sufficient for classification
2.14.	Oxidising solids				Reason for no classification: conclusive but not sufficient for classification

2.15.	Organic peroxides				Reason for no classification: conclusive but not sufficient for classification
2.16.	Substance and mixtures corrosive to metals				Reason for no classification: conclusive but not sufficient for classification
3.1.	Acute toxicity - oral	GHS07, Acute Tox. 4, H302			
	Acute toxicity - dermal				Data lacking
	Acute toxicity - inhalation				Data lacking
3.2.	Skin corrosion / irritation	GHS05, Skin Corr. 1C, H314			
3.3.	Serious eye damage / eye irritation	GHS05, Eye Damage 1, H318			
3.4.	Respiratory sensitisation				Data lacking
3.4.	Skin sensitisation				Data lacking
3.5.	Germ cell mutagenicity				Reason for no classification: conclusive but not sufficient for classification
3.6.	Carcinogenicity				Data lacking
3.7.	Reproductive toxicity	GHS08, Repr. 1B, H360D			
3.8.	Specific target organ toxicity – single exposure				Reason for no classification: conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure				Reason for no classification: conclusive but not sufficient for classification
3.10.	Aspiration hazard				Reason for no classification: conclusive but not sufficient for classification
4.1.	Hazardous to the aquatic environment				Reason for no classification: conclusive but not sufficient for classification
5.1.	Hazardous to the ozone layer				Data lacking

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Signal word:
Danger

Pictogramms:
GSH05, GSH07, GSH08

Hazard statements:
H314: Causes severe skin burns and eye damage
H302: Harmful if swallowed
H360D: May damage the unborn child.

Precautionary statements:
No subject for Annex entry.

Proposed notes assigned to an entry: none

Table 4: Proposed classification according to DSD

Hazardous property	Proposed classification	Proposed SCLs	Current classification [1)]	Reason for no classification [2)]
Explosiveness				Reason for no classification: conclusive but not sufficient for classification
Oxidising properties				Reason for no classification: conclusive but not sufficient for classification
Flammability				Reason for no classification: conclusive but not sufficient for classification
Other physico-chemical properties				Reason for no classification: conclusive but not sufficient for classification
Thermal stability				Reason for no classification: conclusive but not sufficient for classification
Acute toxicity	Xn; R22			
Acute toxicity – irreversible damage after single exposure				Reason for no classification: conclusive but not sufficient for classification
Repeated dose toxicity				Reason for no classification: conclusive but not sufficient for classification

Irritation / Corrosion	C; R34			
Sensitisation				Data lacking
Carcinogenicity				Data lacking
Mutagenicity – Genetic toxicity				Reason for no classification: conclusive but not sufficient for classification
Toxicity to reproduction – fertility				Reason for no classification: conclusive but not sufficient for classification
Toxicity to reproduction – development	Repr. Cat. 2; R61			
Toxicity to reproduction – breastfed babies. Effects on or via lactation				Data lacking
Environment				Reason for no classification: conclusive but not sufficient for classification

[1)] Including SCLs
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Indication of danger:
T- Toxic

R-phrases:
R34 – Causes burns
R22 – Harmful if swallowed
R61 – May cause harm to the unborn child

S-phrases:
S22 – Do not breathe dust
S36/37/39 – Wear suitable protective clothing, gloves and eye/face protection
S26 – In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S45 – In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible)
S53 – Avoid exposure – obtain special instructions before use

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Imidazole has not been included in Annex I to Directive 67/548/EEC or Annex VI Tables 3.1 and 3.2 of the Regulation (EC) No. 1272/2008/EC (CLP Regulation). In October 2006, the TC C&L (the Technical Committee on Classification and Labelling of Dangerous Substances) agreed on classification for Acute toxicity (Xn; R22) and Corrosivity (C; R34) on the basis of the German classification proposal (ECBI/59/06). In September 2007, the TC C&L agreed to the classification for Repr. Cat 2; R61.

2.2 Short summary of the scientific justification for the CLH proposal

Acute toxicity

Current classification: no classification in Annex VI of CLP

Proposed classification: Acute Tox. 4 (CLP) and Xn; R22 (DSD)

Acute toxicity: oral

In an acute oral toxicity study, the LD_{50} in rats was determined to be 970 mg/kg bw (BASF SE, 1956a). Groups of up to 5 animals (sex not specified) were treated with doses of 500, 700, 1000, 1260, 2000, 4000 and 5000 mg/kg bw and were observed for 7 days after dosing. From 1260 mg/kg bw onwards, the substance was lethal to all treated animals. At 1000 mg/kg bw and 700 mg kg bw mortality was 2/5 and 1/5, respectively. Deaths occurred within one day. The symptoms were described as convulsions and disequilibria with lateral posture. Apathy and accelerated respiration was noted in survivors. There was no difference in toxicity between this test with high purity imidazole when compared to the test with 95 % imidazole (LD_{50} rat 960 mg/kg bw) which was performed under the same test conditions (BASF SE, 1956b). Based on these results, imidazole is considered to be harmful if swallowed.

Irritation/corrosion

Current classification: no classification in Annex VI of CLP

Proposed classification: Skin Corr. 1C, H314; Eye Damage 1, H318 (CLP) and C; R34 (DSD)

Skin irritation/corrosion

In a patch test, the clipped dorsal skin of six rabbits was exposed to a 2 x 2 cm patch loaded with 0.5 ml of an aqueous paste of imidazole (concentration 80 %) for 1 or 4 hours (BASF SE, 1979a). Upon removal of the patch, the treated skin area was washed with polyethylene glycol 400 and subsequently with a 1:1 mixture of polyethylene glycol 400 and water. Immediately after 4-hour exposure, the 2 exposed rabbits exhibited severe reddening of the area of exposure and beyond, accompanied by severe oedema. Soft necrosis and marked oedema were observed 24 hours after application. Mild oedema and necrosis with a parchment-like or leathery appearance were still visible at the end of the 8-day post-exposure observation period. No signs of absorptive intoxication were observed after 4 hours of exposure. Imidazole was considered corrosive based on the results obtained after 4-hours of exposure.

After the 1 hour exposure under occlusive dressing, mild erythema was seen in all (4/4) animals. Mild erythema and mild oedema were observed on the following two days of the study. The oedema resolved completely by day 8 of the post-exposure observation period. Residual signs included patchy, superficial necrotic lesions in addition to scaling. On the basis of the results obtained after 1 hour exposure, no substance specific destruction of skin tissue, namely, visible necrosis through the epidermis and into the dermis, was observed.

Serious eye damage/eye irritation

Application of 0.1 g unchanged imidazole to the rabbit's eye affected iris, conjunctiva, cornea, and the nictating membrane of the animals (BASF SE, 1979b). Grade 2 reddening and swelling of the conjunctiva was noted along with chemosis, which aggravated and persisted to grade 3 until day 8. Corneal opacity grade 2 persisted until the end of the observation period on day 8. The affected corneal area comprised more than three quarters. The observed manifestations of irreversible tissue damage and persistent large size cornea opacity indicate that imidazole is severely irritating to corrosive to the rabbit eye.

Toxicity to reproduction

Current classification: no classification in Annex VI of CLP

Proposed classification: Repr. 1B, H360D (CLP) and Repr. Cat. 2; R61 (DSD)

Developmental toxicity/teratogenicity

In a prenatal developmental study conducted in accordance with OECD TG 414, imidazole (purity 99.8 %) was administered by oral gavage to Wistar rats from day 6 to 19 of gestation (BASF SE, 2002b). The dose levels were 0 (vehicle control water), 20, 60 or 180 mg/kg bw/d. During the study, the dams were assessed for clinical observations, body weight and food consumption, and corrected body weight was determined upon necroscopy. Dams were examined for gross pathological changes, the number of corpora lutea in the ovaries, conception rate, the number of live fetuses and pre- and post-implantation losses. The fetuses were weighed, sexed and macroscopically examined for external alterations. One half of all fetuses were fixed and examined for effects on the inner organs, while the other half of fetuses were fixed and stained for skeletal and cartilage evaluation.

No signs of maternal toxicity, fetal or developmental toxicity were noted at 20 and 60 mg/kg bw per day. At 180 mg/kg bw/d a significantly reduced food intake by -13 % was noted when the treatment was started. This was reflected by a statistical significantly reduced body weight gain on gestational days 6 to 8 (-45 %) and 17 to 20 (-34 %). However, terminal body weight was comparable in all groups, and corrected terminal body weight gain was also comparable in all groups. The effect on body weight gain on gestational days 17 to 20 is due to a significant decrease of the gravid uterus weight (-26 %), high rate of resorptions and distinctly lower mean fetal body weight, rather than maternal toxicity. The number of live fetuses per litter was significantly reduced and the post-implantation loss was 43 % compared to only 8 % in the control being statistically significant. The mean fetal body weight was reduced by 14 %. Further, the incidence of external malformations (anasarca and/or cleft palate) was significantly increased. About 10 % of the high dose fetuses were affected (13/132 fetuses; in 7/22 litters) while no such changes were observed in the control. Skeletal malformations were also statistically significantly increased: 7.8 % affected fetuses per litter (7/73 fetuses in 5/21 litters) were noted in the high dose group compared to 1.1 % in the control. The incidences of shortened scapula, bent radius, bent ulna, malpositioned and bipartite

sternebrae were statistically significantly increased. Soft tissue variations (dilated renal pelvis and ureter) were significantly increased in fetuses from high dose dams compared to controls (27 % vs. 6.4 %). The incidences of skeletal variations, mainly delays of the ossification process, were statistically significantly increased from 91 % in the control group to 98.4 % in the high dose group. In historical control animals, the mean occurrence of skeletal variations is 92.6 % (range 87.0 – 98.1 %). The NOAEL for maternal toxicity, developmental toxicity and teratogenicity was 60 mg/kg bw/d. The LOAEL for maternal toxicity, developmental toxicity and teratogenicity can be set at 180 mg/kg bw/d.

In summary, it can be concluded that imidazole caused developmental toxicity and teratogenicity in in a prenatal developmental toxicity study in the rat according to OECD TG 414.

2.3 Current harmonised classification and labelling

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

No classification.

2.3.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

No classification.

2.4 Current self-classification and labelling

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

Classification

Acute Tox. 4, H302

Skin Corr. 1C, H314

Eye Damage 1, H318

Repr. 1B, H360D

Labelling

GHS05: corrosion

GHS07: exclamation mark

GHS08: health hazard

H302, H314, H360D, Dgr

2.4.2 Current self-classification and labelling based on DSD criteria

Classification

Repr. Cat 2; R61,

Xn; R22

C; R34

Labelling

T

R: 61-22-34

S: 22-26-36/37/39-45-53

A summary of the different available self-classifications from notifications under CLP can be found in the table below.

