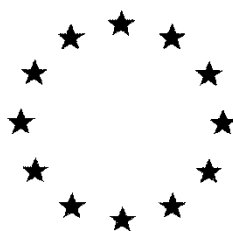


European Commission

**Second Programme for the Renewal of the Inclusion of Active Substances
in Annex I of Council Directive 91/414/EEC**



**Draft Renewal Assessment Report prepared according to the Commission
Regulation (EU) N° 1141/2010**

Amitrole

Volume 3 – Annex B.6

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B.6. TOXICOLOGY AND METABOLISM

Amitrole was first included in annex I on March, 5th, 2001 (Directive 2001/21/EC). The directive came into force on January, 1st 2002. In support to this inclusion was submitted a complete dossier according to the requirements of the Directive 91/414/EEC.

This section will present only the new data available since amitrole was included as well as the data that were specifically required by the RMS or identified in the monograph and /or in the review report as points to be addressed at national level.

Regarding the mammalian toxicology, no concern had been identified in level 4 of the monograph and no further data were requested. New dermal and eye irritation tests on amitrole have been conducted since then and are summarized in the following section. Two new GLP studies were also conducted to confirm the absence of genotoxicity. A study investigating maternotoxicity in the female pregnant rabbit was also submitted.

In the review report (6839/VI/97-final) and directive 2001/21/EC, it was asked to the member states to pay particular attention to the protection of operators. The operator risk assessment is addressed in this renewal.

Furthermore, according to the regulation (EC) N° 1272/2008, amitrole is currently classified as:



Warning

H 361d: Suspected of damaging the unborn child

H 373: May cause damage to organs (thyroid) through prolonged or repeated exposure

H 411: Toxic to aquatic life with long lasting effects

According to the provisional criteria set in annex II, article 3.6 of regulation (EC) N° 1107/2009 it may be considered as a potential endocrine disruptor. A position paper submitted to address the relevance of potential endocrine disrupting properties for human is discussed under point B.6.8.2.

B.6.1. ABSORPTION, DISTRIBUTION, EXCRETION AND METABOLISM IN MAMMALS (TOXICOKINETICS)

B.6.1.1. Single dose, oral route in rats

No new data have been submitted. Please refer to original EU dossier.

B.6.1.2. Single dose, oral route in man and rabbit, and intraperitoneal in rat

No new data have been submitted. Please refer to original EU dossier.

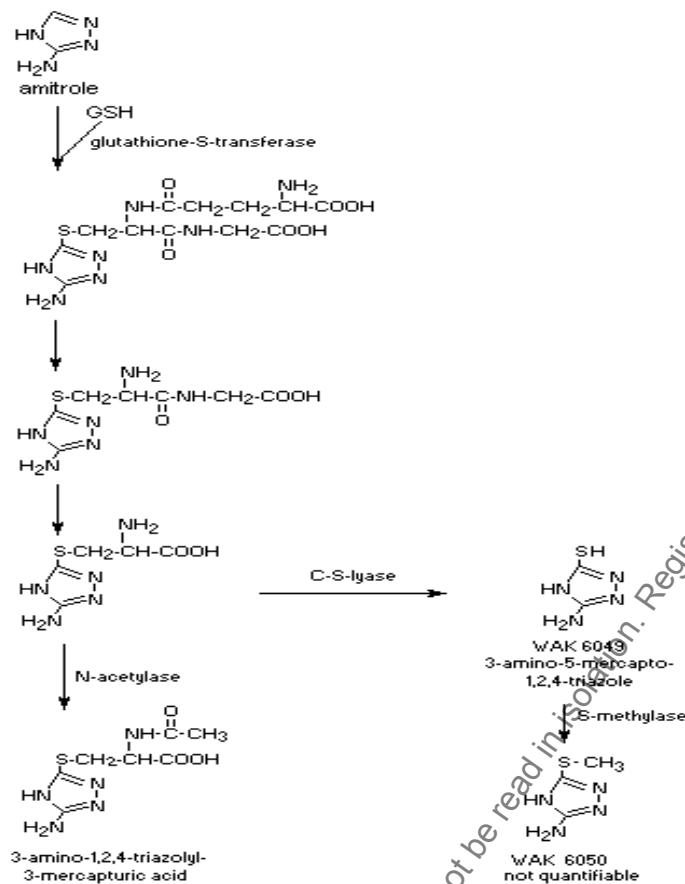
B.6.1.3. Absorption, distribution, metabolism and elimination in mammals: summary

Pharmacokinetic studies were conducted in male and female mice, rats and rabbits.

Following oral administration, amitrole is readily absorbed from the gastro-intestinal tract of mammals. It is rapidly excreted from the body mainly as the parent compound. The main route of excretion in humans and laboratory animals is via the urine and the majority of excretion takes place during the first 24h.

This molecule is a polar molecule which is mostly excreted unchanged in urine. Phase II reactions can occur and consist in conjugation to glutathione through the glutathione transferase. Other enzymes like N-acetylase or CS lyase are also involved. N-acetylase leads to the formation of 3-amino-1,2,4-triazolyl-5-mercaptopuric acid in mammals whereas the CS lyase action gives rise to 3-amino-5-mercapto-1,2,4-triazole.

The reactions are shown in figure 1.

Figure 1. Proposed metabolic pathway of amitrole in rats**B.6.2. ACUTE TOXICITY INCLUDING IRRITANCY AND SKIN IRRITATION****B.6.2.1. Acute oral toxicity in rats**

No new data have been submitted. Please refer to original EU dossier.

B.6.2.2. Acute percutaneous toxicity

No new data have been submitted. Please refer to original EU dossier.

B.6.2.3. Acute inhalation toxicity

No new data have been submitted. Please refer to original EU dossier.

B.6.2.4. Skin irritation

Report: IIA 5.2.4/01 [REDACTED] 2001. Amitrole : acute dermal irritation in rabbits. [REDACTED]

Guidelines

OECD Guideline 404 (1992) and EC method B.4 (1992)

GLP

Yes (certified laboratory)

Deviations

The temperature and relative humidity recorded in the animal room were sometimes outside of the target ranges. This minor deviation was not considered to have compromised the validity or integrity of the study.

Acceptability: Yes

Executive Summary

The potential of amitrole to induce skin irritation was assessed by applying 500 mg of the test item in its original form for 4 hours to the closely-clipped skin of one flank of three male New Zealand White rabbits. The test item was held in contact with the skin by means of a semi-occlusive dressing.

Cutaneous reactions were observed approximately 1h, 24h, 48h and 72h after removal of the dressing.

The mean value scores for erythema and oedema were calculated for each animal.

The interpretation of results were carried out according to the classification criteria laid down in Directive 67/548/EC and regulation (EC) n°1272/2008.

As a result, a very slight erythema was noted in all animals on day 1. It persisted up to day 2 in one animal and up to day 3 in another one. No other cutaneous reactions were observed.

Mean score over 24h, 48h and 72h for each animal were 0.7, 0.0 and 0.3 for erythema and 0.0, 0.0 and 0.0 for oedema.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: amitrole technical grade

Description: whitish flakes

Lot/Batch #: 067

Purity: 92.3%

CAS No.: 61-82-5

Stability of test compound: at least 2 years at room temperature

2. Test animals

Species, strain: New Zealand white rabbits

Sex: male

Weight at dosing: 2.6 +/- 0.1 Kg

Age at dosing: not specified

Source: CEGAV, Saint Mars d'Egrenne, France

Acclimatisation period: at least 5 days before the beginning of the study

Diet: free access (UAR, Villettaud, Epinay sur Orge, France)

Water: *ad libitum*

Housing: individually in polystyrene cage (48.2 cm x 58 cm x 36.5 cm). Each cage was equipped with a food container and a water bottle.

3. Environmental conditions

Temperature: 18 +/- 3 °C

Humidity: 30-70%

Air changes: around 12 cycles/hour of filtered, non-recycled air

Photoperiod: 12 h light/12h dark

B. STUDY DESIGN AND METHODS

1. In life dates: 28 August 2001 - 31 August 2001

2. Animal assignment, treatment and observations

The day before treatment, both flanks of each animal were clipped using electric clippers and the skin of each animal was examined. Only animals with healthy intact skin were used. Skin was clipped thereafter whenever necessary.

The test item was applied for 4 hours to 3 animals. A single dose of 500 mg of the test item in its original form was placed on a moistened gauze pad which was then applied to the right flank of the animals. The test item and

the gauze pad were held in contact with the skin by means of an adhesive hypoallergenic aerated semi-occlusive dressing and a restraining bandage.

The untreated skin served as control.

After removal of the dressing, any residual test item was wiped off by means of a dry gauze pad.

The skin was observed approximately 1 hour, 24, 48 and 72 hours after removal of the dressing. The study was ended on day 4 in the absence of persistent irritation reaction.

3. Statistics

The data did not warrant statistical analysis.

II. RESULTS AND DISCUSSION

A. FINDINGS

A very slight erythema (grade 1) was noted in all animals on day 1. It persisted up to day 2 in one animal and up to day 3 in another one.

No other cutaneous reactions were observed.

Mean scores over 24h, 48h and 72h for each animal were 0.7, 0.0 and 0.3 for erythema and 0.0, 0.0 and 0.0 for oedema.

Table B.6.2.4-1: Individual cutaneous examinations and mean values of the scores recorded for each animal (24, 48 and 72 hours)

Rabbit number	Dermal irritation	Scores				Mean irritation score at 24, 48 and 72h
		1h	24h	48h	72h	
887	Erythema	1	1	1	0	0.7
	Oedema	0	0	0	0	0
888	Erythema	1	0	0	0	0
	Oedema	0	0	0	0	0
889	Erythema	1	1	0	0	0.3
	Oedema	0	0	0	0	0

III. CONCLUSIONS

Under these experimental conditions, the test item amitrole is not irritating when applied topically to rabbit.

B.6.2.5. Eye irritation

Report: IIA 5.2.5/01 2002. Technical amitrole. Acute Eye irritation study with rinse in the rabbit.

Guidelines

OECD Guideline 405 (1987) and EC method B.5 (1992)

GLP

Yes (certified laboratory)

Deviations

Eyelids were held closed for approximately 30 seconds in order to avoid any loss of the test substance instead of one second as specified in the guideline.

Irrigation was performed as soon as 30 seconds after the beginning of instillation of the test substance. As the study author did not mention that corrosive or irritating effects appeared immediately after instillation, this is not

considered to be in accordance with EC method B.5 and OECD guideline n°405: “The eyes of the test animals should not be washed for at least 24 hours following instillation of the test substance, except for solids (...), and in case of immediate corrosive or irritating effects. At 24 hours a washout may be used if considered appropriate” “If the solid test substance has not been removed from the eye of the test animal by physiological mechanisms at the first observation time point of 1 hour after treatment, the eye may be rinsed with saline or distilled water”.

Acceptability: No

Executive Summary

3 animals were used in this study.

0.1 g of technical amitrole grown in a mortar was instilled into the conjunctival sac of the left eye of each of the three rabbits used. The untreated eye served as a control.

Conjunctival, iris or corneal lesions were evaluated approximately one hour after instillation of the test substance, then 24h, 48h and 72h after instillation.

Mean indices were calculated based on the results obtained for each rabbit at time 24h, 48h and 72h.

Under these experimental conditions, with rinse, amitrole was found to be non irritant for the eye of the rabbit.

Therefore no classification is required under DSD and CLP regulation.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Description: Technical amitrole

Lot/Batch #: 067

Purity: 92.3%

CAS No.: 61-82-5

Stability of test compound: at least 2 years at room temperature

2. Test animals

Species, strain: New Zealand Albino Rabbit

Sex: female

Weight at dosing: between 2.7-2.9 Kg

Age at dosing: not specified

Source: CEGAV, Saint Mars d'Egrenne, France

Acclimatisation period: at least 5 days

Diet: free access (UAR 112 foodstuff, Villemoisson, Epinay sur Orge, France)

Water: *ad libitum*

Housing: individually in cages of standard size.

3. Environmental conditions

Temperature: 17-21°C

Humidity: 45-65%

Air changes: approximately ten times per hour (non-recycled and filtered air)

Photoperiod: 12h light (from 7:30 am to 7:30 pm) and 12h darkness

B. STUDY DESIGN AND METHODS

1. In life dates: 12 November 2001 - 15 November 2001

2. Animal assignment, treatment and observations

0.1 g of technical amitrole was instilled into the conjunctival sac of the left eye of each of the three rabbits used. The conjunctival sac was opened by gently pulling the lower lid away from the eyeball. Eyelids were held closed for approximately 30 seconds in order to avoid any loss of the test substance.

The non-treated right eye was used as control.

The treated eye was gently irrigated, approximately 30 seconds following instillation of the test substance, with 100 mL of sterile isotonic saline solution, during approximately 30 seconds. The contralateral eye was similarly rinsed and used for control purpose.

Conjunctival, iris or corneal lesions were evaluated approximately one hour after instillation of the test substance, then 24h, 48h and 72h after instillation. Confirmation of the presence or absence of corneal opacity was carried out (except for reading at time 1 hour) by examination after instillation of a 2% aqueous solution of sodium fluorescein and rinsing with sterile and pyrogen free isotonic saline.

Mean indices were calculated based on the results obtained for each rabbit at time 24h, 48h and 72h.

3. Statistics

The data did not warrant statistical analysis.

II. RESULTS AND DISCUSSION

C. FINDINGS

The only one finding observed in all animals at 1-hour observation was erythema. No other sign was observed at any time point. Mean of the scores obtained at 24, 48 and 72 hours was thus 0 for conjunctival chemosis, 0 for erythema, 0 for iridal effect and 0 for corneal effect.

III. CONCLUSIONS

Under these experimental conditions, with rinse, technical amitrole was found to be non irritant for the eye of the rabbit.

B.6.2.6. Skin sensitization

No new data have been submitted. Please refer to original EU dossier.

B.6.2.7. Summary of acute toxicity including irritancy and skin sensitisation of amitrole

Technical amitrole is of low oral and dermal toxicity in rats ($LD_{50} > 5000$ mg/kg b.w and > 2500 mg/kg b.w. respectively).

The acute toxicity was also very low when administered by intravenous route in rats and mice.

Acute inhalative exposure to the highest technically producible aerosol concentration (439 mg/m^3) was tolerated by rats without any adverse effect. The 4-hour LC_{50} was set to be greater than 0.439 mg/L (highest attainable concentration).

Technical amitrole is not irritant to the skin and slightly irritant to the eyes. Amitrole was shown not to be a skin sensitizer in a Magnusson and Kligmann test.

B.6.3. SHORT-TERM TOXICITY

B.6.3.1. Oral 28-day study

No new data have been submitted. Please refer to original EU dossier.

B.6.3.2. Oral 90- day study

No new data have been submitted. Please refer to original EU dossier.

B.6.3.3. Oral 6 month toxicity in dogs

No new data have been submitted. Please refer to original EU dossier.

B.6.3.4. Oral 12 month toxicity in dogs

No new data have been submitted. Please refer to original EU dossier.

B.6.3.5. Dermal 21-day study

No new data have been submitted. Please refer to original EU dossier.

B.6.3.6. Summary of short-term toxicity of amitrole

Repeated oral administration of amitrole results mainly in effects on thyroid. It is currently well known that thyroid toxicity of amitrole is due to inhibition of thyroid peroxidase. Consequently rats treated with amitrole have low serum T4 and T3 levels with a parallel increase in TSH levels. Amitrole interferes with the production of thyroid hormones thus leading to hypertrophy and hyperplasia of the follicular cells of the thyroid. That can result in tumours of this gland in rodents after a prolonged administration. Evidence for reversibility of the thyroid effect in the rat was observed when the exposure was of short duration.

After 4 weeks of administration by oral route, thyroid effects are observed at levels greater than 1.5 mg/kg bw/d in rats (LOAEL of 5 mg/kg bw/d).

Three short-term studies were conducted in rats (one 10-week study and two 90-day studies), two one-year studies were carried out in dogs and a 90-day study was performed in monkeys.

In the 10-week study, the NOAEL was set at 1.5 mg/kg bw/d in females and less than 1.5 mg/kg bw/d in males, based on increase of TSH levels, follicular cell hypertrophy, small vesicles and increase vascularity observed from 12 mg/kg bw/d in females and 1.5 mg/kg bw/d in males. In the two 90-day studies (one performed in males only and the other in females only), the NOAEL were set at 0.1 mg/kg bw/d in males and females, based on thyroid effects (histological changes in the thyroid regarding appearance of follicular cells, content of colloid and capillary density in males; in females: increased iodine uptake shortly after injection, increased thyroid weight and histopathological changes of the thyroid).

Although no thyroid effects were observed in a 1-year dog study (NOAEL of 12.5 mg/kg bw/d, the highest dose level where only pale coloured pancreas was noted), thyroid parameters were affected from the LOAEL of 13 mg/kg bw/d in the other 1-year dog study. This consisted in enlarged thyroid, increased absolute and relative thyroid weight and follicular cells hyperplasia. The NOAEL was established at 0.3 mg/kg bw/d in this study.

A 90-day toxicity study was performed in cynomolgus monkey by oral route. The NOAEL were set at less than 30 mg/kg bw/d in males and at 100 mg/kg bw/d in females, based on thyroid effects observed from 30 mg/kg bw/d in males and 330 mg/kg bw/d in females.

Overall a NOAEL of 0.1 mg/kg bw/d for thyroid effects by oral route can be considered in the short-term studies.

By dermal route a NOAEL of 100 mg/kg bw/day was established in a 28-day study in rats based on thyroid effects (slightly higher TSH level and slightly decreased T3 and T4 levels, higher mean absolute and relative thyroid weight, morphological indication of gland hyperactivity) observed at 500 mg/kg bw/day. In rabbits no adverse effect was reported until the dose of 100 mg/kg bw/day.

By inhalation, a NOEC of 0.1 mg/L was established in a 28-day study in rats based on thyroid effects (decreased T3 and T4 level, thyroid hyperplasia and elevated thyroid weight) observed at 0.32 mg/L. However this NOEC was established based on the aerosol concentration in the inhalation chamber. The particle size distribution was not evaluated so that no information is given regarding the respirable fraction. Therefore the fraction of the aerosol concentration that was effectively inhaled cannot be determined in order to derive a systemic NOEL.

B.6.4. GENOTOXICITY**B.6.4.1. In vitro genotoxicity testing****B.6.4.1.1. Bacterial studies**

Report: IIA 5.4.1 Wallner, B. 2009. Reverse mutation assay using bacteria (*Salmonella typhimurium* and *Escherichia coli*) with amitrole technical. BSL Bioservice report n° 092153

Guidelines

OECD Guideline 471 and EC method B.13/14

GLP

Yes (certified laboratory)

Deviations: None

Acceptability: Yes

Executive Summary

Amitrole was investigated for its potential to induce gene mutations according to the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using *Salmonella typhimurium* strains TA98, TA 100, TA1535, TA1537 and tester strain *E.coli* WP2 uvrA.

Two independent experiments were conducted with and without metabolic activation. The concentrations, including the controls, were tested in triplicate.

The following concentrations were used: 31.6 – 100 – 316 – 1000 – 2500 and 5000 µg/plate

No precipitation of the test item was observed in any tester strain used in experiments I and II (with and without metabolic activation).

No toxic effects of the test item were noted in any of the five tester strains used up to the highest dose group evaluated with and without metabolic activation in experiments I and II.

No biologically relevant increases in revertant colony numbers of any of the five tester strains were observed following treatment with amitrole technical at any concentration level, neither in the presence nor in the absence of metabolic activation in experiments I and II.