Table 5: Summary of self-classifications from Inventory notifications for the different hazard classes and categories for CAS number 288-32-4 (accessed on 07-11-2012)

Classification		**Labelling**		**Specific Concen-tration limits**	**Number of Notifiers**
Hazard Class and Category Code(s)	**Hazard Statement Code(s)**	**Hazard Statement Code(s)**	**Pictograms**		
Acute Tox 3	H311	H311	GHS06		1
Acute Tox 3	H301	H301	GHS06		3
Acute Tox 4	H312	H312	GHS07		1
Acute Tox 4	H332	H332	GHS07		1
Acute Tox 4	H302	H302	GHS07		468
Skin Corr. 1A	H314	H314	GHS05		1
Skin Corr. 1B	H314	H314	GHS05		286
Skin Corr. 1C	H314	H314	GHS05		143
Skin Irrit. 2	H315	H315	GHS07		44
Eye Dam. 1	H318	H318	GHS05		196
Eye Irrit. 2	H319	H319	GHS07		1
STOT SE 3	H336	H336	GHS07		44
Repr. 1B	H360	H360	GHS08		167
Repr. 2	H361	H361	GHS08		206
			EUH071		10
Not classified					3

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

The information available from acute toxicity testing in rats, revealing a LD_{50} value of 970 mg/kg bw, is indicating that imidazole is harmful after acute oral exposure. Imidazole was found to be corrosive to the rabbit skin and caused serious eye damage. Moreover, imidazole revealed teratogenic and developmental toxic effects in rats treated with doses of 180 mg/kg bw/d. The effects observed included reduced number of life fetuses, reduced fetal body weight, external and skeletal malformations and soft tissue variations.

Based on the results obtained from testing imidazole should be classified and labelled GHS07, Acute Tox. 4, H302; GHS05, Skin Corr. 1C, H314; Eye Damage 1, H318; and GHS08, Repr. 1B, H360D according to Regulation 1272/2008/EC (CLP) and Repr. Cat 2; R61, Xn; R22; C; R34 according to Directive 67/548/EEC (DSD). In October 2006, the TC C&L (the Technical Committee on Classification and Labelling of Dangerous Substances at the ECB) agreed on classification for Acute toxicity (Xn; R22) and Corrosivity (C; R34) on the basis of the German classification proposal (ECBI/59/06). In September 2007, the TC C&L agreed to the classification for Repr. Cat 2; R61.

As the substance requires classification and labelling due to CMR properties, action at Community level is required to ascertain a proper handling and RMMs for this substance. Further, it is recommended to harmonize not only the classification for the CMR property, but also for the hazard class Acute toxicity, Skin corrosion/irritation and Serious eye damage/eye irritation as it was noted that in the EU Classification & Labelling Inventory a wide range of different classifications for acute toxicity and irritation/corrosivity was submitted leading to uncertainty about the correct classification and labelling (see table 5). Therefore, a harmonised classification and labelling for this substance is considered a Community-wide action under Article 36 of Regulation (EC) 1272/2008/EC (CLP Regulation), and it is recommended that the classification proposal is considered for inclusion in Annex VI to CLP Regulation.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

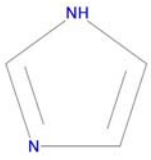
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 6: Substance identity

EC number:	206-019-2
EC name:	Imidazole
CAS number (EC inventory):	288-32-4
CAS number:	288-32-4
CAS name:	1H-Imidazole
IUPAC name:	1H-Imidazole
CLP Annex VI Index number:	--
Molecular formula:	C3H4N2
Molecular weight range:	68.0773

Structural formula:



1.2 Composition of the substance

Table 7: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
• imidazole • EC no.: 206-019-2		≥ 99.5 - ≤ 99.9 % (w/w)	

Current Annex VI entry: No classification

Table 8: Impurities (confidential information)

Impurity	Typical concentration	Concentration range	Remarks
Several		0.1 - 0.5 % (w/w)	

Table 9: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
No additives	--	--	--	--

Current Annex VI entry: Not applicable.

1.2.1 Composition of test material

Not applicable.

1.3 Physico-chemical properties

Table 10: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 1013 hPa	colorless to slightly yellow, crystalline	--	Visual inspection
Melting/freezing point	89.8 °C	BASF SE, 1987	Measured
Boiling point	268.1 °C at 1013 hPa	BASF SE, 1987	Measured
Relative density	1.11 g/cm^3 at 95 °C 1.23 g/cm^3 at 27 °C	BASF SE, 1989 Reaxys Will, 1963	Measured
Vapour pressure	0.00327 hPa at 25 °C	BASF SE, 1987	Measured
Surface tension	not surface active	--	Expert judgement
Water solubility	663 g/l at 20 °C	BASF SE, 1988	Measured
Partition coefficient n-octanol/water	log $P_{o/w}$ -0.02 at 25 °C	BASF SE, 1988	Measured
Flash point	not applicable	--	In accordance with section 1 of REACH Annex XI, the flash point does not need to be tested as the substance is a solid.
Flammability	not easily ignitable	BASF SE, 2006	Measured
Explosive properties	non explosive	BASF SE, 1974	Measured
Self-ignition temperature	480 °C	BASF SE, 1974	Measured
Oxidising properties	no oxidising properties	--	Expert judgement
Granulometry	#1 < 4 µm 0 % #2 < 10 µm 0 % #3 < 100 µm 5.1 %	BASF SE, 2010	Measured
Stability in organic solvents and identity of relevant degradation products	not applicable		The stability of the substance is not considered as critical.
Dissociation constant	7.15 at 25 °C	Serjeant EP, Dempsey B, 1979	Measured
Viscosity	not applicable		Substance is a solid at 20° C and atm. pressure.

2 MANUFACTURE AND USES

2.1 Manufacture

Confidential information.

2.2 Identified uses

Confidential information.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Based on results obtained classification and labelling for physical-chemical properties according to Regulation 1272/2008/EC (CLP) and Directive 67/548/EEC (DSD) is not justified.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

Table 11: Summary table of relevant studies on absorption, metabolism, distribution and elimination

Method	**Results**	**Remarks**	**Reference**
• Rat (Wistar) male • Oral: gavage • Doses: 16.6 mg/kg bw (single dose) • Study was performed prior to the implementation of OECD Guideline 417. • Rat plasma imidazole levels were examined after oral administration. No further pharmacokinetic parameters were studied.	• Toxicokinetic parameters: Mean plasma levels (hrs after dosing): 0.25 h: 0.13 mmol/l (8.8 mg/l) 0.50 h: 0.13 mmol/l (8.8 mg/l) 1.00 h: 0.09 mmol/l (6.1. mg/l) 2.00 h: 0.03 mmol/l (2 mg/l) 4.00 h: not detectable	• 2 (reliable with restrictions) • supporting study • experimental result • Test material (EC name): imidazole	Pagella PG et al. (1983)
• Rat (Wistar) male • Intravenous • Doses: 3 µmol/kg bw (0.204 mg/kg) • Equivalent or similar to OECD Guideline 417 (Toxicokinetics)	• Metabolites identified: yes • Details on metabolites: hydantoin, hydantoic acid	• 2 (reliable with restrictions) • supporting study • experimental result • Test material (EC name): imidazole	Ohta K et al. (1996)

4.1.2 Human information

Table 12: Summary table of relevant studies on absorption, metabolism, distribution and elimination

Method	Results	Remarks	Reference
• Human male • Oral tablet and/or drops • Exposure regime: see "details on exposure" • Doses/conc.: 750 mg of drug (containing 248 mg imidazole), or 3 times 750 mg drug/day for 3 -4 days (10 treatments) • This was a cross-over study with four different groups for tablets and drops (single and multiple dosing). Each group consisted of 18 healthy male volunteers between 18-25 years of age having within 20% of their ideal body weight. Informed written consent was given after the purpose of the study and the nature of the compound was explained.	• Toxicokinetic parameters: • C_{max}: 3.445 mg/l (mean; single dose tablets + drops) (Test No.: #1) • T_{max}: 0.75 h (mean; single dose tablets + drops) (Test No.: #1) • AUC: 14.145 mg h/l (mean; single dose tablets + drops) (Test No.: #1) • Half-life 2nd: 2.73 h (mean; single dose tablets + drops) (Test No.: #1) • C_{max}: 2.705 mg/l (mean; multiple dose tablets + drops) (Test No.: #2) • T_{max}: 0.595 h (mean; multiple dose tablets + drops) (Test No.: #2) • AUC: 8.165 mg h/l (mean; multiple dose tablets + drops) (Test No.: #2) • Half-life 2nd: 1.99 h (mean; multiple dose tablets + drops) (Test No.: #2) • Metabolites identified: yes • Details on metabolites: The metabolites hydantoin and hydantoic acid were present in plasma and urine, although below the limit of detection as • No radioactive label was used.	• 2 (reliable with restrictions) • key study • experimental result • Test material (EC name): imidazole	Kuemmerle H-P et al. (1987)

4.1.3 Summary and discussion on toxicokinetics

Human information

The pharmacokinetic profile, protein binding, relative bioavailability and metabolism of imidazole as the main component of the nonsteroidal antiinflammatory agent imidazole-2-hydroxybenzoate was studied in male subjects after single and multiple oral administration of tablets or drops. Groups of healthy male subjects (aged 18 to 25 years) of ideal body weight (within 20 %), underwent comprehensive medical, biochemical and haematological examination before and after substance

administration. They were given one 750 mg tablet (containing 750 mg imidazole-2-hydroxy-benzoate) or a single dose of 40 drops (containing a total of 400 mg imidazole-2-hydroxybenzoate). In the multiple-dose study, the subjects received three times one tablet or three times 40 drops/day for another two days starting 48 hours after the initial dose. On study day 4, only the morning dose was administered. Very large numbers of blood and urine samples were collected and comprehensive laboratory tests were performed. The maximum concentration (C_{max}) of imidazole observed after single and multiple administration of the two dosage forms (tablets and drops), the times to maximum concentration (T_{max}), and the plasma half-lives are summarised in the following table.

Table 13: Summary table of kinetic parameters after oral administration of imidazole.2-hydroxybenzoate

	Single administration		**Dose**	**Multiple administration**	
	Tablets	**Drops**		**Tablets**	**Drops**
C_{max}[1]	3.59 +/- 0.96	3.3 +/- 1.22	A	2.87 +/- 0.84	2.67 +/- 1.22
			B	3.11 +/- 0.78	2.30 +/- 0.61
T_{max}[2]	0.79 +/- 0.54	0.71 +/- 0.59	A	1.04 +/- 0.5	0.96 +/- 0.67
			B	0.68 +/- 0.51	0.51 +/- 0.52
$T_{1/2}$	2.89 +/- 1.13	2.48 +/- 1.19	A	2.85 +/- 1.25	3.47 +/- 2.64
			B	1.86 +/- 0.78	2.12 +/- 0.91

[1] µg imidazole/ml plasma

[2] time to C_{max}, in hours

A: first dose B: 10th (last) dose

The parameters clearly show that peak plasma concentrations were rapidly attained following single or multiple administration of tablets or drops, thus indicating fast absorption. Plasma levels dropped very rapidly after attainment of the peak plasma concentration. The plasma half-lives of the two dosage forms were similar and no signs of accumulation were observed. Imidazole-2-hydroxybenzoate, the originally administered salt of imidazole and salicylic acid, was not found in

the mono-drug form in either plasma or urine. Renal elimination of imidazole was approx. 10 to 15% of the dose. The protein binding of imidazole was 5 to 15 %. The metabolites hydantoin and hydantoic acid were below the level of detection as no radioactive label was used. The decrease in plasma half-life seen after multiple administrations led the investigators to assume that imidazole had an enzyme inducing effect. The relative bioavailabilities of imidazole after single and multiple administrations were calculated as 138 % and 113 %, respectively. In a pilot study imidazole-2-hydroxybenzoate was applied as a 5 % gel (82 mg imidazole in 5 g gel) to the forearm skin (area about 25 cm^2) of four male volunteers to determine possible systemic influence. Neither imidazole 2-hydroxybenzoate nor imidazole, salicylic acid or salicyluric acid were found in urine up to 12 hours after application. Plasma samples were not examined. No adverse effects were seen either locally or systemically (Kuemmerle et al., 1987).

Further, basic information on plasma half-live of imidazole in men and woman was available. The administration of 750 mg imidazole-2-hydroxybenzoate as a tablet or suppository produced respective peak imidazole plasma concentrations 3.4 +/- 0.26 and 2.78 +/- 0.25 µg/ml in 10 healthy subjects (4 men, 6 women). Maximum plasma concentrations were observed after 86.3 +/- 10.9 minutes (tablet) and 75.2 +/- 5.4 minutes (suppository). The half-lives of elimination from plasma were 1.70 +/- 0.19 hours (tablet) and 1.78 +/- 0.26 hours (suppository). Plasma samples were collected before administration and at 30, 60, 90, 120, 240, 360 and 480 minutes after administration (Noseda et al., 1988).

Non-human information

Following single oral administration of imidazole to Wistar rats (aged 2 months, n = 4-5) at 0.24 mmol/kg bw (equivalent to 16.3 mg/kg bw), plasma imidazole levels were 8.9 µg/ml after 0.25 and 0.5 hours, 6.1 µg/ml after 1 hours and 2.0 µg/ml after 2 hours. Imidazole was no longer detectable in plasma at 4 hours after administration. The limit of detection was 0.02 mmol/l (equivalent to 1.36 µg/ml; Pagella et al., 1983).

Male Wistar rats (180-200g) treated with single intravenous dose of 3 µmol (150 µCi) [2-^{14}C]-imidazole excreted 14.0 +/- 2.0 % of the radioactivity as unchanged imidazole, 38.7 +/- 0.7 % as hydantoin, 31.0 +/- 1.2 % as hydantoic acid and 4.0 +/- 0.4 % as additional, structurally unidentified metabolites in the urine within the first 24 hours after administration. Pretreatment with the cytochrome P450 inhibitor SKF525-A increased the excretion of unmetabolized imidazole while at the same time reducing hydantoin and hydantoic acid, whereas pretreatment with the cytochrome P450 inducers 3-methylcholanthrene and phenobarbitone had no significant effect on urinary metabolites. The residual radioactivity at 24 hours after administration, given as nmol equivalents based on the amount of imidazole/g tissue or per ml body fluid, was located primarily in the liver (approx. 0.35 nmol/g), kidneys (approx. 0.12 nmol/g) and aorta (approx. 0.1 nmol/g). The levels of radioactivity found in plasma, blood, heart, lung, brain, muscle skin and cartilage were all below approx. 0.03 nmol per g or ml. The fatty tissue contained no detectable radioactivity. More detailed studies of the radioactivity retained in the aortic tissue revealed that it was essentially bound to elastin and that binding was enhanced by pretreatment with SKF525-A but was not affected by 3-methylcholanthrene or phenobarbitone. In in-vitro studies, the radioactivity bound to elastin in the aortic tissue was dependent on cupro-ascorbate-catalysed reactions (Ohta et al., 1996)

Conclusion

The available pharmacokinetic studies in rat and human demonstrate that imidazole is rapidly and quantitatively absorbed after oral administration and metabolized in the liver to the main metabolites hydantoin and hydantoic acid. The half-live of elimination from human plasma was between 1.7 and 3.0 hours after a single dose. Imidazole did not accumulate in the body. Renal

excretion was the predominant route of elimination. In the rat, 88 % of the administered radioactivity was eliminated in the urine within 24 hours as imidazole (14 %), hydantoin (39 %), hydantoic acid (31 %) and unidentified metabolites (4 %). After dermal application of imidazole-2-hydroxybenzoate to human volunteers, neither the parent compound nor any metabolite was found in urine, indicating that bioavailability after dermal application is less than after oral administration.

4.2 Acute toxicity

The results of experimental studies are summarised in the following table:

Table 14: Summary table of relevant acute toxicity studies

Method	Results	Remarks	Reference
• Rat • Oral: gavage • Equivalent or similar to OECD Guideline 401 (Acute Oral Toxicity)	LD_{50}: ca. 970 mg/kg bw	• 2 (reliable with restrictions) • key study • experimental result • Test material (EC name): imidazole	BASF SE (1956a)

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

In an acute oral toxicity study, the LD_{50} in rats was determined to be 970 mg/kg bw (BASF SE, 1956a). Groups of up to 5 animals (sex not specified) were treated with doses of 500, 700, 1000, 1260, 2000, 4000 and 5000 mg/kg bw and were observed for 7 days after dosing. From 1260 mg/kg bw onwards, the substance was lethal to all treated animals. At 1000 mg/kg bw and 700 mg kg bw, mortality was 2/5 and 1/5, respectively. Deaths occurred within one day. The symptoms were described as convulsions and disequilibria with lateral posture. Apathy and accelerated respiration was noted in survivors. There was no difference in toxicity between imidazole of high purity when compared to a test with 95 % imidazole (LD_{50} rat 960 mg/kg bw) which was performed under the same test conditions (BASF SE, 1956b).

4.2.1.2 Acute toxicity: inhalation

No information available.

4.2.1.3 Acute toxicity: dermal

No information available.

4.2.1.4 Acute toxicity: other routes

No information available.

4.2.2 Human information

No information available.

4.2.3 Summary and discussion of acute toxicity

In an acute oral toxicity study, the LD_{50} in rats was determined to be 970 mg/kg bw (BASF SE, 1956a). Groups of up to 5 animals (sex not specified) were treated with doses of 500, 700, 1000, 1260, 2000, 4000 and 5000 mg/kg bw and were observed for 7 days after dosing. From 1260 mg/kg bw onwards, the substance was lethal to all treated animals. At 1000 mg/kg bw and 700 mg kg bw, mortality was 2/5 and 1/5 animals, respectively. Deaths occurred within one day and the clinical symptoms were described as convulsions and disequilibria with lateral posture. Apathy and accelerated respiration was noted in survivors. There was no difference in toxicity between imidazole of high purity when compared to a test with 95 % imidazole (LD_{50} rat 960 mg/kg bw) which was performed under the same test conditions (BASF SE, 1956b).

4.2.4 Comparison with criteria

According to the criteria of the DSD (Directive 67/548/EEC), substances should be classified as harmful (Xn) when: LD_{50}, oral, rat > 200 mg/kg ≤ 2000 mg/kg. Based on the oral LD_{50} value of 970 mg/kg obtained from testing in rats, imidazole meets the criteria to be classified Xn; R22.

According to the criteria of the CLP (Regulation 1272/2008/EC), substances should be classified as acutely toxic Category 4 when: LD_{50}, oral, rat > 200 mg/kg ≤ 2000 mg/kg. Imidazole meets the criteria to be classified for Acute toxicity in Category 4 with GHS07 according to Regulation 1272/2008/EC.

4.2.5 Conclusions on classification and labelling

Based on the results obtained in acute oral toxicity test, imidazole should be classified Xn; R22, harmful if swallowed and Category 4, H302, harmful if swallowed in accordance with Directive 67/548/EEC and Regulation 1272/2008/EC. As it has been noted that in the EU Classification & Labelling Inventory different categories for acute toxicity have been allocated (no classification, Category 4 and Category 3), a harmonised classification and labelling for this substance is considered a Community-wide action, and it is recommended that the classification proposal is considered for inclusion in Annex VI to Regulation (EC) No. 1272/2008/EC (CLP Regulation).

4.3 Specific target organ toxicity – single exposure (STOT SE)

4.3.1 Summary and discussion of Specific target organ toxicity – single exposure

In the acute study, mortality was observed from 700 mg/kg bw onwards. The observed clinical findings in animals that died and in survivors were described as convulsions and disequilibria with lateral posture. Apathy and accelerated respiration was noted in survivors. The effects are considered to be secondary due to the high, acutely toxic dosages leading also to mortality.

4.3.2 Comparison with criteria

According to the criteria of the CLP (Regulation 1272/2008/EC), Specific target organ toxicity (single exposure) is defined as specific, non lethal target organ toxicity arising from a single exposure to a substance or mixture (except acute toxicity, skin corrosion/irritation, serious eye damage/eye irritation, skin and respiratory sensitization, CMR properties, aspiration).

The effects observed in the acute toxicity study after single oral exposure to high dosages which lead also to mortality do not require a classification with regard to STOT SE.

4.3.3 Conclusions on classification and labelling

Not required.