The reference mutagens induced a distinct increase of revertant colonies indicating the validity of the experiments.

I. MATERIALS AND METHODS**A. MATERIALS**

Test Material: amitrole technical

Description: crystalline solid colourless

Lot/Batch #: 308024996

Purity: 91.95% w/w

CAS No.: 61-82-5

Stability of test compound: at least 2 years at room temperature

Control materials:

2.1. Positive controls

Without metabolic activation

Tester Strains:	<i>S. typhimurium</i> : TA 100, TA 1535
Name:	Sodium azide, NaN ₃
Supplier:	Merck
Catalogue No.:	106688
Lot No.:	K 28585088
Purity:	at least 99%
Dissolved in:	Aqua dest.
Concentration:	10 µg/plate
Tester Strains:	<i>S. typhimurium</i> : TA 98, TA 1537
Name:	4-nitro-o-phenylene-diamine, 4-NOPD
Supplier:	Fluka
Catalogue No.:	73630
Lot No.:	1364330
Purity:	> 97%
Dissolved in:	DMSO
Concentrations:	10 µg/plate for TA 98, 40 µg/plate for TA 1537
Tester Strain:	<i>E. coli</i> : WP2 uvrA
Name:	Methyl methane sulfonate, MMS
Supplier:	Sigma
Catalogue No.:	M4016
Lot No.:	76296KJ
Purity:	99.0%
Dissolved in:	Aqua dest.
Concentration:	1 µL/plate

With metabolic activation

Tester Strains:	<i>S. typhimurium</i> : TA 98, TA 100, TA 1535, TA 1537 and <i>E. coli</i> : WP2 uvrA
Name:	2-aminoanthracene, 2-AA
Supplier:	Aldrich
Catalogue No.:	A3, 880-0
Lot No.:	S11804-114
Purity:	96%
Dissolved in:	DMSO
Concentrations:	2.5 µg/plate for TA 98, TA 100, TA 1535 and TA 1537; 10 µg/plate for <i>E. coli</i> : WP2 uvrA

2.2. Negative control: solvent: Distillate water

Activation S9: S9 liver microsomal fraction derived from male Wistar rats induced with phenobarbital (80 mg/kg bw) and β -naphthoflavone (100 mg/kg bw) for 3 consecutive days by oral route. Batch control data were provided.

Test bacteria

Species: 4 strains of *Salmonella typhimurium* and one strain of *E. coli* WP2 uvrA

Strains:

TA 98:	
his D 3052; rfa ⁻ ; uvrB ⁻ ; R-factor:	frame shift mutations
TA 100:	
his G 46; rfa ⁻ ; uvrB ⁻ ; R-factor:	base-pair substitutions
TA 1535:	
his G 46; rfa ⁻ ; uvrB ⁻ :	base-pair substitutions
TA 1537:	
his C 3076; rfa ⁻ ; uvrB ⁻ :	frame shift mutations
<i>E. coli</i> :	
WP2 uvrA: trp ⁻ ; uvrA ⁻ :	base-pair substitutions and others

Source: TA98, TA100, TA 1535 and TA1537 are obtained from MOLTOX, Inc. 28607 NC. USA - *E. coli* is obtained from DSMZ Sales, Braunschweig, Germany.

B. STUDY DESIGN AND METHODS

1. In life dates: 29 June 2009 -27 July 2009

2. Treatment and observations:

Pre-experiment for toxicity:

According to the plate incorporation test method, the toxicity of the test item was determined with the strains TA 98 and TA 100 at the following concentrations with three plates each: 3.16 – 10 – 31.6 – 100 – 316 – 1000 – 2500 and 5000 µg/plate.

Exposure concentrations:

Based on the results of the pre-experiment, 5000 µg/plate was chosen as the maximum concentration. Two independent experiments were conducted at the following concentrations: 31.6 – 100 – 316 – 1000 – 2500 and 5000 µg/plate.

Experimental performance:

For the plate incorporation method the following materials were mixed in a test tube and poured over the surface of a minimal agar plate

- 100 µL test solution at each dose level, solvent control or reference mutagen solution (positive control)
- 500 µL S9 mix (for testing with metabolic activation) or S9 mix substitution buffer (for testing without metabolic activation)
- 100 µL bacteria suspension
- 2000 µL overlay agar.

For the pre-incubation method 100µL of the test item preparation is pre-incubated with the tester strains (100 µL) and sterile buffer or the metabolic activation system (500 µL) for 60 minutes at 37°C prior to adding the overlay agar (2000 µL) and pouring onto the surface of a minimal agar plate.

For each strain and dose level, including the controls, three plates were used.

After solidification the plates were inverted and incubated at 37°C for at least 48h in the dark.

The colonies were counted using a ProtoCOL counter (Meintrup DWS Laborgeräte GmbH). If precipitation of the test item precluded automatic counting the revertant colonies were counted by hand. In addition tester strains with low spontaneous mutation frequency like TA 1535 and TA 1537 were counted manually.

Evaluation criteria:

Cytotoxicity: can be detected by a clearing or rather a diminution of the background lawn or a reduction in the number of revertants down to a mutation factor of approximately ≤ 0.5 in relation to the solvent control.

Mutagenicity: A test item is considered as mutagenic if, in at least one tester strain with or without metabolic activation:

- A clear and dose-related increase in the number of revertants occurs and/or
- A biologically relevant positive response for at least one of the dose groups occurs

A biologically relevant response is defined as:

- If in tester strains TA 100 and *E. coli* WP2 uvrA the number of reversions is at least twice as high
- If in tester strains TA 1535, TA 1537 and TA 98 the number of reversions is at least three times higher as compared to the reversion rate of the solvent control.

The biologically relevant response is the criteria for interpretation of the results. No statistical evaluation of the results was necessary.

RESULTS AND DISCUSSION

No precipitation of the test item was observed in any tester strain used in experiments I and II (with and without metabolic activation).

No toxic effects of the test item were noted in any of the five tester strains used up to the highest dose group evaluated with and without metabolic activation in experiments I and II.

No biologically relevant increases in revertant colony numbers of any of the five tester strains were observed following treatment with amitrole technical at any concentration level, neither in the presence nor in the absence of metabolic activation in experiments I and II.

Reductions in the number of revertants were found down to a mutation factor of 0.5 in experiment I in tester strain TA 1537 at a dose of 2500 µg/plate (without metabolic activation) and down to a mutation factor of 0.4 in experiment II in tester strain TA 1535 at a dose of 2500 µg/plate (without metabolic activation). Due to the lack of a dose-response relationship, this was considered as not biologically relevant.

The reference mutagens induced a distinct increase of revertant colonies indicating the validity of the experiments.

CONCLUSIONS

During the described mutagenicity test and under the experimental conditions reported, amitrole technical did not cause gene mutations by base pair changes or frameshifts in the genome of the tester strains used.

Amitrole is considered to be non mutagenic in this bacterial reverse mutation assay.

B.6.4.1.2. Mammalian cell studies

B.6.4.1.2.1. Gene mutation assays

No new data have been submitted. Please refer to original EU dossier.

B.6.4.1.2.2. Clastogenicity

Report: IIA 5.4.2 Oppong-Nketiah, M.2009. *In vitro* mammalian chromosome aberration test in human lymphocytes with amitrole technical. BSL Bioservice report n° 092154

Guidelines

OECD Guideline 473 (1997) and EC method B.10 (2008)

GLP

Yes (certified laboratory)

Deviations: None

Acceptability: Yes

Executive Summary

A chromosome aberration test assay was carried out in order to investigate a possible potential of amitrole technical for its ability to induce structural chromosome aberrations in Human lymphocytes. The chromosomes (cells) were prepared 24h after start of treatment with the test item. Two experiments were conducted:

Experiment I: the cells were treated for 4 h with the test item, with and without metabolic activation. The metaphases were prepared 24h after the treatment. The following concentrations were evaluated: 1000, 2500 and 5000 µg/mL.

Experiment II: the cells were treated for 4h with metabolic activation and for 24h without metabolic activation. With metabolic activation the cells were prepared 24h after treatment and without metabolic activation the cells were prepared at the end of the treatment period.

The following concentrations were evaluated:

- Without metabolic activation: 500, 1000 and 2500 µg/mL
- With metabolic activation: 3000, 4000 and 5000 µg/mL

Two cultures were set up in parallel and 100 metaphases per culture were scored for structural chromosomal aberration.

No precipitation of the test item was noted with and without metabolic activation at the concentrations evaluated in experiments I and II.

Toxic effects of the test item were observed only in experiment II without metabolic activation at a concentration of 2500 µg/mL.

In both experiments,

- No biologically relevant increase of the aberration rates was noted after treatment with the test item with and without metabolic activation. The aberration rates in all dose groups were within the historical control data of the negative control.
- No biologically relevant increase in the frequencies of polyploidy cells was found after treatment with the test item, with and without metabolic activation, compared to the controls.

Ethylmethanesulfonate (400 and 900 µg/mL) and cyclophosphamide (5 and 7.5 µg/mL) were used as positive controls and induced distinct and biologically relevant increases in cells with structural chromosomal aberrations.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: amitrole technical

Description: crystalline solid colourless

Lot/Batch #: 308024996

Purity: 91.95%

CAS No.: 61-82-5

Stability of test compound: at least 2 years at room temperature

2. Control materials:

2.1. Negative control: culture medium

2.2. Positive controls

-S9: EMS (Ethylmethanesulfonate): 400 and 900 µg/mL

+S9: CPA (cyclophosphamide): 5 and 7.5 µg/mL

3. Activation: S9 liver microsomal fraction derived from male Wistar rats induced with phenobarbital (80 mg/kg bw) and β-naphtoflavone (100 mg/kg bw) for 3 consecutive days by oral route

4. Test system: Blood samples were obtained from healthy donors not receiving medication. Blood from only one single donor was used in each experiment in order to reduce the inter-individual variability.

B. STUDY DESIGN AND METHODS

1. **In life dates:** 29 June 2009 -15 September 2009

2. **Treatment and observations:**

The test item was dissolved in culture medium (RPMI 1640) before treatment. No precipitation of the test item was indicated at a concentration of 5000 µg/mL.

Experimental design:

Pre-experiment for toxicity: the highest dose group evaluated was 5000 µg/mL. The relative mitotic index was used for assessing the cytotoxicity.

Exposure concentrations (Main experiment):

Duplicate cultures were treated at each concentration. The selection of the concentrations used in the experiments I and II was based on data from the pre-experiment.

Experiment I: the cells were treated for 4h with the test item with and without metabolic activation. The following concentrations were evaluated: 1000, 2500 and 5000 µg/mL. The metaphases were prepared 24h after the treatment.

Experiment II: the cells were treated for 4h with metabolic activation and prepared 24h after the treatment. The cells were treated for 24h without metabolic activation and prepared at the end of the treatment.

The following concentrations were evaluated:

- Without metabolic activation: 500, 1000 and 2500 µg/mL
- With metabolic activation: 3000, 4000 and 5000 µg/mL

Two cultures were set up in parallel and 100 metaphases were scored for structural chromosomal aberration per culture.

Experimental performance:

Blood cultures were set up within 4h after collection in plastic vessels. The following volumes were added to the culture vessels: 8.45 mL culture medium, 0.90 mL whole blood, 0.50 mL PHA, 0.10 mL antibiotic solution and 0.05 mL heparin.

All incubations were done at 37°C in humidified atmosphere with 5% CO₂.

Treatment:

Experiment I: short-time exposure 4h (with and without S9 mix)

After 48h the culture medium was replaced with serum-free medium containing the test item without S9 or with serum-free medium containing the test item with 50 µL/mL S9 mix.

After 4h the cells were spun down by gentle centrifugation for 10 minutes. The supernatant with the dissolved test item was discarded and the cells were resuspended in PBS. The washing procedure was repeated once as described. After washing, the cells were resuspended in complete cell culture medium. The cells were prepared 24h after beginning of treatment.

Experiment II

- Long time exposure 24h (without S9 mix)

After 48h, the culture medium was replaced with complete medium (with 15%FCS) containing the test item without S9. The treated cells were prepared at the end of the treatment.

- Exposure time with metabolic activation

The cells were prepared as described for experiment I (with metabolic activation).

Preparation of the cultures:

At least 2h before harvesting colcemid was added to the cultures (final concentration of 0.2 µg/mL). The cultures were harvested by centrifugation 24h after the beginning of treatment. The supernatant was discarded and the cells were resuspended in approximately 5 mL hypotonic solution (0.4% KCl). The cells suspension was left at 37°C for 20 minutes. After removal of the hypotonic solution by centrifugation the cells were fixed with 3+1 methanol + glacial acetic acid. The fixation procedure was repeated twice. Slides were prepared by dropping the cell suspension onto a clean microscopic slide. The cells were stained with Giemsa and according to the Fluorescent plus Giemsa technique.

Proliferation index:

BrdU labelling was used for the negative control and the highest dose group to detect a possible cell cycle delay after treatment with the test item. The proliferation index was determined by scoring the number of the first, second and third metaphases in 100 cells per culture.

Evaluation criteria

A product is clastogenic if is observed:

- A clear and dose-related increase in the number of cells with aberrations,
- A biologically relevant response for at least one of the dose groups which is higher than the laboratory negative control range (up to 4.0% aberrant cells).

The biologically relevant response is the criteria for interpretation of the results. No statistical evaluation of the results was necessary.

II. RESULTS AND DISCUSSION**Precipitation**

No precipitation of the test item was noted with and without metabolic activation at the concentrations evaluated in experiments I and II.

Toxicity

Toxic effects of the test item were observed only in experiment II without metabolic activation at a concentration of 2500 µg/mL. Indeed a decrease of the relative mitotic index was observed at the concentration of 2500 µg/mL down to 44%. With metabolic activation no toxic effect of the test item were noted at the concentrations evaluated.

BrdU-labeling

In experiment I, the values of the proliferation index of the negative control were 1.70 (without S9) and 1.25 (with S9). The proliferation index of the highest dose group evaluated (5000 µg/mL) was 1.52 (without S9) and 1.11 (with S9). No biologically relevant decrease of the proliferation index was indicated.

Table 10: Experiment I Proliferation Index determined by BrdU-Labeling

Dose Group	Concentration [µg/mL]	Treatment Time	Proliferation Index	Slide 1			Slide 2		
				1. Mitosis	2. Mitosis	3. Mitosis	1. Mitosis	2. Mitosis	3. Mitosis
without metabolic activation									
C	0	4 h	1.70	33	67	0	27	73	0
6	5000	4 h	1.52	46	54	0	51	49	0
with metabolic activation									
C	0	4 h	1.25	70	30	0	84	20	0
6	5000	4 h	1.11	89	11	0	89	11	0

In experiment II, the values of the proliferation index of the negative control were 1.62 (without S9) and 1.21 (with S9). The proliferation index of the highest dose groups evaluated (1000 and 2500 µg/mL) were 1.63 and 1.52 respectively, without S9. With metabolic activation the proliferation index of the highest dose group evaluated (5000 µg/mL) was 1.22. No biologically relevant decrease of the proliferation index was indicated.

Table 11: Experiment II Proliferation Index determined by BrdU-Labeling

Dose Group	Concentration [µg/mL]	Treatment Time	Proliferation Index	Slide 1			Slide 2		
				1. Mitosis	2. Mitosis	3. Mitosis	1. Mitosis	2. Mitosis	3. Mitosis
without metabolic activation									
C	0	24 h	1.62	30	70	0	47	53	0
6	1000	24 h	1.63	43	57	0	31	69	0
7	2500	24 h	1.52	51	49	0	46	54	0
with metabolic activation									
C	0	4 h	1.21	76	24	0	82	18	0
6	5000	4 h	1.22	77	23	0	79	21	0

Clastogenicity

In both experiments, no biologically relevant increase of the aberration rates was noted after treatment with the test item with and without metabolic activation. The aberration rates in all dose groups were within the historical control data of the negative control.

Summary of aberration rates:Experiment I

Dose Group	Concentration [µg/mL]	Treatment Time	Fixation Interval	mean % aberrant cells	
				incl. Gaps	excl. Gaps
without metabolic activation					
C	0	4 h	24 h	3.0	1.0
4	1000	4 h	24 h	2.0	2.0
5	2500	4 h	24 h	1.0	1.0
6	5000	4 h	24 h	0.5	0.5
EMS	900	4 h	24 h	20.0	17.0
with metabolic activation					
C	0	4 h	24 h	4.5	1.0
4	1000	4 h	24 h	1.0	0.0
5	2500	4 h	24 h	3.0	1.5
6	5000	4 h	24 h	3.5	1.5
CPA	7.5	4 h	24 h	12.5	11.0

Experiment II

Dose Group	Concentration [µg/mL]	Treatment Time	Fixation Interval	mean % aberrant cells	
				incl. Gaps	excl. Gaps
without metabolic activation					
C	0	24 h	24 h	1.0	0.5
5	500	24 h	24 h	3.5	1.0
6	1000	24 h	24 h	5.5	1.5
7	2500	24 h	24 h	4.5	2.0
EMS	400	24 h	24 h	24.0	17.0
with metabolic activation					
C	0	4 h	24 h	4.0	1.0
4	3000	4 h	24 h	2.0	1.5
5	4000	4 h	24 h	3.5	1.5
6	5000	4 h	24 h	5.0	3.0
CPA	5	4 h	24 h	24.5	18.5

Historical control data of the testing facility for the negative control (2004-2008):

Without metabolic activation: incl. gaps: 0.0 – 6.0% / excl. gaps: 0.0 – 4.0%

With metabolic activation: incl. gaps: 0.0 – 7.5% / excl. gaps: 0.0 – 4.0%

Historical control data of the testing facility for the positive controls (2004-2008):

Without metabolic activation: incl. gaps: 10.0 – 33.0% / excl. gaps: 8.0 – 33.0%

With metabolic activation: incl. gaps: 10.0 – 33.0% / excl. gaps: 8.0 – 30.0%

Polyploid cells

No biologically relevant increase in the frequencies of polyploidy cells was found after treatment with the test item compared to the controls.

Positive controls

EMS (400 and 900 µg/mL) and CPA (5 and 7.5 µg/mL) were used as positive controls and induced distinct and biologically relevant increases in cells with structural chromosomal aberrations, within the historical control data.

III. CONCLUSIONS

During the described *in vitro* chromosomal aberration test and under the experimental conditions reported, the test item amitrole technical did not induce structural chromosomal aberrations in human lymphocytes cells. **Therefore amitrole is not considered to be clastogenic in this test.**

B.6.4.1.3. Additional information in vitro

No new data have been submitted. Please refer to original EU dossier.

B.6.4.2. In vivo genotoxicity testing

B.6.4.2.1. In vivo micronucleus test

No new data have been submitted. Please refer to original EU dossier.

B.6.4.2.2. Rat liver UDS assay

No new data have been submitted. Please refer to original EU dossier.

B.6.4.2.3. Dominant lethal mutations in male rats

No new data have been submitted. Please refer to original EU dossier.

B.6.4.3. Summary of genotoxicity of amitrole

Amitrole has been extensively tested in *in vitro* and *in vivo* genotoxicity studies. If positive results were obtained in some *in vitro* tests, no genotoxicity was found *in vivo*.

By weight of evidence, amitrole is not a genotoxic compound. This was confirmed by recent *in vitro* gene mutation and chromosomal aberration tests.