4.4 Irritation

4.4.1 Skin irritation

4.4.1.1 Non-human information

Table 15: Summary table of relevant skin irritation studies

Method	*Results*	*Remarks*	*Reference*
• Tissue studied: skin • Rabbit (Vienna White) • Coverage: occlusive (shaved) • Equivalent or similar to OECD Guideline 404 (Acute Dermal Irritation / Corrosion)	**Corrosive** Erythema score (4h exposure), Mean of d1, 2 and 8 • 4 of max. 4 (animal #1), findings not fully reversible within 8d, comprehensive, parchment-like skin necrosis at the end of the observation period • 4 of max. 4 (animal #2), findings not fully reversible within 8d, comprehensive, leathery skin necrosis at the end of the observation period Erythema score (1h exposure): Mean of d1, 2 and 8 • 2.67 of max. 4 (animal #1) findings not fully reversible within 8d, necrotic spots on the skin surface at the end of the observation period • 0 of max. 4 (animal #2) desquamation at the end of the observation period • 1.33 of max. 4 (animal #3) necrotic spots on the skin surface at the end of the observation period • 1.67 of max. 4 (animal #4) not fully reversible within 8d, necrotic spots on the skin surface and desquamation at the end of the observation period Edema score (4h exposure): Mean of d1, 2 and 8 • 2 of max. 4 (animal #1) not fully reversible within 8d, light edema at the end of the observation period • 2.33 of max. 4 (animal #2) not fully reversible within 8d, light edema at the end of the observation period Edema score (1h exposure): • < 1 of max. 4 (animal #1) fully reversible within 8d • 0 of max. 4 (animal #2) fully reversible within 8d • 1.33 of max. 4 (animal #3) fully reversible within 8d • 1.33 of max. 4 (animal #4) fully reversible within 8d	• 2 (reliable with restrictions) • key study • experimental result • Test material (EC name): imidazole	BASF SE (1979a)

4.4.1.2 Human information

No information available.

4.4.1.3 Summary and discussion of skin irritation

Skin irritation/corrosion

In a patch test, the clipped dorsal skin of six rabbits (White Vienna) was exposed to a patch (2 cm x 2 cm) loaded with 0.5 ml of an aqueous paste of imidazole (imidazole concentration 80 %) for 1 hour (4 animals) or 4 hours (2 animals) ("corrosion test"). Upon removal of the patch, the treated skin area was washed with polyethylene glycol 400 and subsequently with a mixture of polyethylene glycol 400 and water (1:1). Immediately after 4-hour exposure, the 2 exposed rabbits exhibited severe reddening of the area of exposure and beyond, accompanied by severe oedema. Soft necrosis and marked oedema were observed 24 hours after application. Mild oedema and necrosis with a parchment-like or leathery appearance were still visible at the end of the 8-day post exposure observation period. No signs of absorptive intoxication were observed. Imidazole was considered corrosive based on the results obtained after 4 hours of exposure. After the 1 hour exposure under occlusive dressing, mild erythema was seen in all (4/4) animals. Mild erythema and mild oedema were observed on the following two days of the study. The oedema resolved completely by day 8 of the post-exposure observation period. Residual signs included patchy, superficial necrotic lesions in addition to scaling. On the basis of the results obtained after 1 hour exposure, no substance specific destruction of skin tissue, namely visible necrosis through the epidermis and into the dermis, was observed (BASF SE, 1979a).

4.4.1.4 Comparison with criteria

According to the criteria of the DSD (Directive 67/548/EEC), a substance is classified as corrosive if, when it is applied to healthy intact animal skin, it produces full thickness destruction of skin tissue on at least one animal. Risk phrase R34 "Causes burns" shall be assigned if, when applied to healthy intact animal skin, full thickness destruction of skin occurs as a result of up to 4 hours exposure, or if this result can be predicted.

According to the criteria of the CLP (Regulation 1272/2008/EC), a substance is classified as corrosive if it produces destruction of skin tissue, namely, visible necrosis through the epidermis and into the dermis, in at least 1 tested animal after exposure up to 4 hour duration. Subcategory 1C is applied where responses occur after exposures between 1 hour and 4 hours and observations up to 14 days.

The skin irritation/corrosive potential of imidazole was tested in a skin/irritation/corrosion study equivalent or similar to OECD Guideline 404. Animals were treated for 1 and 4 h with a subsequent observation period of 8 days. After treatment of 1 hour mild erythema and mild oedema were observed during the first two days but resolved until the end of the observation period. After treatment of 4 hours soft necrosis and marked oedema were observed 24 h after application. Mild oedema and necrosis with a parchment-like or leathery appearance were still visible at the end of the 8-day post exposure observation period.

In conclusion, imidazole meets the criteria to be classified C; R34 according to Directive 67/548/EEC and GHS05, skin corrosive Category 1C, H314 according to Regulation 1272/2008/EC.

4.4.1.5 Conclusions on classification and labelling

Based on the results obtained in animal tests, imidazole was classified C; R34 (causes burns) and Skin corrosive Category 1C, H314 (causes severe skin burns and eye damage) in accordance with Directive 67/548/EEC and Regulation 1272/2008/EC. As it has been noted that in the EU Classification & Labelling Inventory different hazard categories for irritation/corrosivity have been allocated (no classification, Skin Corr. 1A, 1B, 1C and Skin Irrit. 2, respectively), a harmonised classification and labelling for this substance is considered a Community-wide action and it is recommended that the classification proposal recommended that the classification proposal is considered for inclusion in Annex VI to Regulation (EC) No. 1272/2008/EC (CLP Regulation).

4.4.2 Eye irritation

4.4.2.1 Non-human information

4.4.2.2 Table 16: Summary table of the eye irritation study

Method	Results	Remarks	Reference
• Tissue studied: eye • Rabbit (Vienna White) • Equivalent or similar to OECD Guideline 405 (Acute Eye Irritation / Corrosion)	**Category 1** (irreversible effects on the eye) (based on Regulation 1272/2008/EC) Cornea score: Time point: 24, 48 and 72 hours after substance instillation • 2 of max. 4 (animal #1) not fully reversible within 8d • 2 of max. 4 (animal #2) not fully reversible within 8d • 2 of max. 4 (animal #3) not fully reversible within 8d Iris score: Time point: 24, 48 and 72 hours after substance instillation • 1 of max. 2 (animal #1) (not fully reversible within: 8d) • 1 of max. 2 (animal #2) (not fully reversible within: 8d) • 1 of max. 2 (animal #3) not fully reversible within 8d Conjunctivae score: Time point: 24, 48 and 72 hours after substance instillation • 2 of max. 3 (animal #1) not fully reversible within 8d • 2 of max. 3 (animal #2) not fully reversible within 8d • 2 of max. 3 (animal #3) not fully reversible within 8d Chemosis score: Time point: 24, 48 and 72 hours after substance instillation • 2 of max. 4 (animal #1) not fully reversible within 8d • 2 of max. 4 (animal #2) not fully reversible within	• 2 (reliable with restrictions) • key study • experimental result • Test material (EC name): imidazole	BASF SE (1979b)

	8d • 2 of max. 4 (animal #3) not fully reversible within 8d Secretion: Time point: 24, 48 and 72 hours after substance instillation • 2.33 of max. 3 (animal #1) not fully reversible within 8d • 1.33 of max. 3 (animal #2) not fully reversible within 8d • 3 of max. 3 (animal #3) not fully reversible within: 8d		

4.4.2.3 Human information

No information available.

4.4.2.4 Summary and discussion of eye irritation

Eye irritation/corrosion

Application of 0.1 g unchanged imidazole (purity 99 %) to the rabbit eye (BASF SE, 1979b) affected iris, conjunctiva, cornea, and the nictating membrane of the animals. Grade 2 reddening and swelling of the conjunctiva was noted along with chemosis which aggravated and persisted to grade 3 until day 8. Corneal opacity of grade 2 persisted until the end of the observation period on day 8. The affected corneal area comprised more than 3/4. The observed manifestations of irreversible tissue damage and persistent large size cornea opacity indicate that imidazole is severely irritating to corrosive to the rabbit eye.

4.4.2.5 Comparison with criteria

According to the criteria of the DSD (Directive 67/548/EEC), a substance is assigned R41 “Risk of serious damage to eyes” if it produces severe ocular lesions which occur within 72 hours after exposure and which persist for at least 24 hours. Ocular lesions are also severe when they are still present at the end of the observation time. In addition, when a substance is classified as corrosive and assigned R34 or 35, the risk of severe damage to eyes is considered implicit and R41 is not included in the label.

According to the criteria of the CLP (Regulation 1272/2008/EC), serious eye damage means the production of tissue damage in the eye which is not fully reversible within 21 days of application.

The eye irritation/corrosive potential of imidazole was tested in an eye irritation/corrosion study equivalent or similar to OECD Guideline 405. Application of 0.1 g unchanged imidazole to the rabbit's eye affected iris, conjunctiva, cornea, and the nictating membrane of the animals. The substance caused irreversible tissue damage and persistent large size cornea opacity.

In conclusion, imidazole bears the risk of severe damage to eyes according to the criteria in Directive 67/548/EEC and is classified in Category 1 (irreversible effects on the eye) according to Regulation 1272/2008/EC.

4.4.2.6 Conclusions on classification and labelling

Based on the results on animal tests, imidazole bears the risk of severe damage to eyes according to the criteria in Directive 67/548/EEC and is classified in Category 1 (irreversible effects on the eye) according to Regulation 1272/2008/EC. As the substance is also corrosive to the skin, it is assigned the symbol "C" and the risk phrase R34 (causes burns) according to DSD and the pictogram GHS05 and the hazard statement H314 (causes severe skin burns and eye damage) according to Regulation 1272/2008/EC.

As it has been noted that in the EU Classification & Labelling Inventory different categories for Serious eye damage/eye irritation were allocated (no classification, Eye Dam. 1, Eye Irrit. 2), a harmonised classification and labelling for this substance is considered a Community-wide action and it is recommended that the classification proposal is recommended that the classification proposal is considered for inclusion in Annex VI to Regulation (EC) No. 1272/2008/EC (CLP Regulation).

4.4.3 Respiratory tract irritation

4.4.3.1 Non-human information

No information available.

4.4.3.2 Human information

No indication that would require classification and labelling with regard to this endpoint.

4.4.3.3 Summary and discussion of respiratory tract irritation

No information available.

4.4.3.4 Comparison with criteria

Not applicable.

4.4.3.5 Conclusions on classification and labelling

Not required.

4.5 Corrosivity

4.5.1 Non-human information

Available studies indicating corrosivity to the skin are summarised in section 4.4.1 Skin irritation.

4.5.2 Human information

No information available.

4.5.3 Summary and discussion of corrosivity

See summary and discussion on irritation in section 4.4.1.3

4.5.4 Comparison with criteria

According to the criteria of the DSD (Directive 67/548/EEC), a substance is classified as corrosive if, when it is applied to healthy intact animal skin, it produces full thickness destruction of skin tissue on at least one animal. Risk phrase R34 “Causes burns” shall be assigned if, when applied to healthy intact animal skin, full thickness destruction of skin occurs as a result of up to 4 hours exposure, or if this result can be predicted.

According to the criteria of the CLP (Regulation 1272/2008/EC), a substance is classified as corrosive if it produces destruction of skin tissue, namely, visible necrosis through the epidermis and into the dermis, in at least 1 tested animal after exposure up to 4 hour duration. Subcategory 1C is applied where responses occur after exposures between 1 hour and 4 hours and observations up to 14 days.

The skin irritation/corrosive potential of imidazole was tested in a skin/irritation/corrosion study equivalent or similar to OECD Guideline 404. Animals were treated for 1 and 4 h with a subsequent observation period of 8 days. After treatment of 1 hour mild erythema and mild oedema were observed during the first two days but resolved until the end of the observation period. After treatment of 4 hours soft necrosis and marked oedema were observed 24 h after application. Mild oedema and necrosis with a parchment-like or leathery appearance were still visible at the end of the 8-day post exposure observation period.

In conclusion, imidazole meets the criteria to be classified C; R34 according to Directive 67/548/EEC and GHS05, skin corrosive Category 1C, H314 according to Regulation 1272/2008/EC.

4.5.5 Conclusions on classification and labelling

Based on the results obtained in animal tests, imidazole was classified C; R34 (causes burns) and Category 1C, H314 (causes severe skin burns and eye damage) in accordance with Directive 67/548/EEC and Regulation 1272/2008/EC. As it has been noted that in the EU Classification & Labelling Inventory different hazard categories for irritation/corrosivity have been allocated (no classification, Skin Corr. 1A, 1B, 1C and Skin Irrit. 2, respectively), a harmonised classification and labelling for this substance is considered a Community-wide action and it is recommended that the classification proposal recommended that the classification proposal is considered for inclusion in Annex VI to Regulation (EC) No. 1272/2008/EC (CLP Regulation).

4.6 Sensitisation

4.6.1 Skin sensitisation

No information available.

4.6.1.1 Non-human information

No information available.

4.6.1.2 Human information

No information available.

4.6.1.3 Summary and discussion of skin sensitisation

No information available.

4.6.1.4 Comparison with criteria

Not applicable.

4.6.1.5 Conclusions on classification and labelling

Not applicable.

4.6.2 Respiratory sensitisation

4.6.2.1 Non-human information

No information available.

4.6.2.2 Human information

No information available.

4.6.2.3 Summary and discussion of respiratory sensitisation

No information available.

4.6.2.4 Comparison with criteria

Not applicable.

4.6.2.5 Conclusions on classification and labelling

Not required.

4.7 Repeated dose toxicity

The results of experimental studies are summarised in the following table:

Table 17: Summary table of relevant repeated dose toxicity studies

Method	Results	Remarks	Reference
• Rat (Wistar) male/female • Subchronic (oral: gavage) • 0, 20, 60, 180 mg/kg bw/d (actual ingested) • Exposure: 90 days Test substance was administered daily by gavage using 3 and 5 ml syringes for about 13 weeks. • OECD Guideline 408 (Repeated Dose 90-Day Oral Toxicity in Rodents)	• NOAEL: 60 mg/kg bw/d nominal, males/females • LOAEL: 180 mg/kg bw/d nominal, males/females • treatment related adverse effects at 180 mg/kg bw/d; lesions identified liver (slight centrilobular liver cell hypertrophy) and kidney as the target organs (alpha 2-microglobulin accumulation)	• 1 (reliable without restriction) • key study • experimental result • Test material (EC name): imidazole	• BASF SE (2002a) • BASF SE (2004)
• rat (Sprague-Dawley) male/female • subacute (oral: gavage) • 0; 62.5; 125; 250; 500 mg/kg bw/d • Exposure: 28 days (5x/wk)	NOAEL: 62.5 mg/kg bw/d (nominal) (male/female)	• 2 (reliable with restrictions) • supporting study • Test material (EC name): imidazole	BASF SE (1976)

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

Subchronic effects of imidazole were investigated in a 90-day study in Wistar rats according to OECD TG 408. This study examined the systemic and specific organ toxicity, ophthalmologic effects, effects on male and female reproductive organs, and effects on behaviour and sensomotoric capabilities which were examined in a series of tests delineated as the Functional Observation Battery (FOB). Imidazole was given daily by gavage dissolved in water at 20, 60 and 180 mg/kg bw/d. Liver and the male kidney were identified as target organs in the animal groups receiving 180 mg/kg bw/d as substantiated by significantly increased relative liver weights in males (+7.5 %) and females (+2.6 %) which correlated with minimal to slight centrilobular liver cell hypertrophy in males (9/10 animals affected) and females (2/10). In the kidneys, the absolute and relative weights in high-dose males were significantly increased which was accompanied by an accumulation of alpha 2-microglobulin in the epithelia and lumina of the proximal tubules of the male rat renal cortex. The alpha 2-microglobulin was detected by Mallory Heidenhain staining technique and specificity for alpha 2-microglobulin could be demonstrated by immunohistochemical staining (BASF SE, 2004). The accumulation of this protein appears to be a unique feature of male rats and is not known to occur in other species, including man. Additionally, significant changes in parameters of blood chemistry were noted in high dose animals as substantiated by decreased serum globulin and chloride in male rats, and total protein, albumin, globulin, and chloride in females. No

other substance-related effect was noted in the 90-day study at 180 mg/kg bw/d; i. e. mortality, clinical observation for signs of toxicity, body weight, body weight development and food consumption, clinical chemistry other than noted above, pathology and histopathology of the numerous organs examined were not affected. Also, no effects were noted during ophthalmologic examinations or the FOB tests. Male and female reproductive organs were not affected (including histopathology), as were sperm quality parameters (sperm number, motility, and morphology were determined in testis, epididymides and estrus cycle). No substance-related effect was noted at the intermediate and at the low dose level. Therefore, the no observed adverse effect level (NOAEL) was 60 mg/kg bw per day in both sexes under the conditions of this study (BASF SE, 2002a). The lowest observed adverse effect level (LOAEL) can be set at 180 mg/kg bw/d for males and females based on the findings in the liver (minimal to slight centrilobular hypertrophy) of both sexes and the kidney effects (alpha 2-microglobulin accumulation) in males.

In addition to the liver and the kidney, red blood cells were identified as a target in a 4-week rat study (oral gavage, groups at 0, 62.5, 125, 250, and 500 mg/kg bw per day) in Sprague Dawley rats when hemoglobin was significantly decreased in females at a dose of 125 mg/kg bw per day and above. Hematocrit and the numbers of erythrocytes were also significantly decreased ($p < 0.05$) in females at a dose of 250 mg/kg bw per day and above. In male rats hemoglobin and hematocrit were significantly reduced only at the high dose (BASF SE, 1976). The effect on red blood cells was, however, not confirmed in the more recent 90-day guideline study described above when rats received up to 180 mg/kg bw per day.

4.7.1.2 Repeated dose toxicity: inhalation

No data available.

4.7.1.3 Repeated dose toxicity: dermal

No data available.

4.7.1.4 Repeated dose toxicity: other routes

No data available.

4.7.1.5 Human information

No information available.

4.7.1.6 Other relevant information

No information available.

4.7.1.7 Summary and discussion of repeated dose toxicity

Oral route:

Subchronic effects of imidazole were investigated in a 90-day study in Wistar rats according to OECD TG 408. This study examined systemic and specific organ toxicity, ophthalmologic effects, effects on male and female reproductive organs, and effects on behaviour and sensomotoric capabilities which were examined in a Functional Observation Battery (FOB). Imidazole was given daily by gavage at 20, 60 and 180 mg/kg bw/d. Liver and the male kidney were identified as target

organs in the animal groups receiving 180 mg/kg bw/d as substantiated by significantly increased relative liver weights in males (+7.5 %) and females (+2.6 %) which correlated with minimal to slight centrilobular liver cell hypertrophy in males (9/10 animals affected) and females (2/10). In the kidneys, the absolute and relative weights in high-dose males were significantly increased which was accompanied by an accumulation of alpha 2-microglobulin in the epithelia and lumina of the proximal tubules of the male rat renal cortex. The alpha 2-microglobulin was detected by Mallory Heidenhain staining technique and specificity could be demonstrated by immune-histochemical staining (BASF SE, 2004). The accumulation of this protein appears to be a unique feature of male rats and is not known to occur in other species, including man. Additionally, significant changes in parameters of blood chemistry were noted in high dose animals as substantiated by decreased serum globulin and chloride in male rats, and total protein, albumin, globulin, and chloride in females. No other substance-related effects were observed. In particular, no effects were noted during ophthalmologic examinations or the FOB tests. Male and female reproductive organs were not affected as shown by histopathology, as were sperm quality parameters and estrus cycle unchanged. No substance-related effect was noted at the intermediate and at the low dose level. Therefore, the no observed adverse effect level (NOAEL) was 60 mg/kg bw/d in both sexes and the LOAEL was 180 mg/kg bw/d under the conditions of this study (BASF SE, 2002a).

In addition to the liver and the kidney, red blood cells were identified as a target in a 4-week rat study (oral gavage, groups at 0, 62.5, 125, 250, and 500 mg/kg bw per day) in Sprague Dawley rats when hemoglobin was significantly decreased in females at a dose of 125 mg/kg bw per day and above. Hematocrit and the numbers of erythrocytes were also significantly decreased in females at a dose of 250 mg/kg bw per day and above. In male rats hemoglobin and hematocrit were significantly reduced only at the high dose (BASF SE, 1976). The effect on red blood cells was, however, not confirmed in the more recent 90-day guideline study described above when rats received up to 180 mg/kg bw per day.

4.7.1.8 Summary and discussion of repeated dose toxicity findings relevant for classification according to DSD

The administration of imidazole to rats by gavage for 90 days caused treatment-related findings at the highest dose level only (180 mg/kg bw/d), the lesions identifying the liver (minimal to slight centrilobular liver cell hypertrophy) and kidneys (alpha 2-microglobulin accumulation) as the target organs. The NOAEL was 60 mg/kg bw/d and is, thus, comparable with the NOAEL found in the 28-day rat study using the same route of administration.

4.7.1.9 Comparison with criteria of repeated dose toxicity findings relevant for classification according to DSD

The cut off-value for R48/22 is 50 mg/kg bw/d in the DSD (in a 90-day repeated dose study). Regarding the dose levels leading to toxicity in a reliable oral 90-day study (LOAEL 180 mg/kg bw/d) as well as the quality of findings it can be concluded that imidazole is not subject to classification for repeated dose or specific target organ toxicity according to Directive 67/548/EEC.