B.6.5. LONG-TERM TOXICITY AND CARCINOGENESIS

B.6.5.1. Long-term (2 years) oral toxicity in the rat

No new data have been submitted. Please refer to original EU dossier.

B.6.5.2. Carcinogenicity study in the rat

No new data have been submitted. Please refer to original EU dossier.

B.6.5.3. Carcinogenicity study in the mouse

No new data have been submitted. Please refer to original EU dossier.

B.6.5.4. Summary of long-term toxicity and carcinogenicity studies

Long term and/or carcinogenicity studies have been performed in rats, mice and golden hamsters.

A first 2-year oral toxicity and carcinogenicity in the rat was considered as informative; it was indeed difficult to assess it because of several changes in dosing regimen. Nevertheless, thyroid appeared as the target organ (thyroid hyperplasia were seen in all treated groups, increased incidence of thyroid tumours (mainly follicular adenomas)).

In a carcinogenicity study in rats fed with dose levels of 0, 1, 10 and 100 ppm, a slight decrease in survival time, an increase in the incidence of (benign and malignant) thyroid tumours, and an increase of the incidence of (mainly benign) pituitary gland tumours was observed at 100 ppm. In this study a thyroid function was also performed in a small number of animals. At 100 ppm (5 mg/kg bw/d) thyroid weight was increased during the whole study period as was the percentage of accumulation of radioiodine in the thyroid. The NOAEL was set at 10 ppm (equivalent to 0.5 mg/kg bw/d).

In a 18-month carcinogenicity study in mice at level of 0, 1, 10 and 100 ppm in the diet, increased incidence of tumours was not observed. In this study a thyroid function was also performed in a small number of animals. At 100 ppm (15 mg/kg bw/d) an increase in thyroid weight and in iodine accumulation in the thyroid was observed. The NOAEL was set at 10 ppm (equivalent to 1.5 mg/kg bw/d).

In a 18-month carcinogenicity study in Syrian hamsters at level of 0, 1, 10 and 100 ppm in the diet, the NOAEL was set at 10 ppm (equivalent to 1 mg/kg bw/d) based on decreased body weight gain at 10 mg/kg bw/d. No effect on thyroid was observed. There was no evidence of carcinogenic potential.

The Scientific Committee on Plants evaluated amitrole in 2000 in the context of the inclusion of amitrole in Annex I to council directive 91/414/EEC (SCP/AMITR/002-Final, 13 July 2000) and concluded that:

"In conclusion, since

- a. the thyroid carcinogenic effect of amitrole appears to be mediated through thyroid-pituitary disruption to which humans are less sensitive*
 - b. there does not seem to be a role for increased TSH in human thyroid carcinogenicity,*
- the thyroid tumours observed in rats after long term amitrole treatment appear to be of little or no relevance for humans."*

Corroborating this opinion, IARC Overall evaluation (2001) downgraded amitrole to Group 3 (not classifiable as to its carcinogenicity to humans) with supporting evidence from relevant data.

It was concluded that the thyroid tumours observed in rats were not considered relevant for human and that amitrole does not need to be classified for carcinogenicity.

B.6.6. REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

B.6.6.1. Multigeneration study

No new data have been submitted. Please refer to original EU dossier.

B.6.6.2. Developmental toxicity studies

B.6.6.2.1. Rat

No new data have been submitted. Please refer to original EU dossier.

B.6.6.2.2. Rabbit

Report: IIA 5.6.11 [REDACTED] 2000. Study on maternotoxicity in the pregnant female rabbit following oral administration (gavage). [REDACTED]

Guidelines

No formal guideline but principles of the OECD 414 were followed when relevant

GLP: Yes

Acceptability: Yes

I MATERIALS AND METHODS**A MATERIAL****1. Test Material:** amitrole technical

Description: whitish flakes

Lot/Batch #: 067

Purity: 92.3%

CAS No.: 61-82-5

2. Vehicle:

Purified water obtained by reverse osmosis using a Milli-Ro 8 plus apparatus (Millipore SA, Saint Quentin en Yvelines, France).

B. STUDY DESIGN AND METHODS**2. In life dates:** 9 May 2000-9 June 2000**3. Dosage forms**

The test substance was administered as a solution in the vehicle. The test substance dosage forms were prepared once a week, stored at room temperature away from light.

4. Test system**Animals**

Number: 10 female rabbits per group

Strain: New Zealand white (INRA A 9077) specific pathogen free (S.P.F)

Breeder: Charles River France (Elevage scientifique des Dombes, Châtillon-sur-Chalaronne, France)

Age/weight: approx. 18-19 weeks old at the beginning of treatment period and mean body weight of 3412g (From 2810g to 3940g). The females were sexually mature and primigravid.

Mating: females were mated at breeder's facilities. The day of confirmed mating was assessed visually and designated as day 0 *post coitum*.

Acclimation: for a period of at least 5 days before the beginning of the treatment period

Allocation to study: During the acclimation period the animals were randomly allocated to the groups

Identification: individually by ear tattoo

Environmental conditions

Temperature: 18±3°C

Relative humidity: 50±20°C

Lighting: 12h dark/12h light (07:00-19:00)

Ventilation: about 12 cycles/hour of filtered, non recycled air

Housing

The animals were individually housed in stainless steel cage (64 cmx48 cmx41 cm)

Food and water

The animals had free access to diet “type 110” (UAR, Villemoisson, Epinay-sur-Orge, France). Each batch of diet was analyzed by the supplier for composition and contamination levels.

The animals had free access to tap water filtered using a 0.22µ filter (Millipore SA, Velizy, France) and contained in bottles. Bottles were changed at least once a week.

Treatment**Treatment groups**

Group	Number of mated females	Dose-level (mg/kg/day)
1	10	0
2	10	3
3	10	15
4	10	75

Duration: Each animal was given the test substance or control dosage forms once a day, at approximately the same time, from day 6 to day 18 post coitum inclusive (organogenesis).

Route of administration: oral route by gavage using a plastic syringe fitted with a plastic gavage tube.

Observations

Clinical signs (including evidence of abortion/resorption) and mortality were checked daily.

Body weight, food consumption and water consumption were recorded every 2 days.

On day 5, 19 and 29 post-coitum a blood sample was taken from every female and blood biochemistry investigations relating to the thyroid function (including thyroid hormones) were carried out.

On day 29 post-coitum the dams were sacrificed and subjected to macroscopic examination. The thyroid were sampled, weighted and examined microscopically.

The fetuses were removed by hysterectomy. The litter parameters were recorded: number of corpora lutea, implantation sites, early and late resorptions, dead and live fetuses.

The fetuses were weighted and submitted to external examination. The thyroids were then samples and examined microscopically.

II RESULTS AND DISCUSSIONS**Clinical signs**

No treatment-related clinical signs were observed at any dose level.

Mortality

No death imputable to the treatment was recorded.

Abortions

No abortion was observed in any group.

Body weight, food consumption and water consumption

The body weight gain, the food and water consumption were similar in the control and the 3 and 15 mg/kg bw/d treatment groups.

In the 75 mg/kg bw/d group, the body weight gain, food consumption and water consumption were lower than in the control group (body weight gain: - 66%, $p > 0.01$; food consumption: -37%, $p > 0.01$; water consumption: - 24%).

Blood chemistry

There were no notable changes at 3 and 15 mg/kg bw/d.

At 75 mg/kg bw/d, there was an higher cholesterol level (+225%, $p > 0.01$) and a lower level of the thyroid hormones T3 and T4 (T3: -51%, $p < 0.01$; T4: -29%, $p > 0.01$).

Hysterectomy data

All the hysterectomy parameters were similar in the control and in the treated groups: pre-and post-implantation loss, number of fetuses and fetal weight.

Fetal external examination

There were no treatment-related external malformations or variations in the fetuses of the 3 and 15 mg/kg bw/d treatment groups.

At 75 mg/kg bw/d, 3/66 fetuses displayed a domed-head, probably related to dilatation of cerebral ventricles/hydrocephaly. A relationship to treatment could not be ruled out.

PathologyOrgan weights (dams)

No notable difference in thyroid weights between the control and treated females was observed.

Macroscopic post-mortem examination (dams)

No macroscopic changes (including in the thyroids) that could be attributed to treatment was recorded.

Microscopic examination of the thyroids (dams and fetuses)

No difference was recorded between control and the 3 mg/kg bw/d treated group.

Changes indicative of thyroidal hyperactivity were noted in all the dams (follicular cell hyperplasia, decrease in eosinophil colloid, presence of low or high cuboidal epithelium and decrease in the follicular size). The severity of the morphological changes was similar between the animals given 3 mg/kg bw/d and the controls. However, slightly higher severity was seen in the treated animals given 15 or 75 mg/kg bw/d but without evidence of dose-relationship, which might be explained by great inter-individual variations.

No sign of thyroidal hyperactivity was reported for the fetuses either in the control or in the treated groups.

III CONCLUSIONS

The test substance, amitrole technical, when administered to pregnant female rabbits, daily from day 6 to day 8 post coitum, at 3, 15 and 75 mg/kg bw/d was not maternotoxic at 3 and 15 mg/kg bw/d.

However at 75 mg/kg bw/d a clear maternotoxicity was recorded.

Signs of thyroidal hyperactivity (of slightly higher severity) were noted at 15 and 75 mg/kg bw/d among the dams.

No sign of thyroidal hyperactivity was recorded among the fetuses of any treatment groups.

Consequently the No Observed Effect Level for maternal effects was set at 3 mg/kg bw/d.

A NOAEL for developmental effects can be set at 15 mg/kg bw/d based on fetal malformations observed at 75 mg/kg bw/d (3 out of 66 fetuses from 2 litters displayed a domed-head, probably related to dilatation of cerebral ventricles/hydrocephaly).

B.6.6.3. Summary of reproductive and developmental toxicity study

In two generations studies it was shown that amitrole was not toxic to F0 and F1 generation up to 2 ppm and did not induce any effects on the reproductive performance of F0 and F1, nor impaired the development of F1 and

F2 offspring up to 15 ppm. The dose of 15 ppm was slightly toxic for the F0 (males only) and F1 parental animals as shown by minimal increase of thyroid activity. The 112.5 ppm dose level was severely toxic for the F0 and F1 parents, impaired their fertility and disturbed the development of F1 and F2 litters. Nevertheless all findings on reproduction correlated with severe systemic parental toxicity, so that a primary effect on reproduction was excluded.

A NOAEL was set at 15 ppm (0.9 mg/kg bw/d) for reproductive toxicity and at 2 ppm (0.12 mg/kg bw/d) for parental toxicity.

Teratogenicity studies were conducted in rats (by oral route) and rabbits (by oral and dermal routes).

In rats exposed by gavage to dose levels of 0, 100, 500 and 1000 mg/kg bw/d, slight maternotoxicity was observed at 500 and 1000 mg/kg bw/d (reduced body weight gain and food consumption, increased thyroid weight). Reduced fetal body weight/litter and reduced skeletal ossifications were observed in the high-dose group. Increased incidences of enlarged and/or dark thyroids were seen in foetuses at 500 and 1000 mg/kg bw/d. The NOAEL for maternotoxicity and embryo/fetotoxicity was set at 100 mg/kg bw/d. Amitrole was considered not to be teratogenic in rats at doses levels up to 1000 mg/kg bw/d.

In rabbits exposed by gavage to dose levels of 0, 4, 40 and 400 mg/kg bw/d, a dose related increased incidence of abortions was observed in all treated groups. Decrease body weight gain during the gestation period was observed at 40 and 400 mg/kg bw/d and increased liver weight at 400 mg/kg bw/d. Embryo/foetotoxicity were observed at 40 and 400 mg/kg bw/d. Increased incidences of irreversible structural changes were also found at these dose levels, which involved mainly the head and limbs. The NOAEL for maternotoxicity, embryo-foetotoxicity and teratogenicity was set at 4 mg/kg bw/d.

Based on this study, the classification of amitrole as reprotoxic category 3 (**Xn, Repr. Cat. 3 R63**) was agreed at European level by a specialised experts group (ECBI/59/00 - Add. 1 – Rev.2). Amitrole was thus classified reprotoxic category 2 according to the criteria of the regulation (EC) n°1272/2008 (**H361d**: Suspected of damaging the unborn child).

In a more recent study rabbits were exposed by gavage to dose levels of 0, 5, 20 and 80 mg/kg bw/d from gestation day 6 to 18. All dams were sacrificed on day 29. There was no treatment-related maternal death. In the 80 mg/kg bw/d group, the body weight gain, the feed consumption and water intake were reduced during the first week of treatment so that the NOAEL for maternal toxicity was set at 20 mg/kg bw/d. No teratogenic effect was observed.

The same dose levels were used in a special study to further characterize the toxicity in pregnant rabbits. The NOAEL for maternal toxicity was 5 mg/kg bw/d based on decreased absolute and relative liver weights and statistically significantly decreased albumin and protein levels seen at 20 and 80 mg/kg bw/d. At 80 mg/kg bw/d, there were reductions in food consumption, increased creatine kinase levels, decreased triiodothyronine and thyroxine levels and thyroid follicular cell hypertrophy.

A study to further investigate maternotoxicity in pregnant rabbits was performed in 2000 (see summary above, Richard 2000). In this study rabbits received the test substance as an aqueous solution, by oral gavage, at 0, 3, 15 or 75 mg/kg bw/d from day 6 to 18 *post-coitum*. At the highest dose level of 75 mg/kg bw/d, effects observed in dams were: reduced body weight gains, food consumption and water intake, decreased T3, T4 and cholesterol levels and morphological changes indicative of thyroid hyperactivity, which were also evident at 15 mg/kg bw/d. The NOAEL for maternal toxicity was thus set at 3 mg/kg bw/d. At 75 mg/kg bw/d, 3/66 fetuses from 2 different litters displayed a domed-head, probably related to dilatation of cerebral ventricles/hydrocephaly. A relationship to treatment with the test substance cannot be ruled out. The developmental NOAEL could thus be set at 15 mg/kg bw/d. This finding supports the classification as Xn, Repr Cat 3 R63 proposed before this study report termination.

In a dermal teratogenicity study in rabbits at dose levels of 0, 1000, 1500 and 2000 mg/kg bw/d, maternotoxicity (decreased body weight and food consumption, thin appearance and anorexia) was observed at 2000 mg/kg bw/d. At this level, irreversible structural changes (anencephaly and microphthalmia) were observed. The NOAEL for maternotoxicity, embryo/fetotoxicity and teratogenicity after dermal exposure was 1500 mg/kg bw/d.

B.6.7. DELAYED NEUROTOXICITY STUDY

No new data have been submitted. Please refer to original EU dossier.

B.6.8. FURTHER TOXICOLOGY STUDIES**B.6.8.1. Toxicity studies on metabolites**

No new data have been submitted. Please refer to original EU dossier.

B.6.8.2. Supplementary studies on the active substance**Literature review**

The applicant provided a literature review report on the toxicology of amitrole.

Objective of the review

The review was made in order to identify scientific peer-reviewed open literature on the toxicology of the active substance amitrole which may affect the assessment on human health and/or animal health.

Criteria of relevance applied

Publications meeting the relevance criteria are those showing new/unknown effects or information potentially contradictory to the regulatory data package for the active substance, its relevant metabolites and/or the plant protection products on human health, and/or animal health, which could impact the endpoints or the risk assessment parameters.

Publications providing information that do not challenge the existing regulatory data package will be treated as not meeting the relevance criteria.

The reliability assessment for relevant studies was done according to Klimisch et al¹.

Results

From the registration of amitrole, none of the published papers gives new/unknown information compared to data provided in the first monograph.

Position paper on the relevance of potential endocrine disrupting properties of amitrole for humans

According to the regulation (EC) N° 1272/2008, amitrole is currently classified as:

**Warning**

H 361d: Suspected of damaging the unborn child

H 373: May cause damage to organs (thyroid) through prolonged or repeated exposure

H 411: Toxic to aquatic life with long lasting effects

According to the provisional criteria set in annex II article 3.6 of regulation (EC) N° 1107/2009 it may be considered as a potential endocrine disruptor.

A position paper concerning the relevance of potential endocrine disrupting properties of amitrole for humans was provided. The summary of the applicant is reported below.

Report: IIA 5.11 Anonymous, 2012. Amitrole: why it should not be considered as an endocrine disruptor to Human? Nufarm's position paper.

¹ Klimisch, H.J., Andreas, M., Tillmann, U. 1997. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. Regulatory toxicology and pharmacology 25.1-5. Article n° RT961076

"In April 2004, Amitrole was considered as reprotoxic based on the results of one study in rabbits where malformations were observed at high materno-toxic dose (Directive 2004/73/EC). It is therefore classified as reprotoxic category 3 according to the criteria set out in directive 67/548/EEC or category 2 according to the criteria of the regulation N° 1272/2008.

On the other hand, it is known to have thyroid effects in rodents.

According to the provisional criteria set in Commission regulation 1107/2009/EC, amitrole **may** be therefore considered as a potential endocrine disruptor.

However due to large interspecies differences between rats and humans in the thyroid function, the extrapolation from rodent data to human should be considered as non-relevant.

In a workshop held in Germany from 11/11 to 13/11/2009 (Federal Institute for Risk Assessment, 2010), the BfR defined an Endocrine Disruptor (ED) as "an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations".

They proposed the following tier approach for determining whether a chemical can be considered as an ED:

- **to analyse the toxicological data obtained according to the requirements of the directive 91/414/EC and of all other available data.**

Amitrole was adequately tested according to the requirements of the directive 91/414/EC, all the available data were reviewed when amitrole was included in the Annex I of the directive 91/414/EC. Most studies were designed for investigating the major adverse effect of amitrole, an impairment of the thyroid function. In the different studies, when needed, T3, T4 and TSH levels were measured, thyroid weight was determined and the histopathological data included extensive examination of the pituitary-thyroid axis.

- **to establish the mechanism/mode of action of the chemical.**

In the sub-chronic and long-term studies, amitrole was shown to generate adverse thyroid effects.

The mechanism(s) of action of amitrole is well established : Amitrole acts on thyroid by blocking the iodine uptake and inhibiting thyroid peroxidase.

The inorganic form of iodine, iodide, is quickly and efficiently absorbed from the gastrointestinal tract and enters the follicular cells through the basal membrane. This uptake is rapidly followed by an oxidation reaction catalyzed by Thyroid peroxidase (TPO) resulting in synthesis of DIT (diiodotyrosine) and MIT (monoiodotyrosine). Within the protein thyroglobulin, iodine tyrosyl moieties (DIT and MIT) are combined to form the thyroid hormones T3 and T4 (cf figure 1).

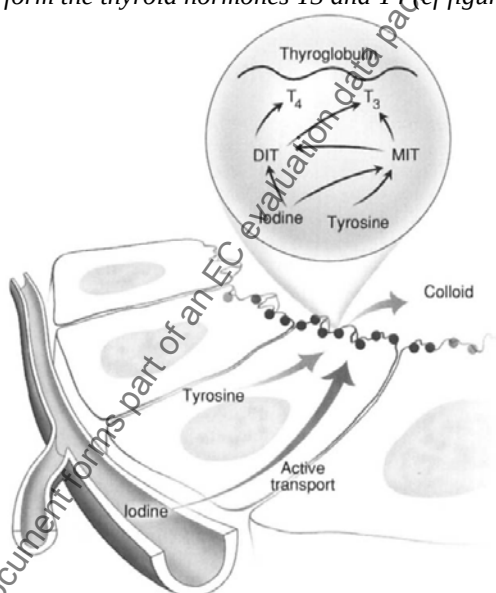


Figure 1: Uptake of iodide into the follicular cell by NIS. Oxidation reaction (catalysed by Thyroid Peroxidase) resulting in synthesis of DIT = diiodotyrosine; MIT = monoiodotyrosine; T₃ = triiodothyronine; T₄ = thyroxine (from Clifton-Bligh and Delbridge 2005).