4.7.1.10 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification according to DSD

Based on the available data imidazole is not subject to classification for repeated dose or specific target organ toxicity according to Directive 67/548/EEC.

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

The administration of imidazole to rats by gavage for 90-days caused only minor treatment-related findings at the highest dose level (180 mg/kg bw/d) tested, the lesions identifying the liver and kidneys as the target organs with at least some effects considered as rat specific lesions. The NOAEL was 60 mg/kg bw/d and is thus comparable with the NOAEL found in the 28-day rat study using the same route of administration.

4.8.2 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

The cut-off values for STOT RE Cat. 2 are $10 < C \leq 100$ mg/kg bw/d in the CLP Regulation (in a 90-day oral repeated dose study). Regarding the dose levels leading to toxicity in a reliable oral 90-day study as well as the quality of findings it can be concluded that imidazole is not subject to classification for repeated dose or specific target organ toxicity according to Regulation 1272/2008/EC.

4.8.3 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

Based on the available data imidazole is not subject to classification for repeated dose specific target organ toxicity according to Regulation 1272/2008/EC.

4.9 Germ cell mutagenicity (Mutagenicity)

The results of experimental studies are summarised in the following table:

Table 18: Summary table of relevant in vitro and in vivo mutagenicity studies

Method	Results	Remarks	Reference
Point mutation assay • Bacterial reverse mutation assay (Ames test) • Salmonella typhimurium TA1535, TA100, TA1537, TA98 • Metabolic activation: with and without • Doses 1st experiment: Standard plate test: 0, 20, 100, 500, 2500, 5000 µg/plate tested with all tester strains. Solvent was aqua dest. 2nd experiment: Preincubation test: 0, 20, 100, 500, 2500, 5000 µg/plate tested with all tester strains. • 3 plates per dose and control. • OECD Guideline 471 (Bacterial Reverse Mutation Assay) (adopted 26-May-1983)	• negative for all S. typhimurium strains tested with and without metabolic activation	• 1 (reliable without restriction) • key study • experimental result • Test material (EC name): imidazole	BASF SE (1992)
Point mutation assay • Bacterial reverse mutation assay (Ames test) • S. typhimurium tester strains TA97, TA98, TA100, TA102 • Metabolic activation: with and without • Doses: 0.625, 1.25, 2.5, 5, 10 mg/plate • Equivalent or similar to OECD Guideline 471 (Bacterial Reverse Mutation Assay)	• negative for all tested S. typhimurium strains with and without metabolic activation	• 2 (reliable with restrictions) • supporting study • Test material (EC name): imidazole	Forster R et al. (1992)
Point mutation assay • Mammalian cell gene mutation assay • Chinese hamster lung fibroblasts (V79) • Metabolic activation: with and without • Doses: Experiment 1 (4h treatment) without S9 mix: 21.9, 43.8, 87.5, 175.0, 350.0, 700.0 µg/mL with S9 mix: 21 .9, 43.8, 87.5,	• negative • Test results: • negative for Chinese hamster lung fibroblasts (V79) with and without metabolic activation; cytotoxicity: not observed (up to 10 mM)	• 1 (reliable without restriction) • key study • experimental result • Test material (EC name): imidazole	HARLAN (2010)

175.0, 350.0, 700.0 µg/mL Experiment 2 (24h and 4h treatment) without S9 mix: 21.9, 43.8, 87.5, 175.0, 350.0, 700.0 µg/mL (24h) with S9 mix: 43.8, 87.5, 175.0, 350.0, 525.0, 700.0 µg/mL (4h) 700.0 µg/mL is equivalent to the limit concentration of 10 mM test substance • OECD Guideline 476 (In vitro Mammalian Cell Gene Mutation Test)			
DNA damage and repair assay • Unscheduled DNA synthesis in mammalian cells in vitro • Rat hepatocytes • Doses: 0.25, 0.5, 1, 2, 4 mg/ml • Equivalent or similar to OECD Guideline 482 (Genetic Toxicology: DNA Damage and Repair, Unscheduled DNA Synthesis in Mammalian Cells In Vitro)	• negative • cytotoxicity at approx. 1 mg/ml 50 % cell survival	• 2 (reliable with restrictions) • key study • experimental result • Test material (EC name): imidazole	Forster R et al. (1992)
In vivo Micronucleus assay • Male/female mouse (NMRI) • Oral: gavage • 500, 1000, 2000 mg/kg bw (suspended in 10 ml olive oil/kg bw) (nominal conc.) • OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test)	• negative (male/female); • clinical signs of systemic toxicity were present at all dose levels	• 1 (reliable without restriction) • key study • experimental result • Test material (EC name): imidazole	BASF SE (1993)

4.9.1 Non-human information

4.9.1.1 *In vitro* data

Imidazole was tested in the standard Ames test and in the pre-incubation Ames test conducted under GLP and according to the OECD TG 471. The substance was tested with *Salmonella typhimurium* TA 1535, TA 100, TA 1537, and TA 98 both in the presence and absence of metabolic activation in concentrations up to 5000 µg/plate. No mutagenic or bacteriotoxic effect was noted up to the limit test concentration (BASF SE, 1992).

Furthermore, imidazole and its metabolites hydantoin, hydantoic acid, and N-acetyl-imidazole were also negative in a standard plate Ames test equivalent to the OECD TG 471 with *S. typhimurium*

TA 97, TA 98, TA 100, and TA 102 in the presence and absence of metabolic activation. Test substance concentrations were up to and including 10 000 µg/plate without reaching cytotoxicity (Forster et al., 1992).

In addition, a mammalian gene mutation test according to OECD 476 and GLP in V79 Chinese hamster cells (HPRT locus) was conducted with imidazole. The assay was performed in two independent experiments, using two parallel cultures each. The first main experiment was performed with and without liver microsomal activation and a treatment period of 4 hours. The second experiment was performed with a treatment time of 4 hours with and 24 hours without metabolic activation. The maximum concentration was 700.0 µg/ml, corresponding to a molar concentration of about 10 mM of the test item. 7,12-dimethylbenz(a) anthracene (DMBA) and Ethylmethane sulfonate (EMS) were used as positive controls in experiment with and without metabolic activation, respectively. Positive as well as negative controls gave expected results. Imidazole did not induce gene mutations at the HPRT locus in V79 cells. Therefore, imidazole is considered to be non-mutagenic in this HPRT assay (Harlan, 2010).

Imidazole did not induce Unscheduled DNA Synthesis (UDS) in rat primary hepatocytes. The test method used was equivalent to the OECD TG 482. The test substance concentrations (0.25, 0.5, 1, 2, 4 mg/ml) reached the cytotoxic concentration range. Cell survival was 50 % at 1 mg/ml (Forster et al., 1992).

4.9.1.2 *In vivo* data

Imidazole hydrochloride was tested in a micronucleus test in accordance with the OECD TG 474 under GLP conditions in mice, dosed once by gavage with 500, 1000, and 2000 mg/kg bw/d. The salt imidazole hydrochloride dissociates into protonated imidazole and chloride in the stomach following oral gavage and did not induce micronuclei at any dose or any harvesting time, which were set at 16, 24, and 48 hrs after dosing. The animals showed signs of systemic toxicity at 500 mg/kg bw and above confirming the systemic availability of the test item which is in line with the toxicokinetic data. The number of polychromatic and normochromatic erythrocytes was not statistical significantly different from the control, therefore it may be concluded, that imidazole was not toxic to the bone marrow. Imidazole was found to be not clastogenic or aneugenic in this test (BASF SE, 1993).

4.9.2 Human information

No information available.

4.9.3 Other relevant information

No information available.

4.9.4 Summary and discussion of mutagenicity

1.) *In vitro* studies:

Imidazole was tested in the standard Ames test and in the pre-incubation Ames test conducted under GLP and according to the OECD TG 471. The substance was tested with *Salmonella typhimurium* TA 1535, TA 100, TA 1537, and TA 98 both in the presence and absence of metabolic activation in concentrations up to 5000 µg/plate. No mutagenic or bacteriotoxic effect was noted (BASF SE, 1992).

Furthermore, imidazole and its metabolites hydantoin, hydantoic acid, and N-acetyl-imidazole were also negative in a standard-plate Ames-test equivalent to the OECD TG 471 with *S. typhimurium* TA 97, TA 98, TA 100, and TA 102 in the presence and absence of metabolic activation. Test substance concentrations were up to and including 10 000 µg/plate without reaching cytotoxicity (Forster et al., 1992).

In addition, a mammalian gene mutation test according to OECD 476 and GLP in V79 Chinese hamster cells (HPRT locus) was conducted with imidazole. The first main experiment was performed with and without liver microsomal activation and a treatment period of 4 hours. The second experiment was performed with a treatment time of 4 hours with and 24 hours without metabolic activation. The maximum concentration was 700.0 µg/mL,corresponding to a molar concentration of about 10 mM of the test item. Imidazole did not induce gene mutations and was considered to be non-mutagenic in the HPRT assay (Harlan, 2010).

Imidazole did not induce Unscheduled DNA Synthesis (UDS) in rat primary hepatocytes. The test method used was equivalent to the OECD TG 482. The test substance concentrations (0.25, 0.5, 1, 2, 4 mg/ml) reached the cytotoxic concentration range. Cell survival was 50 % at 1 mg/ml (Forster et al., 1992).

2.) *In vivo* study:

Imidazole hydrochloride was tested in a micronucleus test in accordance with the OECD TG 474 under GLP conditions in mice, dosed once by gavage with 500, 1000, and 2000 mg/kg bw/d. The substance did not induce micronuclei at any dose or any harvesting time at 16, 24, and 48 hrs after dosing. The animals showed signs of toxicity at 500 mg/kg bw and above. The number of polychromatic and normochromatic erythrocytes was not statistical significantly different from the control. Imidazole was found to be neither clastogenic nor aneugenic in the mouse micronucleus test (BASF SE, 1993).

4.9.5 Comparison with criteria

There is no hint for any mutagenic properties of imidazole, neither *in vitro* nor *in vivo*. No mutagenicity was observed in two Ames tests and a HPRT Test with V79 cells. No unscheduled DNA synthesis was induced in primary rat hepatocytes. No clastogenic or aneugenic effects were found in the mouse micronucleus test *in vivo*. Therefore, imidazole is considered to be non-mutagenic and there is no classification required both according to Directive 67/548/EEC and Regulation No. 1272/2008/EC.

4.9.6 Conclusions on classification and labelling

No genetic toxicity *in vitro* and *in vivo*. No classification and labelling is required.

4.10 Carcinogenicity

4.10.1.1 Non-human information

4.10.1.2 Carcinogenicity: oral

No information available.

4.10.1.3 Carcinogenicity: inhalation

No information available.

4.10.1.4 Carcinogenicity: dermal

No information available.

4.10.1.5 Human information

No information available.

4.10.1.6 Other relevant information

No mutagenic effects noted *in vitro* and *in vivo* mutagenicity tests.

4.10.1.7 Summary and discussion of carcinogenicity

Based on data from valid *in vitro* and *in vivo* mutagenicity tests, a genotoxic carcinogenic potential is not expected or indicated.

4.10.1.8 Comparison with criteria

Not applicable.

4.10.1.9 Conclusions on classification and labelling

No classification and labelling with regard to carcinogenic effects required.

4.11 Toxicity for reproduction

The results of experimental studies are summarised in the following table:

Table 19: Summary table of relevant reproductive toxicity studies

Method	Results	Remarks	Reference
• OECD Guideline 414 (Prenatal Developmental Toxicity Study) • Rat (Wistar) • Oral: gavage • 0, 20, 60, 180 mg/kg bw/d (nominal conc.) • Exposure: Test substance was administered by oral gavage once a day from implantation to one day prior to expected parturition, i.e. d 6-19 p.c. (7 d/wk)	• NOAEL (maternal toxicity): 60 mg/kg bw/d (decreased food consumption, body weight gain and uterus weight at 180 mg/kg bw/d) • NOAEL (fetotoxicity): 60 mg/kg bw/d (reduced mean fetal weight and increased number of resorptions at 180 mg/kg bw/d) • NOAEL (teratogenicity): 60 mg/kg bw/d (increased rate of variations and malformations at 180 mg/kg bw/d)	• 1 (reliable without restriction) • key study • experimental result • Test material (EC name): imidazole	BASF SE (2002b)
• In-vitro study (teratogenicity screen). • Whole embryo culture (rat and mouse embryos)	• Some indication of developmental effects in presence of high mortality	• Investigative study • 3 (not reliable)	Daston GP et al. (1989)
• Effects of imidazoles on testosterone secretion and testicular intestinal fluid formation after s.c. injection	• Imidazole suppressed testosterone secretion and TIF formation	• Investigative study • reporting and methodology does not comply with OECD TG guideline standards • 3 (not reliable)	Adams ML et al. (1998)

4.11.1 Effects on fertility

4.11.1.1 Non-human information

There is no one- or two-generation reproductive toxicity study available. However, in an OECD TG 408 conforming 90-day gavage study in rats, male and female reproductive organs were examined (including histopathology), as were sperm quality parameters and morphology determined in testis and epididymides. In this study, no changes in weight and histopathology of reproductive organs (uterus, ovaries, oviducts, vagina, female mammary gland, left testes, left epididymis, prostate gland, seminal vesicles) were found at all dose levels. Moreover, the test substance did not cause any effects on sperm parameters (motility, morphology, head count in cauda epididymis and testis)

and oestrus cycle. From these results, it can be concluded that imidazole had no adverse effect on the reproductive organs up to the highest tested dose of 180 mg/kg bw/d (BASF SE, 2002a).

In an investigative, non-guideline study, increasing doses (three doses between 10 and 300 mg/kg bw) of imidazole were injected in adult rats (10 rats per group), and samples of serum and testicular intestinal fluid (TIF) were collected 2 hours later (Adams ML et al., 1998). It was reported that imidazole suppressed the two major regulating aspects of testicular function (testosterone secretion and TIF formation) at 30 mg/kg bw and higher and can suppress LH secretion regulating systems in the pituitary in rats at 300 mg/kg bw. The authors concluded that the findings support the hypothesis that imidazoles can suppress male reproductive function. With regard to hazard assessment, the findings reported by Adams et al. (1998) are considered to be of limited relevance because the subcutaneous injection does not represent a relevant exposure route. In addition, the precise s.c. injection site is not indicated in the publication, only one time point (2 hours after treatment) was studied and no microscopical examination of the testes was performed. By contrast, there was no indication of any adverse effect on male reproductive organs and sperm quality in the 90-day oral gavage study according to OECD TG 408 mentioned above.

4.11.1.2 Human information

No information available.

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

In a prenatal developmental study conducted in accordance with OECD TG 414, imidazole (purity 99.8 %) was administered by oral gavage to Wistar rats from day 6 to 19 of gestation. The dose levels were 0, 20, 60 or 180 mg/kg bw/d. A standard dose volume of 10 ml/kg bw was used for each group. The control group, consisting of 25 females, was dosed with the vehicle only (doubly distilled water). During the study the dams were assessed for clinical observations, body weight and food consumption, and corrected body weight was determined upon necropsy. On day 20 post coitum, dams were sacrificed and examined for gross pathological changes (including weight determinations of the unopened uterus and the placentae), the number of corpora lutea in the ovaries, conception rate, the number of live fetuses and pre- and post-implantation losses. The fetuses were weighed, sexed and macroscopically examined for external alterations. One half of all fetuses were fixed and examined for effects on the inner organs, while the other half of fetuses were fixed and stained for skeletal and cartilage evaluation.

The following substance-related findings were obtained (see also tables 19 and 20). There were no signs of maternal toxicity, fetal or developmental toxicity noted at the low and mid dose (20 and 60 mg/kg bw/d). At 180 mg/kg bw/d, transient salivation in 6 females was observed between days 15 to 19 p.c. and vaginal hemorrhage in one dam on day 20 p.c. The food intake was significantly reduced (-13 %) when the treatment was started. This was reflected by a statistical significantly reduced body weight gain on gestational days 6 to 8 (-45 %) and 17 to 20 (-34 %). However, terminal body weight was comparable in all groups, and corrected terminal body weight gain was also comparable in all groups. The effect on body weight gain on gestational days 17 to 20 is due to a significant decrease of the gravid uterus weight (-26 %), high rate of resorptions (see below) and distinctly lower mean fetal body weight (see below), rather than maternal toxicity. The number of live fetuses per litter was significantly reduced and the post-implantation loss was 43 % compared

to only 8 % in the control being statistically significant. Three of 24 pregnant dams resorbed all implants and had no live fetuses at necropsy.

Table 20: Group mean reproductive and fetal data

Dose levels (mg/kg bw/d)	**0**	**20**	**60**	**180**
Pregnancies on day 20	22	24	23	24
Conception rate	88	96	92	96
Dams with viable fetuses	22	22	23	21
Gravid uterus weight (g)	51.3 (9.52)	45.6 (16.65)	50.0 (11.57)	38.2 (16.81)**
Implantations/dam	9.7 (1.67)	9.0 (2.1)	9.7 (1.69)	9.3 (1.88)
Pre-implantation loss (%)	10.7 (14.07)	18.5 (20.84)	14.5 (13.33)	15.7 (12.46)
Post-implantation loss (%)	7.9 (10.13)	14.8 (28.31)	9.6 (11.77)	43.4 (34.09) **
Resorptions (total)	0.8 (1.05)	0.9 (1.2)	0.9 (1.12)	3.8 (3.06) **
Live fetuses/dam	8.9 (1.61)	8.8 (1.68)	8.8 (1.93)	6.3 (3.15) **
Fetal weight (g) (all viable)	3.7 (0.27)	3.6 (0.2)	3.6 (0.32)	3.2 (0.27) **
Placental weights (mg) (all viable fetuses)	460 (62)	450 (46)	490 (57)	560 (173) **
Sex ratio (% males)	53	41	52	46
SD in brackets ** p < = 0.01 (Dunnett test) (two-sided)				

Examination of the live fetuses from high dose dams revealed no changes with respect to sex distribution. The mean fetal body weight was reduced by 14 % due to a higher number of stunted fetuses (so-called runts). Further, the incidence of external malformations (anasarca and/or cleft palate) was significantly increased. About 10 % of the high dose fetuses were affected (13/132 fetuses; in 7/21 litters (= 33 %)) while no such changes were observed in the control. Skeletal malformations were also statistically significantly increased: 7.8 % affected fetuses per litter (7/73 fetuses in 5/21 litters (=24 %)) were noted in the high dose group compared to 1.1 % in the control. The incidences of shortened scapula, bent radius, bent ulna, malpositioned and bipartite sternebrae were statistically significantly increased. Soft tissue variations (dilated renal pelvis and ureter) were significantly increased in fetuses from high dose dams compared to controls (27 % vs. 6.4 %). The incidences of skeletal variations, mainly delays of the ossification process, were statistically significantly increased from 91 % in the control group to 98.4 % in the high dose group. In historical control animals the mean occurrence of skeletal variations is 92.6 % (range 87.0 - 98.1 %).

In summary, there were statistically significantly increased rates of total malformations (10.8 % versus 0.6 % affected fetuses/litter in the control group), variations (70.4 % versus 52.0 % affected

fetuses/litter in the control group) and unclassified skeletal cartilage observations (69.8 % versus 50.8 % affected fetuses/litter in the control group.