By blocking the iodine uptake and inhibiting thyroid peroxidase, amitrole inhibits the thyroid hormone synthesis which results in a decrease in T3 and T4 blood levels and causes an increase in TSH production in pituitary gland. This hormone imbalance leads to cell hypertrophy / hyperplasia and can lead to thyroid tumour formation in rodents.

- **to investigate the relevance of the mechanism of action to humans. If a mechanism of toxicity in animals is identified that is clearly not relevant to humans, the decision procedure might be left at this step.**

Several interspecies differences were observed between human (and primates) and rodent such as iodide uptake, TPO activity, presence of a specific binding protein (TBG) with a high affinity for thyroid hormones, half-lives of T3 and T4, TSH levels and thyroid histology.

Indeed, the effects of substances like amitrole on thyroid function are expected to be of different nature in rodents and in primates including human for two sets of reasons:

- 1) **Different sensitivity of the thyroid:** Indeed, amitrole acts by blocking the iodine uptake and inhibiting thyroid peroxidase.
- a) **Iodide uptake:** Iodide is taken up from the extracellular space into the thyroid follicular cells by an active transport process. In the rat, the NIS protein is expressed at high density while in humans, NIS is seen in a "patchy" pattern.

- b) **Primates were shown to be far less sensitive to TPO inhibition than rodents are.**

Thyroxine peroxidase is the enzyme catalyzing the oxidation of inorganic iodide to iodine tyrosyl moieties that are combined to form thyroid hormones.

The inhibition of monkey TPO requires approximately 50 and 450 times the concentration of PTU and sulfamonomethoxine, respectively, than rat TPO does.

This striking inequality might explain the greater susceptibility of the rat to the anti-thyroid effects of such compounds compared to the primate.

- 2) **General physiology of the hypothalamus/pituitary/thyroid axis:**

In Human thyroid, the follicles are uniformly large with abundant colloid and are lined by relatively flattened follicular epithelial cells. In contrast, the rodent thyroid has large follicles only in the periphery of the gland. The interior is comprised of comparatively small follicles with small amounts of colloid surrounded by a more cuboidal follicular epithelium.

- a) **In rats there is a high hormone turnover** partially due to the absence or low concentration of the TBG, then a high proportion of free hormone with a high hormone clearance.

Once released in the peripheral blood, more than 99% of thyroid hormones are bound to different serum proteins: thyroxine-binding globulin (TBG), transthyretin (prealbumin) and albumin which prevents thyroid hormones from metabolic degradation and decreases renal elimination.

TBG is a glycoprotein containing only one binding site and is responsible for the transport of more than 75% of thyroid hormones (T3 & T4) in human.

In rats, TBG levels vary considerably with age. TBG is not found in rats between the ages of 2 and 7 months, the age range typically used in basic toxicology studies and is found at very low levels after 7 months. So that the major way for thyroid hormones transportation in blood is through binding to albumin which is a none specific carrier with a low affinity.

As hormone binding is generally accompanied by slower metabolic degradation of the hormone, the biological half-life of T4 and T3 in human plasma are longer than that of rats (5 and 10 times longer for T3 and T4 respectively).

- b) **The faster turnover of thyroid hormones in the rat results in an increased need for thyroid hormone production** (10 fold higher than in the adult human), which is maintained by higher circulating levels of TSH. All these make the rat more sensitive to any event which will impact hormone synthesis or catabolism.

These species-specific, physiological differences in the thyroid indicate that the rat is clearly more susceptible to thyroid perturbation than primates and humans are.

This difference in sensitivity has been also acknowledge by the Scientific Committee on Plants (SCP) in its opinion of 06/06/2000 who has already recognized that the thyroid tumours found in rats after long-term administration of amitrole was of little or no relevance to humans.

Due to all these interspecies differences between rodents and Human, the mode of action of amitrole in rodents is not considered as relevant for Human.

- **to make a decision on the endocrine properties of the chemical.**

Human exposure to amitrole is low.

Amitrole is a non-selective herbicide that is used on non-cropped areas, on cultivated areas after harvest and before sowing and/or planting as well as in orchards and vines (soil applications). Depending on crops, Amitrole can be applied two times in a year: at the end of winter (end of February/Beginning of March-April) in vineyards and in autumn (October-November) in orchards/olives.

It is applied with a tractor-mounted / trailed boom sprayer with hydraulic nozzles (spraying downward). A field operator study conducted on 30 operators showed that the 90th percentile of the results was below the Acceptable Operator Exposure Level (AOEL) (Thouvenin, I. 2012 see point IIIA7.3.3 of the European dossier). As the AOEL is really low (0.001 mg/kg bw/d) the operator exposure being below this AOEL can also be considered as very low.

The AOEL is based on thyroid effects observed after repeated administrations of amitrole to rats over 90 days. As rats are known to be more sensitive than humans to thyroid impairment this AOEL is consequently very low, thus ensuring a large margin of safety for the operators which are all the less likely to develop thyroid effects that they are exposed only once a year.

Maximum Residue Limits were set up by the Regulation 149/2008/EC (29/01/2008). Most of the MRL were fixed at 0.01 mg/kg. The only exceptions are olives (table and oil), 0.05 ppm, oilseeds as well as tea, coffee, herbal infusions and cocoa (0.02 ppm). Although the MRL exceeds in some cases the trigger value given in the regulation 396/2005/EC – 0.01 ppm – the dietary exposure of humans is once again limited (max of 50 % of the ADI set at 0.001 mg/kg).

- **Conclusion**

Taking into account the non-relevance to humans of the mode of action of amitrole on thyroid and the very low exposure of operators and / or of consumers, no endocrine effects are expected to occur in Human at the level of reasonably expected exposure and well beyond.

As a consequence, amitrole should not be considered as an endocrine disruptor for Human.”

RMS assessment on endocrine disrupting properties:

The mechanism of action of amitrole on thyroid is well known and it is accepted that humans are less sensitive than rodents. Amitrole exhibits thyroid effects in rat, mouse, dog, monkey and rabbit after oral, inhalation and dermal exposure, as well as in birds and fishes. A non-relevance of thyroid effects to humans cannot be completely ruled out.

B.6.9. MEDICAL DATA AND INFORMATION

B.6.9.1. Medical surveillance on manufacturing plant personnel

According to the notifier, each worker exposed to amitrole is medically followed every year. The following tests are performed:

- A blood analysis: complete blood count and blood biochemistry
- A lung X-ray photograph
- A clinical examination

The applicant reported two accidents occurring at the manufacturing plant during the handling of amitrole:

- One worker was packing amitrole in bags. When he closed one bag a cloud of amitrole was emitted. He breathed it in and eventually ate some of the product. He was sent to hospital for control and was released in the afternoon. No particular treatment was administered.

- Another worker, in order to close a bag of 25kg of amitrole, displaced air contained in the bag by pressing with dimensions bag. Amitrole dust was projected out of the bag. And felt into his eyes. In order to avoid any irritation, the eyes were washed with Diphoterine (lava eye). The worker went to see an ophthalmologist for check-up. No medical treatment was necessary.

B.6.9.2. Clinical cases and poisoning incidents

The notifier reported that a case of fatal poisoning was reported since the first review of amitrole². A 54-year-old man was hospitalized because of unexplained coma with myoclonic jerks and vascular collapse. Despite symptomatic treatment with mechanical ventilation and vascular filling, life-threatening shock occurred with oliguria, profound metabolic acidosis and cardiac arrest.

Hyperchloremia (141 mmol/L) with reversed anion gap (-19) suggested interference with chloride measurement caused by halogens (Br,F,I) or other anions such as thiocyanate. Eventually a weed killer, Radoxone TL containing amitrole and ammonium thiocyanate, was found at the patient's house. Thiocyanate and amitrole blood levels were 750 mg/L and 138 mg/L respectively more than 12 hours after ingestion. After prolonged cardiopulmonary resuscitation, continuous venovenous hemodiafiltration was performed. Despite hemodynamic recovery the patient died 48 hours later of postanoxic coma.

The conclusions of this paper were that amitrole, a systemic nonselective herbicide, is often associated with ammonium thiocyanate which enhances its activity. Experimental studies and previous fatal cases suggest a predominant toxicity of thiocyanate. Early diagnosis is important.

B.6.9.3. Observations on exposure to the general population

No new data. Please refer to original EU dossier.

B.6.9.4. Clinical signs and symptoms of poisoning and details on clinical tests

No new data. Please refer to original EU dossier.

B.6.9.5. First aid measures and therapeutic regimes

No new data. Please refer to original EU dossier.

B.6.9.6. Expected effects of poisoning

No new data. Please refer to original EU dossier.

B.6.10. SUMMARY OF MAMMALIAN TOXICITY AND OVERALL EVALUATION

B.6.10.1. Summary of mammalian toxicity

Absorption, distribution, metabolism and excretion

Pharmacokinetic studies were conducted in male and female mice, rats and rabbits.

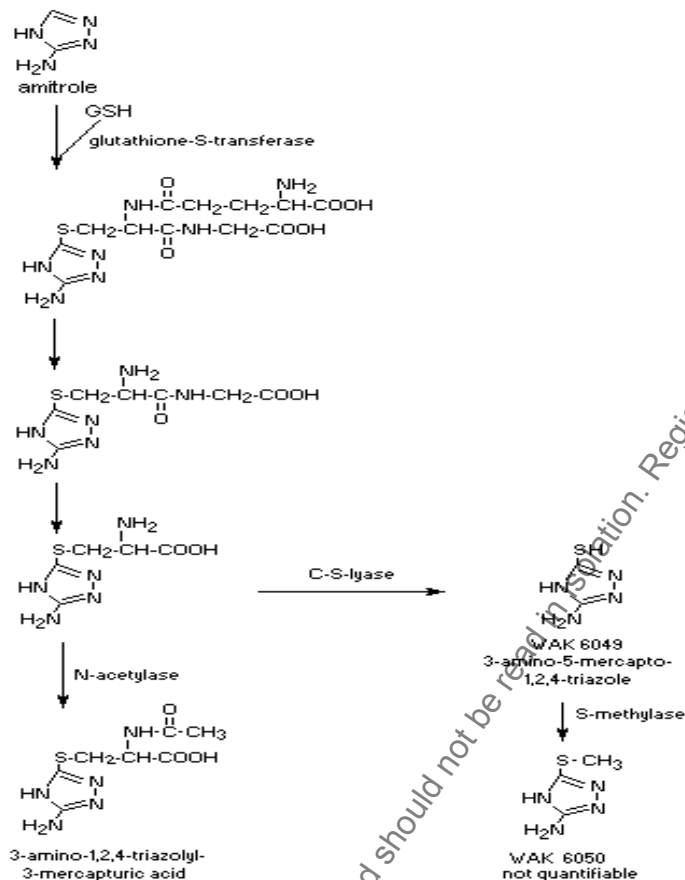
Following oral administration, amitrole is readily absorbed from the gastro-intestinal tract of mammals. It is rapidly excreted from the body mainly as the parent compound. The main route of excretion in humans and laboratory animals is via the urine and the majority of excretion takes place during the first 24h.

This molecule is a polar molecule which is mostly excreted unchanged in urine. Phase II reactions can occur and consist in conjugation to glutathion through the glutathione transferase. Other enzymes like N-acetylase or CS

² Annick Legras, Didier Skrobala, Yves Furet, Pascal Kintz, Elisabeth Forveille, Pierre-Francois Dequin and Dominique Perrotin. Herbicide: Fatal Ammonium Thiocyanate and Aminotriazole Poisoning. 1996. Clinical toxicology. Vol. 34, No. 4, Pages 441-446

lyase are also involved. N-acetylase leads to the formation of 3-amino-1,2,4-triazolyl-5-mercaptopuric acid in mammals whereas the CS lyase action gives rise to 3-amino-5-mercapto-1,2,4-triazole. The reactions are shown in figure 1.

Figure 1. Proposed metabolic pathway of amitrole in rats



Acute toxicity

Technical amitrole is of low oral and dermal toxicity in rats ($\text{LD}_{50} > 5000 \text{ mg/kg b.w}$ and $> 2500 \text{ mg/kg b.w}$, respectively).

The acute toxicity was also very low when administered by intravenous route in rats and mice.

Acute inhalative exposure to the highest technically producible aerosol concentration (439 mg/m^3) was tolerated by rats without any adverse effect. The 4-hour LC_{50} was set to be greater than 0.439 mg/L (highest attainable concentration).

Technical amitrole is not irritant to the skin and slightly irritant to the eyes. Amitrole was shown not to be a skin sensitizer in a Magnusson and Kligmann test.

Short-term toxicity

Repeated oral administration of amitrole results mainly in effects on thyroid. It is currently well known that thyroid toxicity of amitrole is due to inhibition of thyroid peroxidase. Consequently rats treated with amitrole have low serum T4 and T3 levels with a parallel increase in TSH levels. Amitrole interferes with the production of thyroid hormones thus leading to hypertrophy and hyperplasia of the follicular cells of the thyroid. That can result in tumours of this gland in rodents after a prolonged administration. Evidence for reversibility of the thyroid effect in the rat was observed when the exposure was of short duration.

After 4 weeks of administration by oral route, thyroid effects are observed at levels greater than 1.5 mg/kg bw/d in rats (LOAEL of 5 mg/kg bw/d).

Three short-term studies were conducted in rats (one 10-week study and two 90-day studies), two one-year studies were carried out in dogs and a 90-day study was performed in monkeys.

In the 10-week study, the NOAEL was set at 1.5 mg/kg bw/d in females and less than 1.5 mg/kg bw/d in males, based on increase of TSH levels, follicular cell hypertrophy, small vesicles and increase vascularity observed from 12 mg/kg bw/d in females and 1.5 mg/kg bw/d in males. In the two 90-day studies (one performed in males only and the other in females only), the NOAEL were set at 0.1 mg/kg bw/d in males and females, based on thyroid effects (histological changes in the thyroid regarding appearance of follicular cells, content of colloid and capillary density in males; in females: increased iodine uptake shortly after injection, increased thyroid weight and histopathological changes of the thyroid).

Although no thyroid effects were observed in a 1-year dog study (NOAEL of 12.5 mg/kg bw/d, the highest dose level where only pale coloured pancreas was noted), thyroid parameters were affected from the LOAEL of 13 mg/kg bw/d in the other 1-year dog study. This consisted in enlarged thyroid, increased absolute and relative thyroid weight and follicular cells hyperplasia. The NOAEL was established at 0.3 mg/kg bw/d in this study.

A 90-day toxicity study was performed in cynomolgus monkey by oral route. The NOAEL were set at less than 30 mg/kg bw/d in males and at 100 mg/kg bw/d in females, based on thyroid effects observed from 30 mg/kg bw/d in males and 330 mg/kg bw/d in females.

Overall a NOAEL of 0.1 mg/kg bw/d for thyroid effects by oral route can be considered in the short-term studies.

By dermal route a NOAEL of 100 mg/kg bw/day was established in a 28-day study in rats based on thyroids effects (slightly higher TSH level and slightly decreased T3 and T4 levels, higher mean absolute and relative thyroid weight, morphological indication of gland hyperactivity) observed at 500 mg/kg bw/day. In rabbits no adverse effect was reported until the dose of 100 mg/kg bw/day.

By inhalation, a NOEC of 0.1 mg/L was established in a 28-day study in rats based on thyroids effects (decreased T3 and T4 level, thyroid hyperplasia and elevated thyroid weight) observed at 0.32 mg/L. However this NOEC was established based on the aerosol concentration in the inhalation chamber. The particle size distribution was not evaluated so that no information is given regarding the respirable fraction. Therefore the fraction of the aerosol concentration that was effectively inhaled cannot be determined in order to derive a systemic NOEL.

Genotoxicity

Amitrole has been extensively tested in *in vitro* and *in vivo* genotoxicity studies. If positive results were obtained in some *in vitro* tests, no genotoxicity was found *in vivo*.

By weight of evidence, amitrole is not a genotoxic compound. This was confirmed by recent *in vitro* gene mutation and chromosomal aberration tests.

Chronic toxicity/carcinogenicity

Long term and/or carcinogenicity studies have been performed in rats, mice and golden hamsters.

A first 2-year oral toxicity and carcinogenicity in the rat was considered as informative; it was indeed difficult to assess it because of several changes in dosing regimen. Nevertheless, thyroid appeared as the target organ (thyroid hyperplasia were seen in all treated groups, increased incidence of thyroid tumours (mainly follicular adenomas)).

In a carcinogenicity study in rats fed with dose levels of 0, 1, 10 and 100 ppm, a slight decrease in survival time, an increase in the incidence of (benign and malignant) thyroid tumours, and an increase of the incidence of (mainly benign) pituitary gland tumours was observed at 100 ppm. In this study a thyroid function was also performed in a small number of animals. At 100 ppm (5 mg/kg bw/d) thyroid weight was increased during the whole study period as was the percentage of accumulation of radioiodine in the thyroid. The NOAEL was set at 10 ppm (equivalent to 0.5 mg/kg bw/d).

In a 18-month carcinogenicity study in mice at level of 0, 1, 10 and 100 ppm in the diet, increased incidence of tumours was not observed. In this study a thyroid function was also performed in a small number of animals. At 100 ppm (15 mg/kg bw/d) an increase in thyroid weight and in iodine accumulation in the thyroid was observed. The NOAEL was set at 10 ppm (equivalent to 1.5 mg/kg bw/d).

In a 18-month carcinogenicity study in Syrian hamsters at level of 0, 1, 10 and 100 ppm in the diet, the NOAEL was set at 10 ppm (equivalent to 1 mg/kg bw/d) based on decreased body weight gain at 10 mg/kg bw/d. No effect on thyroid was observed. There was no evidence of carcinogenic potential.

The Scientific Committee on Plants evaluated amitrole in 2000 in the context of the inclusion of amitrole in Annex I to council directive 91/414/EEC (SCP/AMITR/002-Final, 13 July 2000) and concluded that:

“In conclusion, since

- a. the thyroid carcinogenic effect of amitrole appears to be mediated through thyroid-pituitary disruption to which humans are less sensitive*
 - b. there does not seem to be a role for increased TSH in human thyroid carcinogenicity,*
- the thyroid tumours observed in rats after long term amitrole treatment appear to be of little or no relevance for humans.”*

Corroborating this opinion, IARC Overall evaluation (2001) downgraded amitrole to Group 3 (not classifiable as to its carcinogenicity to humans) with supporting evidence from relevant data.

It was concluded that the thyroid tumours observed in rats were not considered relevant for human and that amitrole does not need to be classified for carcinogenicity.

Reproductive toxicology

In two generations studies it was shown that amitrole was not toxic to F0 and F1 generation up to 2 ppm and did not induce any effects on the reproductive performance of F0 and F1, nor impaired the development of F1 and F2 offspring up to 15 ppm. The dose of 15 ppm was slightly toxic for the F0 (males only) and F1 parental animals as shown by minimal increase of thyroid activity. The 112.5 ppm dose level was severely toxic for the F0 and F1 parents, impaired their fertility and disturbed the development of F1 and F2 litters. Nevertheless all findings on reproduction correlated with severe systemic parental toxicity, so that a primary effect on reproduction was excluded.