Table 21: Summary of all classified fetal external, soft tissue, and skeletal observations

Parameter	**No. and (%) fetuses at (mg/kg bw/d)**			
	0	**20**	**60**	**180**
No. litters evaluated	22	22	23	21
No. fetuses evaluated	195	194	202	132
Total malformations, mean (%) (affected fetuses/litter)	0.6 (3.05)	1.1 (3.68)	0.5 (2.32)	10.8 (14.67) **
Total variations, mean (%) (affected fetuses/litter)	52 (11.51)	50 (13.24)	61 (15.8)	70.4 (20.64) **
Unclassified skeletal cartilage observations, mean %, (affected fetuses/litter)	50.8 (29.2)	42.9 (38.11)	51.2 (28.95)	69.8 (28.79) **
External malformations, mean - litter incidence (%) - affected fetuses/litter (%)	 0 0	 0 0	 0 0	 33 ## 9 (15.08) **
Skeletal malformations, mean - litter incidence (%) - affected fetuses/litter (%)	 4.5 1.1 (5.33)	 9.1 2.3 (7.36)	 4.3 0.9 (4.17)	 24 7.8 (15.95) *
Soft tissue variations, mean - affected fetuses/litter (%)	6.4 (16.25)	9.2 (17.02)	22.7 (29.69) *	27.1 (35.05) *
Skeletal variations, mean - affected fetuses/litter (%)	91.1 (14.91)	87.2 (16.1)	94.2 (9)	98.4 (7.27) *
SD in brackets * p < = 0.05 (Wilcoxon-test, one-sided), ** p < = 0.01 (Wilcoxon-test, one-sided) ## p < = 0.01 (Fischer's exact test, one-sided)				

From this prenatal developmental toxicity study, it can be concluded that the oral administration of imidazole to pregnant Wistar rats from implantation to one day prior to the expected day of parturition (days 6 - 19 p.c.) elicited substance-related signs of maternal toxicity at 180 mg/kg bw/d. A total of 6 rats of this group showed transient salivation (being most likely indicative for slight irritations of the upper digestive tract) during some days of the treatment period. Moreover, vaginal haemorrhage occurred in another high dose dam, which resorbed all of its implants, just before scheduled sacrifice. At initiation of dosing the high dose dams showed statistically significant impairments in food consumption (about 13 % below the control) and impaired body weight gains (about 45 % below the control) on days 6 - 8 p.c. Moreover, high dose body weight gains were also statistically significantly diminished (33 % - 34 % below the control) on days 17 - 20 p.c. and the mean gravid uterus weight was distinctly affected (about 26 % below the control) due to a high resorption rate and a markedly lower mean fetal body weight at 180 mg/kg bw/d. According to the

scope of parameters examined in the present prenatal developmental toxicity study, the administration of 180 mg imidazole/kg bw/d to pregnant rats induced adverse effects on the dams. Concerning gestational parameters, there was a high rate of resorptions at the top dose, which led to a clearly elevated post-implantation loss value, but no substance-induced effects on the gestational parameters occurred at 20 or 60 mg/kg bw/d. At the highest dose level (180 mg/kg bw/d) clear signs of developmental toxicity, including indications of teratogenicity, were obtained. Mean placental weights and the number of stunted fetuses were clearly increased, whereas the mean fetal body weights were about 14 % below the corresponding control value. The external, skeletal and consequently the overall malformation rate and the incidences for several soft tissue and certain skeletal variations were statistically significantly increased and clearly above historical control values. At 20 and 60 mg/kg bw/d, however, no substance induced signs of embryo-/fetotoxicity, especially no indications of teratogenicity, were observed. Based on these results, the no observed adverse effects level (NOAEL) for maternal and prenatal developmental toxicity is 60 mg/kg bw/d (BASF SE, 2002b).

4.11.2.2 Human information

No information available.

4.11.3 Other relevant information

Some indication of developmental toxicity was obtained in a whole embryo culture test when rat and mouse embryos were exposed in vitro to imidazole at 30 and 60 µg/ml in vitro. The findings of this teratogenicity screen included reduced yolk sac diameter and crown rump length, and decreased brain size observed in up to 100 % of treated embryos. Mortality was up to 83 % in this exploratory study (Daston et al., 1989).

4.11.4 Summary and discussion of reproductive toxicity

Effects on fertility

One- or two-generation studies for imidazole are not available. However, parameters relevant to assess effects on fertility were included in a 90-day repeated toxicity study conducted according to OECD TG 408 (BASF SE, 2002a), in which Wistar rats (10 animals per sex and dose group) were dosed with 0, 20, 60, 180 mg imidazole/kg bw/d via gavage. In this study no changes in weight and histopathology of reproductive organs (uterus, ovaries, oviducts, vagina, female mammary gland, left testes, left epididymis, prostate gland, seminal vesicles) were found at any dose levels. Moreover, the test substance did not cause any effects on sperm parameters (motility, morphology, head count in cauda epididymis and testis) and estrus cycle.

In an investigative, non-guideline study, three doses between 10 - 300 mg/kg bw) of imidazole were injected in adult rats, and samples of serum and testicular intestinal fluid (TIF) were collected two hours later (Adams ML et al., 1998). It was reported that imidazole suppressed testosterone secretion and TIF formation at 30 mg/kg bw and higher and could suppress LH secretion regulating systems in the pituitary in rats at 300 mg/kg bw. With regard to hazard assessment, these findings are considered to be of limited relevance because subcutaneous injection does not represent a relevant exposure route for the substance, and there was no indication of any adverse effect on male reproductive organs and sperm quality in the 90-day gavage study according to OECD TG 408. Furthermore, the reliability of the publication is poor due to the insufficient methodology applied and the limited documentation of methods and results.

Developmental toxicity

In a prenatal developmental study conducted in accordance with OECD TG 414, imidazole (purity 99.8 %) was administered by oral gavage to Wistar rats from day 6 to 19 of gestation. The dose levels were 0, 20, 60 or 180 mg/kg bw/d. The control group, consisting of 25 females, was dosed with the vehicle water only. The dams were assessed for clinical observations, body weight and food consumption, and corrected body weight was determined upon necropsy. On day 20 p.c., dams were sacrificed and examined for gross pathological changes (including weight determinations of the unopened uterus and the placentae), the number of corpora lutea in the ovaries, conception rate, the number of live fetuses and pre- and post-implantation losses. The fetuses were weighed, sexed and macroscopically examined for external alterations. One half of all fetuses were fixed and examined for effects on the inner organs, while the other half of fetuses were fixed and stained for skeletal and cartilage evaluation.

There were no signs of maternal toxicity, fetal or developmental toxicity noted at 20 and 60 mg/kg bw/d. At 180 mg/kg bw/d, transient salivation in 6 females was observed between days 15 to 19 p.c. and vaginal hemorrhage in one dam on day 20 p.c. The food intake was significantly reduced by -13 % when the treatment was started. This was reflected by a statistical significantly reduced body weight gain on gestational days 6 to 8 (-45 %) and 17 to 20 (-34 %). However, terminal body weight was comparable in all groups, and corrected terminal body weight gain was also comparable in all groups. The effect on body weight gain on gestational days 17 to 20 is due to a significant decrease of the gravid uterus weight (-26 %), high rate of resorptions and distinctly lower mean fetal body weight, rather than maternal toxicity. The number of live fetuses per litter was significantly reduced and the post-implantation loss was 43 % compared to only 8 % in the control being statistically significant. Three of 24 pregnant dams resorbed all implants and had no live fetuses at necropsy.

From this prenatal developmental toxicity study, it can be concluded that the oral administration of imidazole to pregnant Wistar rats from implantation to one day prior to the expected day of parturition elicited substance-related signs of maternal toxicity at the highest dose (180 mg/kg bw/d). A total of 6 rats of this group showed transient salivation (being most likely indicative for slight irritations of the upper digestive tract) during some days of the treatment period. Moreover, vaginal haemorrhage occurred in another high dose dam, which resorbed all of its implants, just before scheduled sacrifice. At initiation of dosing, the high dose dams showed statistically significant impairments in food consumption (about 13 % below the control) and impaired body weight gains (about 45 % below the control) on days 6 - 8 p.c. Moreover, high dose body weight gains were also statistically significantly diminished on days 17 - 20 p.c. and the mean gravid uterus weight was distinctly affected (about 26 % below the control) due to a high resorption rate and a markedly lower mean fetal body weight at 180 mg/kg bw/d. According to the scope of parameters examined in the present prenatal developmental toxicity study, the administration of 180 mg imidazole/kg bw/d to pregnant rats induced adverse effects on the dams. Concerning gestational parameters there was a high rate of resorptions at the top dose, which led to a clearly elevated post-implantation loss value, but no substance-induced effects on the gestational parameters occurred at 20 or 60 mg/kg bw/d. At the highest dose level (180 mg/kg bw/d) clear signs of developmental toxicity, including indications of teratogenicity, were obtained. Mean placental weights and the number of stunted fetuses were clearly increased, whereas the mean fetal body weights were about 14 % below the corresponding control value. The external, skeletal and consequently the overall malformation rate and the incidences for several soft tissue and certain skeletal variations were statistically significantly increased and clearly above historical control values. At 20 and 60 mg/kg bw/d, however, no substance induced signs of embryo-/fetotoxicity, especially no indications of

teratogenicity, were observed. Based on these results, the no observed adverse effects level (NOAEL) for maternal and prenatal developmental toxicity is 60 mg/kg bw/d (BASF SE, 2002).

Some indication of developmental toxicity was obtained in a whole embryo culture test when rat and mouse embryos were exposed *in vitro* to imidazole at 30 and 60 µg/ml *in vitro*. The findings of this teratogenicity screen included reduced yolk sac diameter and crown rump length, and decreased brain size observed in up to 100 % of treated embryos. Mortality was up to 83 % in this exploratory study (Daston et al., 1989).

4.11.5 Comparison with criteria

Imidazole caused developmental toxicity and teratogenicity in the rat in a prenatal developmental toxicity study according to OECD TG 414.

There were no indications of a possible fertility impairing potential from a reliable 90-day oral gavage study in rats up to the highest dose level (180 mg/kg bw/d) with thorough histopathological examination of all male and female reproductive organs, sperm and estrus cycle analysis.

4.11.6 Conclusions on classification and labelling

Based on these results imidazole may cause damage to the unborn child and is classified and labelled Repr. Cat. 2; R61 according to Directive 67/548/EEC and Repr. 1B, H360D, GHS08 according to Regulation 1272/2008/EC.

Imidazole has not been included in Annex I to Directive 67/548/EEC or Annex VI Tables 3.1 and 3.2 of the Regulation (EC) No. 1272/2008/EC (CLP Regulation). In October 2006, the TC C&L (the Technical Committee on Classification and Labelling of Dangerous Substances) agreed on classification for Acute toxicity (Xn; R22) and Corrosivity (C; R34) on the basis of the DE classification proposal (ECBI/59/06). In September 2007, the TC C&L agreed to the classification for Repr. Cat 2; R61.

4.12 Other effects

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

No effects noted in a valid 90-day subchronic toxicity study according to OECD TG 408 (see 4.7.1.1).

4.12.1.2 Immunotoxicity

No information available.

4.12.1.3 Specific investigations: other studies

No information available.

4.12.1.4 Human information

No information available.

4.12.2 Summary and discussion

No information available.

4.12.3 Comparison with criteria

Not applicable.

4.12.4 Conclusions on classification and labelling

Not required.

5 ENVIRONMENTAL HAZARD ASSESSMENT

Not relevant for this dossier. No classification and labelling proposed based on available data.

6 OTHER INFORMATION

This substance has been registered according to the requirements of the REACH legislation. In addition, the substance is currently under evaluation in the framework of the Community Rolling Action Plan (CoRAP). The evaluation has been foreseen for the year 2012 and the listing was based on concerns regarding human health due to CMR properties wide dispersive use and high tonnage.

7 REFERENCES

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8 ANNEXES

None