A NOAEL was set at 15 ppm (0.9 mg/kg bw/d) for reproductive toxicity and at 2 ppm (0.12 mg/kg bw/d) for parental toxicity.

Teratogenicity studies were conducted in rats (by oral route) and rabbits (by oral and dermal routes).

In rats exposed by gavage to dose levels of 0, 100, 500 and 1000 mg/kg bw/d, slight maternotoxicity was observed at 500 and 1000 mg/kg bw/d (reduced body weight gain and food consumption, increased thyroid weight). Reduced fetal body weight/litter and reduced skeletal ossifications were observed in the high-dose group. Increased incidences of enlarged and/or dark thyroids were seen in foetuses at 500 and 1000 mg/kg bw/d. The NOAEL for maternotoxicity and embryo/fetotoxicity was set at 100 mg/kg bw/d. Amitrole was considered not to be teratogenic in rats at doses levels up to 1000 mg/kg bw/d.

In rabbits exposed by gavage to dose levels of 0, 4, 40 and 400 mg/kg bw/d, a dose related increased incidence of abortions was observed in all treated groups. Decrease body weight gain during the gestation period was observed at 40 and 400 mg/kg bw/d and increased liver weight at 400 mg/kg bw/d. Embryo/foetotoxicity were observed at 40 and 400 mg/kg bw/d. Increased incidences of irreversible structural changes were also found at these dose levels, which involved mainly the head and limbs. The NOAEL for maternotoxicity, embryo-foetotoxicity and teratogenicity was set at 4 mg/kg bw/d.

Based on this study, the classification of amitrole as reprotoxic category 3 (**Xn, Repr. Cat. 3 R63**) was agreed at European level by a specialised experts group (ECBI/59/00 – Add. 1 – Rev.2). Amitrole was thus classified reprotoxic category 2 according to the criteria of the regulation (EC) n°1272/2008 (**H361d**: Suspected of damaging the unborn child).

In a more recent study rabbits were exposed by gavage to dose levels of 0, 5, 20 and 80 mg/kg bw/d from gestation day 6 to 18. All dams were sacrificed on day 29. There was no treatment-related maternal death. In the 80 mg/kg bw/d group, the body weight gain, the feed consumption and water intake were reduced during the first

week of treatment so that the NOAEL for maternal toxicity was set at 20 mg/kg bw/d. No teratogenic effect was observed.

The same dose levels were used in a special study to further characterize the toxicity in pregnant rabbits. The NOAEL for maternal toxicity was 5 mg/kg bw/d based on decreased absolute and relative liver weights and statistically significantly decreased albumin and protein levels seen at 20 and 80 mg/kg bw/d. At 80 mg/kg bw/d, there were reductions in food consumption, increased creatine kinase levels, decreased triiodothyronine and thyroxine levels and thyroid follicular cell hypertrophy.

A study to further investigate maternotoxicity in pregnant rabbits was performed in 2000 (see summary above, Richard 2000). In this study rabbits received the test substance as an aqueous solution, by oral gavage, at 0, 3, 15 or 75 mg/kg bw/d from day 6 to 18 *post-coitum*. At the highest dose level of 75 mg/kg bw/d, effects observed in dams were: reduced body weight gains, food consumption and water intake, decreased T3, T4 and cholesterol levels and morphological changes indicative of thyroid hyperactivity, which were also evident at 15 mg/kg bw/d. The NOAEL for maternal toxicity was thus set at 3 mg/kg bw/d. At 75 mg/kg bw/d, 3/66 fetuses from 2 different litters displayed a domed-head, probably related to dilatation of cerebral ventricles/hydrocephaly. A relationship to treatment with the test substance cannot be ruled out. The developmental NOAEL could thus be set at 15 mg/kg bw/d. This finding supports the classification as Xn, Repr Cat 3 R63 proposed before this study report termination.

In a dermal teratogenicity study in rabbits at dose levels of 0, 1000, 1500 and 2000 mg/kg bw/d, maternotoxicity (decreased body weight and food consumption, thin appearance and anorexia) was observed at 2000 mg/kg bw/d. At this level, irreversible structural changes (anencephaly and microphtalmia) were observed. The NOAEL for maternotoxicity, embryo/fetotoxicity and teratogenicity after dermal exposure was 1500 mg/kg bw/d.

Based on thyroid effects observed in several species, amitrole is classified as **Xn, R48/22** (Harmful: danger of serious damage to health by prolonged exposure if swallowed) and **H373** (May cause damage to thyroid through prolonged or repeated exposure, STOT-RE Cat. 2) in the regulation (EC) n°1272/2008.

The sub-chronic, chronic and reproductive toxicological studies are summarized in the following table.

Species / study	Doses	NOAEL	LOAEL / effects	Reference
Rat 28-day Oral (+28-d recovery period)	Amitrole (94.6%) 0, 30, 100, 300 ppm equivalent to 1.5, 5 and 15 mg/kg bw/d	1.5 mg/kg bw/d (30 ppm)	LOAEL: 100 ppm (equivalent to 5 mg/kg bw/d): decreased body weight and food consumption, decrease T3 and T4 levels. All parameters returned to normal levels during the recovery period. 500 ppm (15 mg/kg bw/d): decreased body weight and food consumption, decrease T3 and T4 levels.	
Rat 28-day Dermal	Amitrole 0, 10, 50 the first days then 100 and 500 mg/kg bw/d	100 mg/kg bw/d	LOAEL: 500 mg/kg bw/d: lower lymphocyte counts in females only, slightly higher TSH level and slightly decreased T3 and T4 levels, slightly higher absolute and relative thyroid weight, indication of gland hyperactivity.	
Rabbit 28-day Dermal	Amitrole 0, 25 and 100 mg/kg bw/d	>100 mg/kg bw/d	No adverse effect was observed in any of the treated groups	
Rat 28-day Inhalation	Amitrole (94.59%) 1 st phase: 0, 1 and 5 mg/L 2 nd phase: 0, 0.1 and 0.32 mg/L	0.1 mg/L (NOEC)	LOAEL: 0.32 mg/L: thyroid effects: decreased T3 and T4 level, thyroid hyperplasia and elevated thyroid weight	
Rat 90-day Oral (gavage)	Amitrole (91.8 %) 0, 1.5 for 8 weeks then 330 mg/kg bw/d for 2 weeks, 12 and 100 mg/kg bw/d	M : <1.5 mg/kg bw/d F : 1.5 mg/kg bw/d	LOAEL: 1.5/330 mg/kg bw/d in males and 12 mg/kg bw/d both sexes: moderate increase (x 2.6) of TSH levels. Follicular cell hypertrophy, small follicles and increase vascularity (less severe in the 12 mg/kg b.w. group) 100 mg/kg b.w: Piloerection, pallor of extremities and hypokinesia observed only in males Decrease in body weight gain and food consumption Lipid metabolism disruption, decrease of T3 & T4 and increase of TSH, higher absolute and relative weight of the thyroid (both sexes) and of the pituitary gland (males), follicular cell hypertrophy, small follicles and increase vascularity.	
Rat (males) 90-day Oral	Amitrole (supplied by American Cyanamid Company) <u>1st experiment</u> 0, 2, 10 and 50 ppm equivalent to 0.11, 0.58 and 2.85 mg/kg b.w. for 13 weeks <u>2nd experiment</u> 0.25 and 0.50 ppm for 11 weeks	0.11 mg/kg bw/d (2 ppm)	LOAEL: 10 ppm equivalent to 0.58 mg/kg bw/d: thyroid effect, smaller follicle size, lower content in colloid, increased vascularity 50 ppm equivalent to 2.85 mg/kg bw/d: decrease iodide intake, very small size of the thyroid follicles, lower content in colloid, increased vascularity	

Species / study	Doses	NOAEL	LOAEL / effects	Reference
Rat (females) 90-day Oral	Amitrole <u>1st experiment</u> 0, 2, 20 and 200ppm for 6 weeks <u>2nd experiment</u> 0, 20, 50 and 200 ppm for 6 weeks <u>3rd experiment</u> 0, 20, 50 and 200 ppm for 13 weeks <u>4th experiment</u> 0, 20, 50, 200 and 500 ppm for 6 weeks (2 ppm iodide in diet)	0.1 mg/kg bw/d (2 ppm)	LOAEL: 20 ppm equivalent to 1 mg/kg bw/d: tendency to higher uptake of ¹³¹ I and slight activation of the gland 6 h after the iodine injection. Then decrease of iodine uptake 24 and 48h after injection. 200 ppm: increased uptake of ¹³¹ I, increased thyroid weight and goitre 6 h after the iodine injection. Then decrease of iodine uptake 24 and 48h after injection.	
Monkey 90-day Oral	Amitrole (91.8%) 0, 30 for 8 weeks then 330 mg/kg for 4 weeks, 100 and 300 mg/kg bw/d Additionnal group: increasing dose level from 1500 to 4000 mg/kg bw/d for 3 weeks	M : < 30 mg/kg bw/d F : 100 mg/kg bw/d	LOAEL males : 30 mg/kg bw/d: thyroid effects: decrease in T3 & T4 levels, increased TSH level, higher absolute and relative thyroid weight, darkness of thyroid 100 mg/kg bw/d in males: in addition to the observations described above, morphological changes indicative of hyperactivity of the thyroid (follicular cells hypertrophy and hyperplasia, increased vascularity) are observed. LOAEL females: 330 mg/kg bw/d: thyroid effects: decrease in T3 & T4 levels, increased TSH level, higher absolute and relative thyroid weight, darkness of thyroid. Morphological changes indicative of hyperactivity of the thyroid (follicular cells hypertrophy and hyperplasia, increased vascularity).	
Dog 1-year Oral	Amitrole 0, 0.25, 1.25, 2.5 and 12.5 mg/kg bw/d	12.5 mg/kg bw/d	The only observed effect was a pale coloured pancreas at 12.5 mg/kg bw/d	
Dog 1-year Oral	Amitrole 0, 10, 500 and 1500 ppm equivalent to 0.3, 13 and 30 mg/kg bw/d	0.3 mg/kg bw/d (10 ppm)	LOAEL: 500 ppm equivalent to 13 mg/kg bw/d: clinical chemistry changes, thyroid parameters slightly affected (enlarged thyroid, increased absolute and relative thyroid weight, follicular cells hyperplasia) 1000 ppm (30 mg/kg bw/d): reduced food consumption, lethargy, alopecia, clinical parameters and hematological changes, organ weight changes, thyroid effects (enlarged thyroid, increased absolute and relative thyroid weight), micropathological lesions: follicular cells hyperplasia, minimal to slight hypertrophy and vacuolisation of pituitary cells	
Rat	Amitrole (94.6%)	Not determined due to	Follicular epithelial hyperplasia in the thyroid in all treated animals, increased incidence of thyroid	

Species / study	Doses	NOAEL	LOAEL / effects	Reference
2-year (toxicity and carcinogenicity) Oral	Group A: control Group B: 5 ppm for 39 weeks then 100 ppm during weeks 40-115 for males and 40-119 for females. Group C: 1 ppm for 39 weeks then 20 ppm Group D: 3 ppm for 39 weeks then 60 ppm Group E: 10 ppm for 39 weeks then 200 ppm Groups C, D, E were treated at pulsed intervals of 4 weeks (4 weeks treated and 4 weeks with basic diet containing no amitrole)	complicated protocol	tumours (mainly follicular adenomas) from 3 ppm in males and from 5 ppm in females.	1981
Rat Carcinogenicity (2-year) Oral	Amitrole (97.0%) 0, 1, 10, 100 ppm	0.5 mg/kg bw/d (10 ppm)	LOAEL: 100 ppm equivalent to 5 mg/kg bw/d: Slight decrease in survival time, increased thyroid weight, increased uptake of ¹³¹ I, elevated incidence of hemorrhages and hyperaemia of the pituitary gland, very high rate of cystically dilated thyroid follicles, presence of both benign and malignant tumours of the thyroid, increased incidence of benign tumours in pituitary gland.	
Mouse Carcinogenicity (18-month) Oral	Amitrole (97.0%) 0, 1, 10, 100 ppm	1.5 mg/kg bw/d (10 ppm)	LOAEL: 100 ppm equivalent to 15 mg/kg bw/d: Increased thyroid weight, increased percentage of iodine accumulation and iodine levels, elevated percentage of protein-bound iodine (PBI) in plasma 9 months after study initiation then decreased percentage of PBI. These effects are significant in males and are also observed in females but are not significant in most cases Slight increase in the number of hyperaemia of the pituitary gland.	
Hamster Carcinogenicity (18-month)	Amitrole (97.0%) 0, 1, 10, 100 ppm	1 mg/kg bw/d (10 ppm)	LOAEL: 100 ppm equivalent to 10 mg/kg bw/d: Decrease body weight from day 400 onward. Increased mortality due to severe amyloidosis in kidneys (observed also in the control group). No treatment-related histopathological changes, no treatment-related carcinogenic effect. No treatment-related effect on thyroid.	
Rat Multi-generation Oral	Amitrole (97.0 - 98.0%) 0, 0.5, 2, 15 and 112.5 ppm equivalent to: F0 males: 0, 0.03, 0.12,	Parental toxicity: 0.12 mg/kg bw/d (2 ppm) Reprotoxicity:	LOAEL : 15 ppm equivalent to 0.9 mg/kg bw/d: Minimal increase of thyroid activity (follicular cells hyperplasia and adenomas) in F0 males only and F1 parents 112.5 ppm: F0 : Decreased food consumption and decreased body weights in males, thyroid effects (thyroid	

Species / study	Doses	NOAEL	LOAEL / effects	Reference
	0.90 and 5.88 mg/kg bw/d F0 females: 0, 0.04, 0.16, 1.23 and 7.83 mg/kg bw/d F1 males: 0, 0.04, 0.16, 1.24 and 12.02 mg/kg bw/d F1 females: 0, 0.05, 0.21, 1.64 and 15.62 mg/kg bw/d	0.9 mg/kg bw/d (15 ppm)	enlargement, follicular cell adenomas), hepatocellular atrophy and microscopic changes in various organs. Lower mating and fertility indexes. F1 (parents): hypoactivity, piloerection and dyspnea, death (30% males and 30% females), decreased food consumption and decreased body weights in males, thyroid effects (thyroid enlargement, follicular cell adenomas), hepatocellular atrophy and microscopic changes in various organs. Lower mating and fertility indexes, longer gestation for F1 F1 and F2 pups: reduced number of implantation sites (→ reduced number of live born pups), decreased body weight, increased thyroid weight, lower absolute and relative weight of various organs	
Rat Teratogenicity Oral	Amitrole (91.83%) 0, 100, 500 and 1000 mg/kg bw/d	Maternal and foetal toxicity: 100 mg/kg bw/d	LOAEL: 500 mg/kg bw/d: reduced body weight gains and food consumption, increased thyroid weight Enlarged and dark thyroid in fetuses 1000 mg/kg bw/d : Reduced body weight gains and food consumption, increased thyroid weight. Reduced fetal body weight per litter, reduction in the degree of ossification of bones, enlarged and dark thyroid No evidence of teratogenesis	
Rabbit Teratogenicity Oral	Amitrole (91.83%) 0, 4, 40 and 400 mg/kg bw/d	Maternal toxicity, foetal toxicity and teratogenicity: 4 mg/kg bw/d	LOAEL: 40 mg/kg bw/d: reduced body weight gains, increased incidence in the abortions, increased number of non viable implants/litter, decreased number of live foetuses/litter, malformations of the head and limbs, increased number of visceral and skeletal variants, thyroid effects (enlarged and dark thyroid) 400 mg/kg bw/d: reduced body weight gains, increased relative liver weight, increased incidence in the abortions, increased number of non viable implants/litter, decreased number of live foetuses/litter, decreased fetal weight/litter, malformations of the head and limbs, increased number of visceral and skeletal variants, thyroid effects (enlarged and dark thyroid)	
Rabbit Teratogenicity Oral	Amitrole (97.5%) 0, 5, 20 and 80 mg/kg bw/d	Maternal toxicity and developmental toxicity: 20 mg/kg bw/d Teratogenicity: 80 mg/kg bw/d	LOAEL: 80 mg/kg bw/d: reduced body weight gains, food consumption and water intake No evidence of teratogenicity	
Rabbit	Amitrole (97.5%)	Maternal toxicity: 5 mg/kg bw/d	20 and 80 mg/kg bw/d: decreased albumin and protein levels, decreased absolute and relative liver weights	

Species / study	Doses	NOAEL	LOAEL / effects	Reference
Teratogenicity (supplementary study) Oral	0, 5, 20 and 80 mg/kg bw/d		80 mg/kg bw/d: reductions in food consumption, increased creatine kinase level, decreased plasma levels of T3 and T4, thyroid follicular cell hypertrophy and increased number of Kupffer cell foci in the liver.	
Rabbit Teratogenicity Oral (study of maternotoxicity)	Amitrole (92.3%) 0, 3, 15 and 75 mg/kg bw/d	Maternal toxicity: 3 mg/kg bw/d Developmental toxicity: 15 mg/kg bw/d	LOAEL: 15 mg/kg bw/d: thyroid hyperactivity (morphological changes) in dams 75 mg/kg bw/d: in dams: reduced body weight gains, food consumption and water intake, decreased T3, T4 and cholesterol levels, morphological changes indicative of thyroid hyperactivity, in fetuses: fetal malformations: domed head probably related to dilatation of cerebral ventricles/hydrocephaly [3/66 fetuses from 2 different litters], a relationship to treatment with the test substance cannot be ruled out	
Rabbit Teratogenicity Dermal	Amitrole (93.9%) 0, 1000, 15000 and 2000 mg/kg bw/d	Maternal toxicity, developmental toxicity and teratogenicity: 1500 mg/kg bw/d	LOAEL: 2000 mg/kg bw/d: dermal irritation, thin appearance and anorexia of does, transient reduction in body weight, decreased food consumption, slightly reduced uterine weight, reduced fetal weight, increased number of early resorption, malformations (anencephaly and microphthalmia)	

Mode of action and mechanism of action leading to toxicity

Amitrole acts on thyroid by blocking the iodide uptake and inhibiting thyroid peroxidase.

The inorganic form of iodine, iodide, is quickly and efficiently absorbed from the gastrointestinal tract and enters the follicular cells through the basal membrane. This uptake is rapidly followed by an oxidation reaction catalyzed by Thyroid Peroxidase (TPO) resulting in synthesis of DIT (diiodotyrosine) and MIT (monoiodotyrosine). Within the protein thyroglobulin, iodine tyrosyl moieties (DIT and MIT) are combined to form the thyroid hormones T₃ and T₄ (cf figure 2).

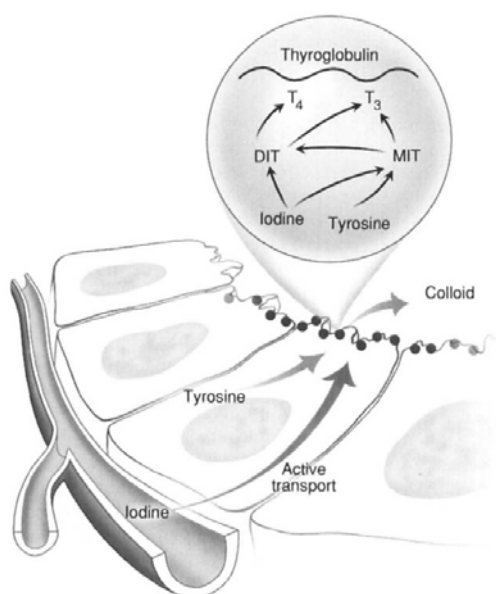


Figure 2: Uptake of iodide into the follicular cell by NIS (Sodium Iodide Symporter). Oxidation reaction (catalysed by Thyroid Peroxidase) resulting in synthesis of DIT = diiodotyrosine; MIT = monoiodotyrosine; T₃ = triiodothyronine; T₄ = thyroxine (from Clifton-Bligh and Delbridge 2005).

By blocking the iodine uptake and inhibiting thyroid peroxidase, amitrole inhibits the thyroid hormone synthesis which results in a decrease in T₃ and T₄ blood levels and causes an increase in TSH (Thyroid Stimulating Hormone) production in pituitary gland. This hormone imbalance leads to cell hypertrophy/hyperplasia and can lead to thyroid tumour formation.

B.6.10.2. Acceptable daily intake (ADI)

The ADI was set at 0.001 mg/kg bw/d based on thyroid effects observed in the 90-day rat studies and by using a safety factor of 100 (Review Report (6839/VI/97-final 22 march 2001)).

B.6.10.3. Acceptable operator exposure level (AOEL)

The AOEL was set at 0.001 mg/kg bw/d based on thyroid effects observed in the 90-day rat studies and by using a safety factor of 100 (Review Report (6839/VI/97-final 22 march 2001)).

B.6.10.4. Acute reference dose (ARfD)

When amitrole was first peer-reviewed (Review Report (6839/VI/97-final 22 march 2001)), the setting of an acute reference dose was not deemed relevant in view of the hazard profile of the substance.

An ARfD should be set based on the lowest NOAEL observed in the short-term and teratology toxicity studies. The most relevant study appear to be the 28-day toxicity study performed in rat, where the NOAEL was 1.5 mg/kg bw/d, based on decreased body weight and food consumption and decreased T3 and T4 levels. Therefore the following ARfD can be derived, with a safety factor of 100:

$$\text{ARfD} = \text{NOAEL} / \text{SF} = 1.5 \text{ mg/kg bw/d} / 100 = 0.015 \text{ mg/kg bw}$$

B.6.11. ACUTE TOXICITY INCLUDING IRRITANCY AND SKIN IRRITATION OF THE PLANT PROTECTION PRODUCT

The representative formulation for the first annex I inclusion was a soluble concentrate (CA0123) containing 229 g/L of amitrole and 215 g/L of ammonium thiocyanate (specific synergist). Further to publication of Directive 2003/53/EC dealing with the restrictions on the marketing and use of nonylphenol and nonylphenol ethoxylate (NPE), a formulation without NPE was developed (CA2210). In order to demonstrate that the toxicological profiles were comparable between these two formulations, an acute oral toxicity study in rats as well as an eye irritation test in rabbits and a skin sensitization assay in guinea pigs (Magnusson and Kligman test method) were performed on CA2210 and are summarized below.

For this current annex I renewal process the representative formulation is still a soluble concentrate containing 229 g/L of amitrole and 215 g/L of ammonium thiocyanate (CA2735). Compared with CA0123 and CA2210, minor changes were made in formulation CA2735 (please refer to the detailed compositions submitted in Volume 4). Therefore the toxicological studies that were conducted on CA0123 and CA2210 are considered to cover the toxicological profile of CA2735.

The results of the 3 new studies, together with the results of the studies performed on CA0123 and detailed in the original draft assessment report, are reported in the following table.

Table B.6.11-1: Summary of acute toxicity studies performed on CA0123 and CA2210

Study type	OECD guidelines	Endpoints obtained with CA0123	References (summarized in the original monograph)	Endpoints obtained with CA2210	References (summarized below)
Acute oral toxicity in rats	401 and 423	LD ₅₀ > 2000 mg/kg bw	1995	LD ₅₀ > 2000 mg/kg bw	2004a
Acute dermal toxicity in rats	402	LD ₅₀ > 2000 mg/kg bw	1995	-	-
Skin irritation test in rabbits	404	Not irritating	1987	-	-
Eye irritation study in rabbits	405	Irritating for eyes (Xi, R36)	1987	Non irritant	1.2004b
Skin sensitization in guinea pigs (Magnusson and Kligman test method).	406	Not sensitizing	1995	Not sensitizing	2004b

CA0123 and CA2210 are considered to be similar to CA2735 in terms of composition. Indeed, wetting agents and antifoam agents included in CA0123 and/or CA2210 formulations are removed from CA2735 and are replaced by water. No new formulant was added to CA2735 formulation. Therefore it is considered adequate to rely on acute toxicity data from CA0123 and CA2210 to support CA2735.

According to these studies, CA0123 has to be classified for eye irritation whereas CA2210 is not irritating for eye. The changes observed in the composition of these 2 formulations are not able to lead to such a difference in terms of eye irritation. As this difference cannot be explained and as CA2735 should be classified as Xi, R41 if the calculation method is considered, taking into account the results of these two studies performed on similar formulations, CA2735 should be classified as Xi, R36 as a worst-case.

Considering the classification of the active substance and the non-active ingredients and their quantities in the preparation and taking into account the results of acute toxicity studies performed on CA0123 and CA2210, as a worst-case, CA2735 formulation requires the following toxicological classification:

Xn, Repr Cat 3 R63 R48/22 R36.

B.6.11.1. Acute oral toxicity in rats

Report: IIIA7.1.1: 2004a. CA2210 - Acute oral toxicity study in the rat: Acute toxic class method.

GLP: Yes
Not published
Guideline: OECD 423

Test substance: CA 2210
Batch n°: RD-816-166B (229 g/L amitrole)

The test substance CA2210 was administered as a single dose by gavage to Sprague-Dawley rats. In a first step three females were treated at the limit dose of 2000 mg/kg bw, followed by three additional females treated at the same dose rate (second step). Animals were monitored daily for 14 days after administration of the test substance. Animals were weighted on days D1 (day of administration), D7, D14 and D15 (day of euthanasia).

No mortality occurred during each step of the study.
One animal presented piloerection on D2 and D3 during the first step of the study.
On D1 (about 35 minutes and 3 hours 35 minutes post dosing), piloerection was noted in animals of the second step of the study.
No other clinical sign was reported.
Mean weight gain was not affected.
No organ or tissue gross findings were seen at necropsy.

Under these experimental conditions, the LD₅₀ is higher than 2000 mg/kg bw in female rats.

B.6.11.2. Acute dermal toxicity

No new data have been submitted. Please refer to original EU dossier.

B.6.11.3. Acute inhalation toxicity in rats

No new data have been submitted. Please refer to original EU dossier.

B.6.11.4. Skin irritation in rabbits

No new data have been submitted. Please refer to original EU dossier.

B.6.11.5. Eyes irritation in rabbits

Report: IIIA7.1.5: 2004b. CA2210 - Acute eye irritation study in the rabbit.

GLP: Yes
Not published
Guideline: OECD 405

Test substance: CA 2210
Batch n°: RD-816-166B (229 g/L amitrole)

The irritating properties of CA2210 were evaluated after a single ocular instillation of the test substance in female New Zealand albino rabbits. 3 animals were used in this study. A quantity of 0.1 mL of undiluted CA2210 was instilled into the conjunctival sac of the left eye of each of the three rabbits used. The untreated right eye served as a control.

The application of the test substance did not induce coloration of the application site and did not interfere with grading of any eye lesion.

Conjunctival, iris or corneal lesions were evaluated approximately one hour after instillation of the test substance, then 24h, 48h and 72h after instillation.

Conjunctival redness was observed in all animals 1 hour after instillation. No other reaction was observed.

Mean scores were calculated based on the results obtained for each rabbit at time 24h, 48h and 72h and were as follows:

Test item	Animal number	Conjunctiva		Iris	Cornea
		Chemosis	Redness		
CA2210	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0

Under these experimental conditions, CA2210 was found to be non irritant for the eye of the rabbit.

B.6.11.6. Skin sensitisation

Report: IIIA7.1.: [REDACTED] 2004b. CA2210 - Skin sensitization study in the guinea pig (Magnusson and Kligman maximisation test method). [REDACTED]

GLP: Yes

Not published

Guideline: OECD 406

Test substance: CA 2210

Batch n°: RD-816-166B (229 g/L amitrole)

The possible delayed sensitising capacity of CA 2210 was evaluated in albino Hartley guinea pigs (10 males and 10 females). A negative control group of 5 males and 5 females was used. The sensitivity and the reliability of the experimental method are verified every 6 months by use of a positive control group in which 5 animals of each sex are treated with dinitrochlorobenzene (DNCB, 1% alcoholic acid).

A preliminary study was performed with 6 males and 6 females in order to determine concentrations for the main study.

Animals of the main study were treated as follows:

Intradermal induction on day 1:

Each animal received 6 injections in the retroscapular region on either side of the vertebral column, over an area of approximately 24 cm² previously clipped free of hair.

Treatment of negative controls

- Site 1: 2 injections of 0.1 mL of complete Freund's adjuvant diluted 50% in sterile and pyrogen-free isotonic sodium chloride solution
- Site 2: 2 injections of 0.1 mL of sterile water
- Site 3: 2 injections of 0.1 mL of an emulsion of equal volume of sterile water and of complete Freund's adjuvant diluted 50% in sterile and pyrogen-free isotonic sodium chloride solution

Treatment of positive controls

- Site 1: 2 injections of 0.1 mL of complete Freund's adjuvant diluted 50% in sterile and pyrogen-free isotonic sodium chloride solution
- Site 2: 2 injections of 0.1 mL of 1% DNCB

- Site 3: 2 injections of 0.1 mL of an emulsion of equal volume of 1% DNCB and of complete Freund's adjuvant diluted 50% in sterile and pyrogen-free isotonic sodium chloride solution

Treatment of test substance animals

- Site 1: 2 injections of 0.1 mL of complete Freund's adjuvant diluted 50% in sterile and pyrogen-free isotonic sodium chloride solution
- Site 2: 2 injections of 0.1 mL of CA2210 at 75% v/v
- Site 3: 2 injections of 0.1 mL of an emulsion of equal volume of CA2210 and of complete Freund's adjuvant diluted 50% in sterile and pyrogen-free isotonic sodium chloride solution. The final concentration of CA 2210 to be injected was the same as for site 2.

Topical induction on day 9:

Cutaneous applications at the 6 injection sites were performed. The treatment group received 0.5 mL of undiluted CA2210 by cutaneous application on a piece of absorbent gauze of 8 cm² held in place for 48 hours. Negative control animals received 0.5 mL of the vehicle and positive control animals received 0.5 mL of 1% DNCB solution.

Expression phase: Animals were left untreated from day 11 to day 21 (11 days).

Topical challenge on day 22: Animals received topical application of 0.5 mL of undiluted CA 2210, corresponding to the MNIC (Maximum Non-Irritant Concentration) to the right flank region over an area of 4 cm² for 24 hours.

Reading of reactions on days 24 and 25: Sensitizing reactions were assessed 24h and 48h after removal of the dressing.

Results:

Animals were monitored daily throughout the period. Behaviour of the animals treated with CA2210 was normal and was not different from that of the control group.

Mean body weight gain in animals treated with CA2210 did not differ significantly from that of animals of the negative control group.

CA2210 did not induce any coloration of the application site. Grading of skin reaction was therefore possible.

Five percent of animals presented a reaction (score 1) at time 24-hour. No reaction was noted in any tested animals at 48-hour nor in any control animals at 24- and 48-hour time point.

100% of the animals of the positive control group are sensitised at times 24- and 48-hour.

Under these experimental conditions, CA2210 was found not to be sensitizing.

B.6.12. DERMAL ABSORPTION

Two dermal absorption studies were available for the first inclusion of the active substance: one in the original draft assessment report (Volume 3.B.5.1.1.2, [REDACTED] and the other one in the addendum to amitrole monograph (Volume 3.B.5.1, Marty J. M., 1999). The dermal absorption value of amitrole was set at 1% during the first EU review of the active substance.

The dermal absorption of an aqueous solution of ¹⁴C-Aminotriazole was investigated *in vivo* in the rat by [REDACTED]. Doses of 0.1, 1 and 10 mg/rat under a volume of 50 µL were applied. The resulting concentrations that were tested were 2, 20 and 200 g/L respectively. According to the original draft assessment report, a dermal absorption of less than 0.1% was determined after 10h based on residues measured in urine, faeces and carcass. Residues found in the skin were not taken into account.

Nevertheless, as no tape-stripping was performed, it should be considered that all these residues may be available for absorption and have to be taken into account for the determination of the percutaneous absorption of amitrole in this study. The dermal absorption of amitrole is set at 4.5% in the undiluted formulation (200 g/L) and at approximately 20% for the diluted formulations (2 and 20 g/L).

³ Puhl.1985. Dermal absorption of 14C-Amitrole in male rats. Report N° 6158-105. Hazleton Lab., Madison, USA

A non-GLP *in vitro* dermal absorption study on human skin was also conducted using ^{14}C -Aminotriazole (Marty, 1999)⁴. The dermal absorption was determined after 4, 6 and 12h of skin contact. Two concentrations were tested: 240 g/L and 12 g/L (aminotriazole dissolved in water). Percutaneous absorption of amitrole was determined by measuring the radioactivity in receptor fluid. As stated in the addendum to the monograph, the absorbed fraction was $3.54\% \pm 1.65\%$ of the dose for high concentration and $2.06\% \pm 0.72\%$ of the dose for low concentration.

Nevertheless, only residues present in the receptor fluid are considered. It is unclear whether the receptor chamber was washed and analysed. The residues present in the skin were not analysed. Moreover study authors indicated that it was difficult to have an homogeneous distribution of the amitrole solution (undiluted and diluted) on the skin surface. For these reasons, it is thus considered that dermal absorption values obtained should be underestimated.

Two new *in vitro* studies were conducted with formulations containing amitrole and were submitted for the renewal of the active substance inclusion. These studies are detailed below.

A GLP study measuring *in vitro* dermal absorption on human and rat skin of Weedazol TL formulation has been carried out (Gelis C., 2007). Two different formulations have been tested:

- CA0123: Weedazol TL containing nonyl-phenol ethoxylated (NPE), i.e. the formulation for the first inclusion of the active substance
- CA2210: Weedazol TL which does not contain NPE. The composition change was done further to the implementation of Commission Directive 2003/53/EC of June 18, 2003 restricting the uses of NPEs in plant Protection Products.

Percutaneous absorption of amitrole was determined by measuring the radioactivity in the following compartments: skin excess (including washing solution, the first two layers of *stratum corneum*, washing of donor receptor, upper seal and scalpel blade), epidermis + partial dermis and receptor fluid (receptor fluid and cleaning of receptor compartment) after an exposure of 6h. The results obtained at the end of the 24-hour sampling period are summarised in the following tables:

Table B.6.12-1: Summary of dermal absorption values for amitrole in product CA0123 (Gelís, 2007)

	% of the applied amount (mean $\pm$ SD)	
	Undiluted formulation (229 g/L)	Diluted formulation (3.66 g/L)
In vitro (human)	22.93 $\pm$ 21.39	18.54 $\pm$ 3.29
In vitro (rat)	30.11 $\pm$ 3.11	16.66 $\pm$ 3.73

Table B.6.12.-2: Summary of dermal absorption values for amitrole in product CA2210 (Gelís, 2007)

	% of the applied amount (mean $\pm$ SD)	
	Undiluted formulation (229 g/L)	Diluted formulation (3.66 g/L)
In vitro (human)	14.70 $\pm$ 9.79	13.52 $\pm$ 11.25
In vitro (rat)	26.66 $\pm$ 18.96	15.14 $\pm$ 7.47

A non-GLP study measuring *in vitro* dermal absorption on human skin of Weedazol TL has been carried out (Gelís C., 2010). Different formulations have been tested:

- CA2210: Weedazol TL which does not contain NPE.
- CA2735: Weedazol TL which is the representative formulation in this inclusion renewal dossier.
- CA2736: amitrole in UHQ water

⁴ Marty, J.P. 1999. In vitro dermal absorption of ^{14}C Amitrole on human skin. Pharmacy University

Each preparation was tested at two concentrations: undiluted (229 g/L of amitrole) and in-use dilution (3.66 g/L). The exposure was of 6 hours and the sampling periods were 24 hours and 48 hours after application. Percutaneous absorption of amitrole was determined by measuring the radioactivity in the following compartments: skin excess (including washing solution, the first two layers of *stratum corneum*, washing of donor receptor, upper seal and scalpel blade), epidermis + partial dermis and receptor fluid (receptor fluid and cleaning of receptor compartment).

Table B.6.12-3: Summary of dermal absorption values for amitrole in product CA2210 (Gelis, 2010)

	% of the applied amount (mean ± SD)	
	Undiluted formulation (229 g/L)	Diluted formulation (3.66 g/L)
In vitro (human) – 24h sampling	36.25 ± 19.81	10.40 ± 2.51
In vitro (human) – 48h sampling	9.30 ± 3.00	6.29 ± 1.10

Table B.6.12-4: Summary of dermal absorption values for amitrole in product CA2735 (Gelis, 2010)

	% of the applied amount (mean ± SD)	
	Undiluted formulation (229 g/L)	Diluted formulation (3.66 g/L)
In vitro (human) – 24h sampling	0.50 ± 0.08	6.76 ± 3.00
In vitro (human) – 48h sampling	0.86 ± 0.46	3.80 ± 4.10

Table B.6.12-5: Summary of dermal absorption values for amitrole in product CA2736 (Gelis, 2010)

	% of the applied amount (mean ± SD)	
	Undiluted formulation (229 g/L)	Diluted formulation (3.66 g/L)
In vitro (human) – 24h sampling	0.26 ± 0.07	6.86 ± 2.71
In vitro (human) – 48h sampling	0.25 ± 0.17	3.30 ± 1.32

Setting of the dermal absorption values to be used for risk assessments of the formulation CA2735:

The applicant proposed to use the “Triple pack” approach in order to estimate the *in vivo* human absorption of the formulated product. Nevertheless, according to EFSA Guidance on Dermal Absorption⁵, the same test material, formulation, vehicle, concentration and a similar exposure time and swabbing technique should be used in the *in vivo* rat study and in the *in vitro* studies.

The only *in vivo* rat study performed is an old study (1985) conducted with the active substance and which did not use the same parameters described above as the new *in vitro* studies conducted on formulations. Therefore this approach could not be considered as adequate.

Dermal absorptions determined in the *in vitro* studies are very different between the tested formulations. CA0123 and CA2210 showed penetration higher than CA2735. This could be explained by the composition of the formulations. Indeed CA0123 and CA2210 contained a surfactant which is known to enhance penetration of hydrophilic substances by reducing the surface tension. Therefore dermal absorption values of amitrole determined from these formulations are not considered adequate for CA2735 formulation which does not contain this formulant.

Although non-GLP, the *in vitro* study on human skin performed with the representative formulation CA2735 is considered as the most relevant study.

⁵ EFSA Panel on Plant Protection Products and their Residues (PPR); Guidance on Dermal Absorption. EFSA Journal 2012;10(4):2665.

Dermal absorption for the undiluted formulation (229 g/L) amounts to $0.50 \pm 0.08\%$ at 24-hour and to $0.86 \pm 0.46\%$ at 48-hour. This value could be rounded to 1% to make up for the non-GLP status of the study. For the spray dilution (3.66 g/L), dermal absorption is $6.76 \pm 2.71\%$ at 24-hour and $3.30 \pm 1.32\%$ at 48-hour. To compensate for a non-GLP study and to take into account the variability within the results greater than 25% (in accordance with EFSA Guidance document), a dermal absorption of 10% is proposed.

Table B.6.12-6: Dermal absorption end-points for the risk assessments

End-Point	Amitrole in CA2735 formulation
Dermal penetration	Undiluted: 1%
	Spray dilutions: 10%

Dermal absorption studies:

Report: IIIA7.6.2/01: Gelis, C. 2007. Comparison of the *in vitro* percutaneous absorption of Weedazol TL old (CA0123) versus new (CA2210) generation. ADME Report n° NUF/WEE/06003R

GLP: Yes

Guideline: OECD 428

Not published

The *in vitro* dermal absorption on human and rat skin of two formulations of Weedazol TL was measured. Two different formulations have been tested:

- CA0123 (batch n°RD877-075A): Weedazol TL containing nonyl-phenol ethoxylated (NPE)
- CA2210 (batch n°RD877-047A): Weedazol TL which does not contain NPE

The formulations were tested at two different concentrations:

1. Undiluted: 229 g/L of amitrole
2. Diluted: 3.66 g/L (corresponding to in-use concentration in the field)

Four static cells were used per species, per dose and per formulation (i.e. a total of 32 cells).

The human and rat skin samples were obtained from several donors.

- The human skin was obtained from abdominal surgery.
- The dorsal hairless rat skin samples were obtained from animals supplied by IFFA CREDO, L'Arbresle, France.

The skin integrity was evaluated before each application by measuring the transepidermal water loss (TEWL).

- The abdominal human skin was included because TEWL was $\leq 13 \pm 3 \text{ g/m}^2/\text{h}$
- The hairless rat skin was included in the study as the TEWL was $\leq 18 \pm 1 \text{ g/m}^2/\text{h}$

The thickness of the skin samples was measured and was shown to be within the acceptance criteria of $300 \pm 100 \mu\text{m}$.

Receptor fluid: due to the good solubility of amitrole in water, the most common receptor fluid was used (PBS 0.01M).

The diffusion chamber and skin were maintained at a mean temperature of $32 \pm 1^\circ\text{C}$.

Before application the formulation was mixed and the homogeneity was tested by using 6 aliquots from the top, middle and the bottom of the container. The coefficient of variation of the 6 values was less than 5%.

The radiochemical purity of radiolabelled amitrole formulations was tested by radio-HPLC.

After control of the skin sample integrity, formulations were applied homogeneously on each skin sample at $10 \mu\text{L}/\text{cm}^2$ corresponding to $2.32 \text{ mg}/\text{cm}^2$ and $36.6 \mu\text{g}/\text{cm}^2$ of amitrole at the highest and lowest doses respectively.

Six hours after application, the tested formulation remaining on the skin surface was removed by washing with water (collected for analysis).

The receptor fluid was collected 1, 2, 4, 7, 8 and 24 h after application.

Percutaneous absorption of ^{14}C -amitrole was determined by measuring the radioactivity in the following compartments:

- Skin excess (including washing solution, the first two layers of *stratum corneum*, remaining skin, washing of donor compartment, upper seal and scalpel lame),
- Epidermis + partial dermis
- Receptor fluid (receptor fluid and washing of receptor compartment, lower seal, absorbent paper)

Results

Recovery

For undiluted formulations, the mean total recoveries for the radioactivity were 98.26% and 100.28% for CA0123 and 101.23% and 102.60% for CA2210, validating the results obtained with human and rat skins respectively.

For diluted formulations, the mean total recoveries for the radioactivity were 100.07% and 101.07% for CA0123 and 98.71% and 100.33% for CA2210, validating the results obtained with human and rat skins respectively

Dermal absorption

As a conservative estimate, according to Sanco/222/2000 rev7 the dermal absorption was considered to be the sum of the radioactivity measured in epidermis, dermis and receptor fluid. The first two layers of the *stratum corneum* were excluded.

Amitrole-absorbed fraction of the **undiluted CA0123** was 22.93% and 30.11% in human and rat skins respectively.

For **undiluted CA2210**, amitrole-absorbed fraction was slightly lower: 14.70% and 26.66% in human and rat skins respectively.

Amitrole-absorbed fraction of the **diluted CA0123** was 18.54% and 16.66 % in human and rat skins respectively.

For **undiluted CA2210**, amitrole-absorbed fraction was slightly lower: 13.52% and 15.14% in human and rat skins respectively.

Table B.6.12-7: Distribution of ^{14}C radioactivity after application on human and rat skin for both undiluted formulations

	Undiluted formulation (229 g/L)			
	CA0123		CA2210	
	Human	Rat	Human	Rat
% of applied amount	(Mean±SD)	(Mean±SD)	(Mean±SD)	(Mean±SD)
Skin excess including the first 2 layers of stratum corneum	75.3±25.1	70.2±2.5	86.5±10.4	75.9±18.2
Epidermis+dermis (ED)	3.67±2.18	6.58±1.81	3.29±1.23	3.06±0.91
Receptor fluid (RF)	19.26±23.47	23.53±4.08	11.41±9.52	23.61±18.93
Dermal delivery (Epidermis+dermis+receptor fluid)	22.93±21.39	30.11±3.11	14.70±9.79	26.66±18.96
Total recovery	98.26±3.83	100.28±0.66	101.23±4.19	102.6±4.11

Table B.6.12-8: Distribution of ^{14}C radioactivity after application on human and rat skin for both diluted formulations

% of applied amount	Diluted formulation (229 g/L)			
	CA0123		CA2210	
	Human (Mean+SD)	Rat (Mean+SD)	Human (Mean+SD)	Rat (Mean+SD)
Skin excess including the first 2 layers of stratum corneum	81.5±4.5	84.4±3.9	85.2±13.7	85.28±0
Epidermis+dermis (ED)	15.07±2.31	10.69±3.39	8.06±2.58	6.33±1.57
Receptor fluid (RF)	3.48±1.17	5.97±2.19	5.46±8.69	8.81±8.36
Dermal delivery (Epidermis+dermis+receptor fluid)	18.54±3.29	16.66±3.73	13.52±11.25	15.14±7.47
Total recovery	100.07±6.98	101.07±3.94	98.71±2.66	100.33±2.67

Report: IIIA7.6.2/02: Gelis, C. 2010. *In vitro* skin penetration of amitrole. ADME Report n° NUF/AMI/10001R

GLP: No

Guideline: OECD 428

Not published

The aim of this non GLP work was to investigate the rate and extent of the *in vitro* dermal absorption of amitrole following topical application of the three preparations Weedazol TL (CA2210), Weedazol TL without adjuvant (CA2735), amitrole in UHQ water (CA2736), to the surface of dermatomed human skin mounted on static cells. Each preparation was tested at 2 concentrations 229 g/L and 3.66 g/L (representative of the in-use concentration).

Three static cells were used per dose and per formulation.

The human skin samples were obtained from abdominal surgery from 2 different donors.

The skin integrity was evaluated before each application by measuring the transepidermal water loss (TEWL). For the abdominal human skin the resulting was $\text{TEWL} \leq 13 \pm 3 \text{ g/m}^2/\text{h}$.

The thickness of the skin samples was measured and was shown to be within the acceptance criteria of $300 \pm 100 \mu\text{m}$.

Receptor fluid: due to the good solubility of amitrole in water, the most common receptor fluid was used (PBS 0.01M).

The diffusion chamber and skin were maintained at a mean temperature of $32 \pm 1^\circ\text{C}$.

Before application the formulation was mixed and the homogeneity was tested by using 6 aliquots from the top, middle and the bottom of the container. The coefficient of variation of the 6 values was less than 5%.

Two duration time were tested: 24h and 48h. A total of 18 cells for the 24h duration and 18 cells for the 48h duration were used.

Formulations were applied homogeneously at $10 \mu\text{L}/\text{cm}^2$ after control of the skin sample integrity.

Six hours after application, the tested formulation remaining on the skin surface was removed by washing with water.

The receptor fluid was collected 2, 8 and 24 or 2, 8, 24 and 48h after application. The cells were dismantled after 24h or 48h.

Percutaneous absorption of ^{14}C -amitrole was determined by measuring the radioactivity in the following compartments:

- Skin excess (including washing solution, the first two layers of *stratum corneum*, remaining skin, washing of donor compartment, upper seal and scalpel lame),
- Epidermis + partial dermis
- Receptor fluid (receptor fluid and washing of receptor compartment, lower seal, absorbent paper)

Results

Recovery

For undiluted formulations, the mean total recoveries for the radioactivity after 24h were 94.69%, 96.58% and 96.99% for CA2210, CA2735 and CA2736 respectively. After 48h the recoveries were of 99.20%, 100.34% and 101.38% for CA2210, CA2735 and CA2736 respectively.

For diluted formulations, the mean total recoveries for the radioactivity after 24h were 97.67%, 97.17% and 99.48% for CA2210, CA2735 and CA2736 respectively. After 48h the recoveries were of 96.47%, 100.04% and 95.41% for CA2210, CA2735 and CA2736 respectively.

Dermal absorption

The dermal absorption was considered to be the sum of the radioactivity measured in epidermis, dermis and receptor fluid. The first two layers of the *stratum corneum* were excluded.

Undiluted formulations:

CA2210 formulation showed a penetration higher (between 2.89% and 25.35%) than the two other formulations (between 0.44 and 1.34% for CA2735 and between 0.12 and 0.44% for CA2736). The dermal penetrations of CA2735 and CA2736 formulations were equivalent.

Moreover, a high variability of the results for the formulation CA2210 was observed. Considering this study and the above study (Gelis, 2007), the dermal absorption of amitrole observed from undiluted CA2210 formulation was between 2.89% and 53.9%.

Diluted formulations:

CA2735 and CA2736 showed the same dermal absorption: 6.76% and 6.86% for CA2735 and CA2736 respectively at 24-hour, 3.80% and 3.30% for CA2735 and CA2736 respectively at 48-hour. Penetration of amitrole from CA2210 formulation is slightly higher: 10.40% at 24-hour and 6.29% at 48-hour.

In conclusion the formulations CA2735 and CA2736 lead to less variability and lower absorption when compare to CA2210.

Table B.6.12-9: Distribution of ^{14}C -amitrole after application of the undiluted formulations (229 g/L) to human skin

Compartment (% of the applied amount)	CA2210		CA2735		CA2736	
	24h	48h	24h	48h	24h	48h
Skin excess excluding the first two layers of the <i>stratum corneum</i>	58.31 ± 18.88	88.97 ± 3.91	95.99 ± 1.19	99.38 ± 2.86	96.70 ± 2.31	101.09 ± 0.87
Two first layers of the <i>stratum corneum</i>	0.13 ± 0.17	0.53 ± 0.14	0.09 ± 0.01	0.10 ± 0.05	0.04 ± 0.02	0.04 ± 0.03
Epidermis + dermis (ED)	2.95 ± 3.58	3.51 ± 1.57	0.41 ± 0.07	0.39 ± 0.32	0.17 ± 0.12	0.17 ± 0.12
Receptor fluid (RF)	33.29 ± 23.37	5.79 ± 1.46	0.10 ± 0.15	0.46 ± 0.67	0.09 ± 0.05	0.08 ± 0.06
Dermal absorption Epidermis + Dermis + Receptor fluid	36.25 ± 19.81	9.30 ± 3.00	0.50 ± 0.08	0.86 ± 0.46	0.26 ± 0.07	0.25 ± 0.17
Total recovery	94.69 ± 1.94	98.80 ± 0.98	96.58 ± 1.23	100.34 ± 2.83	96.99 ± 2.27	101.38 ± 0.90

Table B.6.12-10: Distribution of ¹⁴C-amitrole after application of the diluted formulations (3.66 g/L) to human skin

Compartment (% of the applied amount)	CA2210		CA2735		CA2736	
	24h	48h	24h	48h	24h	48h
Skin excess excluding the first two layers of the <i>stratum corneum</i>	80.98 ± 7.17	89.32 ± 1.09	88.59 ± 6.23	96.00 ± 5.75	90.30 ± 4.04	91.93 ± 7.95
Two first layers of the <i>stratum corneum</i>	6.30 ± 2.92	0.86 ± 0.34	1.82 ± 0.99	0.23 ± 0.27	2.33 ± 1.04	0.28 ± 0.27
Epidermis + dermis (ED)	7.89 ± 6.08	4.81 ± 1.33	6.24 ± 3.20	2.72 ± 3.85	6.20 ± 2.24	2.44 ± 0.46
Receptor fluid (RF)	2.50 ± 3.69	1.48 ± 0.95	0.52 ± 0.35	1.09 ± 0.89	0.66 ± 0.47	0.86 ± 0.87
Dermal absorption Epidermis + Dermis + Receptor fluid	10.40 ± 2.51	6.29 ± 1.10	6.76 ± 3.00	3.80 ± 4.10	6.86 ± 2.71	3.30 ± 1.32
Total recovery	97.67 ± 7.09	96.47 ± 2.22	97.17 ± 2.65	100.04 ± 1.43	99.48 ± 0.83	95.52 ± 6.68

B.6.13. TOXICOLOGICAL DATA ON NON ACTIVE SUBSTANCES

Material safety data sheets are supplied for all the co-formulants in the plant protection product. The non active substance do not trigger additional risk phrase for CA2735.

B.6.14. EXPOSURE DATA

B.6.14.1. Operator exposure

B.6.14.1.1. Operator exposure estimates

Toxicological endpoints of amitrole used for risk assessments are as follows:

AOEL (mg/kg bw/day)	0.001 mg/kg bw/day 90-day rat study, SF = 100 [Amitrole Review Report 6839/VI/97- final, March 22 nd , 2001]
Dermal absorption of undiluted formulation	1% [see B.6.12]
Dermal absorption of in-use dilution	10% [see B.6.12]

- **Summary of the critical GAP(s)** used for operator risk assessment (i.e. risk envelope approach)

Amitrole is a non-selective broad spectrum systemic herbicide. The formulation CA2735 contains 229 g/L of amitrole. This formulation is intended to be registered for use for weed control on cultivated and non cultivated areas.

The formulation is a soluble concentrate packaged in 5 and 20 L containers. Usage information pertinent to operator exposure is summarised in the following table.

To be noted that the applicant only stated that CA2735 is applied with a tractor mounted boom sprayer or with a train or in trucks for non-crop applications. Therefore the RMS noted that applications with a hand-held sprayer or a lance are not conceivable and assessments with these types of equipment will not be done here.

Table B.6.14.1.1-1: Summary of critical use patterns (i.e. worst case) for use of CA2735

Uses	Application Equipment	Application rates		Water volume
		L product/ha	kg a.s/ha	L/ha
Orchards and vineyards	Tractor mounted boom sprayer (under the row or on localized areas)	13	2.977	300-500
Non crop uses (impermeable and permeable surfaces: roadsides, railways, industrial areas)	Train/truck boom sprayer	13	2.977	300-500

Estimations of potential operator exposure have been undertaken for amitrole using the critical uses (table B.6.14.1.1-1) and the following predictive models: German BBA model ⁶ and UK POEM model ⁷.

The estimations were compared to agreed EU AOEL for amitrole, see table B.6.14.1.1-2.

Table B.6.14.1.1-2: Summary of estimated operator exposure to amitrole

Model data	Level of PPE	Total absorbed dose (mg/kg bw/day)	% of AOEL (0.001 mg/kg bw/day)
Tractor mounted boom sprayer application (vineyards, orchards, olive groves) 13 L/ha i.e. 2.977 a.s./ha			
UK POEM • 50 ha/day, 6 h/day • 300 L/ha • 60 kg operator • 20 litres 63 mm closure	No PPE	0.7601	76009 %
	Gloves during mixing/loading and application	0.1197	11975 %
German Model • 20 ha/day • 70 kg operator	No PPE	0.1953	19529 %
	Gloves and coverall during mixing/loading and application	0.0138	1380 %

⁶ Uniform Principles for Safeguarding the Health of Applicators of Plant Protection Products (Uniform Principles for Operator Protection), Mitteilungen aus der Biologischen Bundesanstalt für Land-und Forstwirtschaft, Berlin-Dahlem, Heft 277, 1992. ("German Model").

Estimation of Exposure and Absorption of Pesticides by Spray Operators, Scientific subcommittee on Pesticides and British Agrochemical association Joint Medical Panel Report (UK MAFF), 1986 and the Predictive Operator Exposure Model (POEM) V 1.0, (UK MAFF), 1992, 2007 version. ("UK POEM").

Application to vineyards, orchards and olive groves with a tractor mounted boom sprayer:

According to the GAPs, CA2735 is an herbicide which is applied on the soil under the row (at the foot of the plants or of the trees) with a tractor mounted boom sprayer in vineyards, olive groves and orchards. Due to this band application, it is commonly admitted that the surface which is actually treated represents 1/3 of the total surface (only 1/3 of the total dose, i.e. 0.993 kg as/ha, is applied on the soil). Nevertheless the models will first be used with the default surface and work duration parameters. The resulting exposures may thus be over-estimated.

UK-POEM model

The UK-POEM model predicts that even if gloves are worn by the operator during mixing/loading and application, the systemic exposure will exceed the AOEL for the active substance amitrole (refer to summary in table B.6.14.1.1-2). Exposure to amitrole is estimated to be 0.1197 mg/kg bw/d. This estimate corresponds to 11975% of the AOEL of amitrole.

Calculations could be made using 1/3 of the total dose (0.993 kg as/ha, i.e. 4.3 L/ha) which is applied on the soil for this herbicidal treatment on vineyards, orchards and olives. The estimate will amount to 3962% of the AOEL of amitrole considering the same maximal personal protective equipment.

German BBA model

Calculations according to the German BBA model predict that, even if gloves and coverall are worn by the operator during mixing/loading and application, the systemic exposure will exceed the AOEL for the active substance amitrole (refer to summary in table B.6.14.1.1-2). Exposure to amitrole is estimated to be 0.0138 mg/kg bw/d. This estimate corresponds to 1380% of the AOEL for the active substance amitrole.

Considering that only 1/3 of the dose will be applied on the soil, the estimate will amount to 456% of the AOEL of amitrole considering the same maximal personal protective equipment.

As a conclusion, for uses with a tractor-mounted boom sprayer, the operator exposure calculated according to the UK Predictive Operator Exposure Model (UK POEM) and to the German BBA model showed an unacceptable risk for the operator, even if maximal personal protective equipment are worn during mixing/loading and application.

Application to railways, roadsides and industrial areas with a train/truck mounted boom sprayer:

CA2735 can be applied on main railroads or roadsides and on industrial areas with train or truck mounted sprayers. The notifier did not provide any exposure estimate for these uses. Indeed, he considered that *“the non-crops applications are done either in a train or in trucks so that the operator is not exposed during the application phase. Therefore the worst case is represented by the uses for which the applications are made on tractors without cab (orchards, vineyards, olives)”*. This argument is not considered relevant by the RMS. **In absence of specific data provided in the dossier for these applications, it is considered that risk assessment cannot be finalised and should be performed at Member State level.**

B.6.14.1.2. Measurement of operator exposure

As the operator risk assessed according to the models is not acceptable when using a tractor mounted boom sprayer, a field operator exposure study was performed. This study was conducted in vineyards and orchards in France and in olives in Spain. The operator exposures were assessed using:

- External dosimetry: the amount of amitrole deposited on cotton or cotton/polyester coverall and cotton undergarment, retrieved in hand wash and face/neck wipes was measured
- Biomonitoring: the amount of amitrole excreted in urine was assessed over 7 days in Spain and French vineyard or 5 days in French orchards.

This study was conducted in February in Spain (olives) and in March and November in French vineyards and orchards respectively.

A first field study (Wilson A.J., 1999) was performed in 1999/2000 and was provided for national granting authorizations in several European Member States. Nevertheless, as this study is not considered to be representative for this renewal, it will not be assessed and summarized in this additional report. Indeed this study was performed with an old composition of the preparation containing a surfactant whereas the representative preparation CA2735 does not contain it. As this surfactant is known to enhance dermal penetration of hydrophilic substances by reducing the surface tension, measured exposures may be overestimated. Moreover, the dose rates used in this study were higher than those actually recommended and the number of subjects included in the study was low. The new study (Thouvenin I., 2011) performed on the representative formulation, at the same dose rates than those recommended and with higher number of subjects than the study performed in 1999/2000 is considered to give more relevant information.

Justification of the biomonitoring

Report: IIIA7.3.2: Redondi, S. 2010. Justification of biomonitoring to measure the operator exposure to aminotriazole.

GLP: No

Not published

The biomonitoring provides a direct measure of total internal dose resulting from all routes of exposure under field conditions. This is the reason why this technique was chosen for this field operator exposure study.

Amitrole is a non-selective herbicide, the biotransformation of which has been extensively studied and reviewed. Metabolism has been widely investigated in particular in mammals and showed that amitrole is mainly excreted as unchanged compound in urine (approximately 60-90%) within 48h following its administration whatever is the route of administration. In addition based on the pharmacokinetic data, there is no evidence of persistence in tissues.

Therefore urinary unchanged amitrole appears to be a reliable marker of exposure to amitrole-based products. The conjugated forms (3-amino-5-mercapto-1,2,4-triazole and 3-amino-1,2,4-triazolyl-(5)-mercapturic acid) are minor and account for less than 6% of the radioactivity retrieved in urine.

No data are available concerning urinary excretion of amitrole in humans. Nevertheless, it is considered that the total amount of amitrole in urine samples should be corrected by the 60% recovery of the administered dose excreted unchanged in rat urine as a worst-case.

Field operator exposure study

Report: IIIA7.6.3: Thouvenin, I. 2011. Determination of external and systemic exposure (using biomonitoring in urine) of operators to amitrole during downward application. Staphyt report n° ChR-11-8256

GLP: Yes (Except for the creatinine levels in urine specimens that were determined using equipment which was not calibrated under GLP. As these measurements were not directly used for the calculation of exposure but only for initial selection of specimens to analyse, this is considered to have no significant impact on data quality)

Guideline: OECD Series on Testing and Assessment No.9 "Guidance document on the conduct of studies of occupational exposure to pesticides during agricultural application", 1997

Not published

This outdoor field study was carried out in two European regions: in Spain in February 2011 and in France in March and November 2011. In this study, the dermal and/or systemic exposures of operators to amitrole were concurrently measured during a typical day of activities including mixing/loading and application of an amitrole-based formulation. Applications on olive groves (in Spain), on vineyards and orchards (in France) were performed using a tractor-mounted/trailed boom sprayer.

I. Materials and methods**a. Materials**

Formulation used in the field by operators: CA 2735

Batch n°: 31012355, contains 230.8 g/L of amitrole and 219.5 g/L of ammonium thiocyanate

Monitored molecule: amitrole, CAS # 61-82-5

b. Study design

- 2 sites: France (Bordelais, Cognac, Anjou and Touraine) and Spain (Andalucia)
- Treated crops: Vineyard and orchards in France ; olives in Spain
- Treatment period: at the end of February/beginning of March for vineyards and olives or in autumn (November) for orchards.
- Applied dose:
 - 13L of product /ha equivalent to 3 kg as/ha in France (grapes and orchards)
 - 6L of product/ha equivalent to 1.4 kg as/ha in Spain (Olives)
- Equipment: tractor-mounted/trailed boom sprayer (with or without cab or canopy)
- Application: soil directed application
- Number of subjects: 12 in Spain (among which 9 were biomonitored) and 16 (14 analyzed and 2 not analysed on the basis of the measured creatinine) in France on vineyard (among which 11 were biomonitored) and 12 (10 analyzed, 1 not analysed on the basis of the measured creatinine and another one not analysed due to leakage problems) in France on orchards (only biomonitoring was done on these 10 subjects) were analyzed.

A total of 40 male operators were involved in the field phase of the study but 36 were analyzed. All of them conducted mixing/loading and application and were professional operators, having 3 to 48 years' experience. In 29 cases, in addition to mixing/loading and application, the cleaning of equipment after application was also monitored when this procedure was part of their normal routine at the end of a spraying day.

The following table summarizes application details.

Table B.6.14.1.2-1: Application details

	Olives - Spain	Vineyards - France	Orchards - France
Number of subjects	12	16	12
Biomonitoring	9	11	10
Passive dosimetry	9	11	0
Biomonitoring and passive dosimetry	6	8	0
Actual product quantity applied <i>Arithmetic Mean (Min-Max)</i>	5.8 L/ha (5.1-6.7)	12 L/ha (8-13.8)	12.9 L/ha (5.2-13)
Total working duration <i>Min-Max</i>	172-355 min	79-480 min	189-391 min
Treated surface <i>Arithmetic Mean (Min-Max)</i>	9.8 ha/day (4.7-17.5)	4.2 ha/day (0.6-8.7)	3.6 ha/day (1.5-5.8)
Amount of active substance handled <i>Arithmetic Mean (Min-Max)</i>	13 kg as/day (6.92-20.8)	11.3 kg as/day (1.85-24)	10.5 kg as/day (4.8-15.6)

- Assessed parameters: passive dosimetry and biomonitoring (urinary excreted amitrole)

Passive dosimetry

Dermal exposure to amitrole was measured by analysing of the residues deposited on standardised whole-body dosimeters. These dosimeters correspond to the clothes usually worn by operators. For all operators, the dosimeters consisted in:

- Outer layer of clothes: a long-sleeved and long-legged polyester/cotton coverall;
- Inner layer of clothes (representing the actual body exposure): a full-length cotton undergarment (covering arms, legs and torso);

The dosimeters acted as collection media for the test substance. They were worn throughout the period of monitoring. The polyester/cotton coverall and eventually the underwear were removed for lunch (if taken).

At the end of the working day, all dosimeters were removed with the assistance of a member of the monitoring team. Then the specimens were sectioned, wrapped and were maintained in frozen storage until analysis.

Hand exposure was assessed by the hand wash procedure. Hand washes were conducted at times when the operator would have normally washed his hands for reasons such as eating, using the toilet, etc... and at the end of the working day. Hand wash specimens collected during and at the end of the working day were treated as separate specimens. For each operator, intermediate and terminal specimens were pooled in the analytical facility after thawing to obtain only one hand wash specimen per operator.

Protective gloves (nitrile) were worn by all operators during mixing/loading and for maintenance activity during application. These gloves were not sampled for analysis.

Exposure of the head was assessed by face/neck wipes. Face/neck wipes were taken whenever the operator would have normally washed his face (at the end of the working day). The solution used to perform face/neck wipes was the same as this used for the hand wash procedure. As for body dosimeters, the specimens were kept frozen until analysis.

Biomonitoring

Systemic exposure was measured by analysing the quantity of amitrole excreted as unchanged molecule in 24-hour urine specimens collected over a five or seven-day period.

Each operator collected a complete 24-hour urine specimen for each of 7 days (Spain and French vineyard) or 5 days (French orchards). Sampling started one day prior to exposure to the product by collection of a pre-exposure urine specimen.

Operators had been previously required to have no prior exposure to amitrole or structurally-related compounds like triazole-based products for a minimum of 7 days before the day of exposure monitoring.

During 6 days following exposure they had also to refrain from any contact with amitrole or structurally-related compounds like triazole-based products.

Before each day of collection each operator was given (by the study team) a polypropylene urine collection container (plus a spare) in which to collect a complete 24-hour urine specimen. At collection the total volume of each urine specimen was measured by reading the volume on the graduated collection container.

One or two 30 mL (+/- 5 mL) aliquots were taken, placed in 50 mL screw cap bottles and labelled with the study and sequence number (one primary specimen and one reserve specimen when necessary). One extra specimen of about 1-2 mL was sampled in a small test tube for creatinine measurement. The specimens were placed in a freezer immediately after sub-sampling, prior to shipping for analysis. At the end of each field phase per country the primary (or unique) set of specimens was packed and dispatched frozen to the analytical test site for analysis. The reserve set of specimens (only done in France) remained in deep-frozen storage until receipt of the first set had been confirmed by the analytical test site.

- Field fortification: On each day of field monitoring, field fortification specimens of each matrix to be sampled were performed to show the stability of the active substance on specimens under field, storage and transit conditions.

Rates of fortification for the various matrices were as described below:

- Polyester/cotton coverall: 50 and 5000 µg/1000 cm² cloth
- Cotton undergarment: 5.0 and 500 µg/1000 cm² cloth
- Hand wash: 5.0 and 500 µg/500 mL solution
- Face/neck wipe: 0.5 and 50 µg/2 gauze wipes
- Urine: 25.0 and 2500 µg/L

- Specimen storage and transport: As soon as possible after sampling, all operators' specimens and travel and field fortification specimens were placed in a deep freezer. Storage conditions were monitored and recorded using a min-max thermometer, which was placed inside the freezer. During transport storage conditions were monitored and recorded.

- Analytical phase:

The objectives of the analytical phase of this study were:

- To determine residual concentrations of amitrole in specimens of the matrices fortified with known concentrations of amitrole and to establish their stability under field, storage and transit conditions: *field fortifications*.
- To determine deposits of amitrole in specimens of polyester/cotton coveralls and inner dosimeters and to determine residual concentrations in face wipes and hand wash solutions and in urine specimens: *operator samples*.

II. Results and discussion

a. Analytical method

The limit of quantification (LOQ) of the method was defined as the lowest analysed concentration in a specimen at which the methodology was validated.

The limit of detection (LOD) of the method can be estimated as the lowest analyte concentration detectable above the mean amplitude of the background noise in an untreated sample at the corresponding retention time. An estimate of the LOD can be taken as three times background noise.

Table B.6.14.1.2-2: Limits of quantification (LOQ) and detection (LOD) for each matrix

µg amitrole/specimen	Urine (µg/L)	Polyester/cotton coverall (µg/1000 cm²)	Cotton undergarment (µg/1000 cm²)	Face/neck wipes (µg/2 gauze pads)	Hand wash (µg/500 mL)
LOQ	5	10	1	0.1	1
LOD	0.65	2.41	0.54	0.02	0.31

b. Field recovery

All the mean recoveries at the low and high fortification levels were within the range of 89% to 113% (within the acceptable range of 70% to 120%).

Levels of amitrole found on/in the specimens taken from each operator were corrected when mean field recovery was <95%: correction was then applied to all PE/cotton coverall specimens (94% recovery level at the low and high fortification levels), face/neck wipe specimens when the measured residue level was >5 µg/specimen (89% recovery level) and urine specimens for operators monitored in November 2011 (French orchards).

Table B.6.14.1.2-3: Field recoveries - summary

	Fortification level	n	Mean	SD	CV	Min.	Max.
Polyester/cotton (µg/specimen) coverall	50	8	94	6.3	6.7%	83	101
	5 000	8	94	11.2	11.8%	78	105
Cotton (µg/specimen) undergarment	5	8	97	4.1	4.2%	89	101
	500	8	98	5.4	5.5%	86	104
Hand wash (µg/specimen)	5	8	101	4.3	4.3%	94	107
	500	8	95	5.1	5.3%	87	101
Face-neck wipe (µg/specimen)	0.5	8	95	5.9	6.3%	85	101
	50	8	89	6.5	7.3%	80	99
Urine (ng/mL)	5.0	23	113	10.9	9.6%	92	131
	500	23	110	8.1	7.4%	94	126

French vineyards and Spanish olive groves							
Urine (ng/mL)	5.0	13	94	4.8	5.1%	86	102
French orchards	500	13	91	2.9	3.2%	86	96

c. Field observation

Some incidents were observed during the field study.

Operator 18 took a first formulation container and began to open it (bare hands), then very quickly remembered to shake it for homogenization. As there was no sealed paper below the cap of the container, some droplets of pure product came out and could have reached his hands, which were rinsed approximately only one hour later when he asked for a break to urinate. This was an error (made by the study team) not to wash this operator's hands when the incident occurred and to analyse this specimen apart.

Other incidents included bad handling of protective gloves (e.g. Operator 4), touching of nozzle with bare hands (Operator 8), driving the vehicle with gloves on inducing indirect hand contamination afterwards (Operator 8). Some operators did not wash their hands before urinating on the day of application. Some did not wear protective gloves when manually folding/unfolding the boom.

These incidents were considered as usual and all operators were considered to work according to usual practices (even if not always in accordance with the recommended good agricultural practices).

The specimens from operator 34, who performed all the requested tasks, were not analysed. This operator experienced big tank leakage problems which implied transfer of the spray solution into big containers, repair and then re-filling of the spray solution from the containers. As this incident can be considered as possible to occur, the exclusion of this operator is not considered adequate and it is considered that specimens should have been analysed.

Most operators wore at least one supplementary layer (jacket, sweater...) over the outer dosimeter, for some hours or during all the working day. A dust mask or a mask with cartridge against organic vapours was worn by most operators during at least mixing/loading. These supplementary equipments were not analyzed. The dosimeters specimens of the operators who decided to wear an additional layer of cloths for a long time because they were too cold were not analyzed; only urine specimens were analyzed for these operators.

Some operators were smokers, and some operators answered mobile phone calls on several occasions; these operators may have transferred the contamination present on their hands to their face.

Most operators cleaned (rinsed) the application equipment at the end of the working day. Some of them chose to wear a protective coverall over the poly-cotton coverall during cleaning. When not done by the operator (not used to do this), rinsing was done by a study monitor. The objective was to avoid any further contamination when using the vehicle and sprayer during the next days when biomonitoring was on-going.

d. Exposure calculations

Potential dermal exposure excluding hands is the sum of the residue levels measured in/on whole-body dosimeters (polyester/cotton coverall and cotton undergarment) and face/neck wipes.

Actual dermal exposure excluding hands is the sum of the residue levels measured in/on cotton undergarment and face/neck wipes.

Actual hand exposure is the exposure as determined by hand wash.

Urine residue levels are presented as the total amount of amitrole (expressed as µg/operator) found over the 4 or 6 days of collection (beginning on the application day) for Operators 31 to 42 and Operators 1 to 30, respectively.

All exposure calculations were done using the non-rounded figures.

The exposure results were corrected by the field recovery results lower than 95%. For exposure values between two fortification levels, the correction was done with the nearest recovery value on a log scale.

The limit was therefore defined as the logarithmic mean of the two fortification levels. When the mean field recoveries were higher or equal to 95%, no correction was done.

The BLQ [below the limit of quantification (LOQ)] values were replaced by $\frac{1}{2}$ LOQ for calculations. BLQ values were not corrected for field recoveries.

The BLD [below the limit of detection (LOD)] values were replaced by $\frac{1}{2}$ LOD for calculations. BLD values were not corrected for field recoveries.

Results are presented with arithmetic and geometric means (A.M. and G.M.), standard deviations (ASD and GSD), Min., Max. and 50th, 75th, 90th percentiles. All were calculated by means of Excel 2010 functions.

e. Field specimens

The external specimens taken on twenty operators (Op. 1, 2, 4, 5, 6, 7, 8, 10, 12, 14, 18, 19, 21, 22, 23, 24, 25, 26, 27 and 28) and the urine specimens taken on thirty operators (Op. 1, 3, 4, 5, 8, 9, 10, 11, 12, 14, 15, 18, 19, 22, 25, 26, 27, 28, 29, 30, 32, 33, 35, 36, 37, 38, 39, 40, 41 and 42) were analysed.

The selection of specimens to be analysed was done based on the following criteria:

- External specimens: the passive dosimetry specimens of at least 20 operators had been planned to be analysed. Those who were not selected for analysis were those from Operators 3, 9 and 11 in Spain (because they wore an additional long-sleeved jacket at least in the morning) and from Operators 15, 16, 20, 29 and 30 in French vineyards (because they wore a sleeveless jacket all the working day long or a long-sleeved jacket at least in the morning). No passive dosimetry was planned and done for the operators monitored in orchards.

- Urine specimens: as a check on the operators' compliance with the essential requirement to collect complete 24-hour urine specimens, urine specimens of each day for each operator were analyzed for creatinine concentration. Some operators (Op. 2, 6, 7, 20, 21, 23, 24 and 31) were then excluded when more than a two-fold variation between minimum and maximum values for one operator and/or when their urine collection was visibly incomplete.

Operator 22 was not excluded because the 2-fold difference can be explained by differences in daily urine volume.

Operator 26 was not excluded because the 2-fold difference was due to low creatinine levels measured on pre-exposure day and on the last sampling day which were considered less important for the amitrole residue level collection.

Operator 16 was excluded because of the lack of consistency between urine volume and creatinine levels over the 7-day sampling period.

Dermal exposure:

The partial potential dermal exposure (PPDE) is defined as the sum of the residue levels measured in/on whole-body dosimeters (polyester/cotton coverall and cotton undergarment), face/neck wipes and hand washes. It is 'partial' because it does not include exposure on the protective gloves (which were not analysed).

The outer arms and legs were exposed at a similar level, each part representing 39% (arithmetic mean) of the measured PPDE. The upper and lower parts of the coverall torso were respectively exposed to 5.3 and 7.2% of the dermal distribution.

Hand washes represented 6.8% of the PPDE (arithmetic mean in $\mu\text{g}/\text{operator}$). This was not negligible when compared to body exposure. Actually, the operators wore protective gloves (not analysed) only during mixing/loading and for repair/maintenance during application.

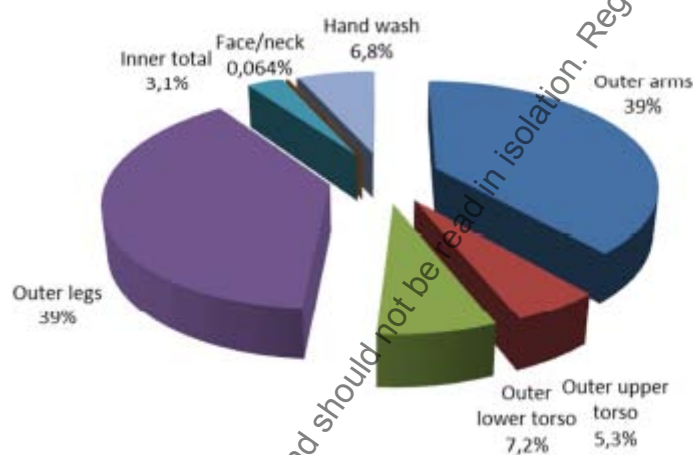
Three operators had a higher hand and/or face/neck exposure level than other operators:

- **Operator 4:** he was observed to touch the outside of the protective gloves with bare hands (incorrect glove handling). Moreover, the highest residue level on the face/neck was measured in this operator: as he answered mobile phone calls on several occasions, he may have transferred the contamination present on his hands to his face.
- **Operator 8:** he touched a nozzle with bare hands after the beginning of application; once he drove the tractor with gloves on and then may have contaminated the wheel he touched afterwards with bare hands. This operator also had the second highest residue level on the face/neck. He may have received projection from the tank during mixing/loading (his face was near the manhole where the product was poured).
- **Operator 18:** he handled the first containers without gloves and some droplets of concentrate product came on his bare hands.

Residue levels measured for these operators are not excluded from calculations.

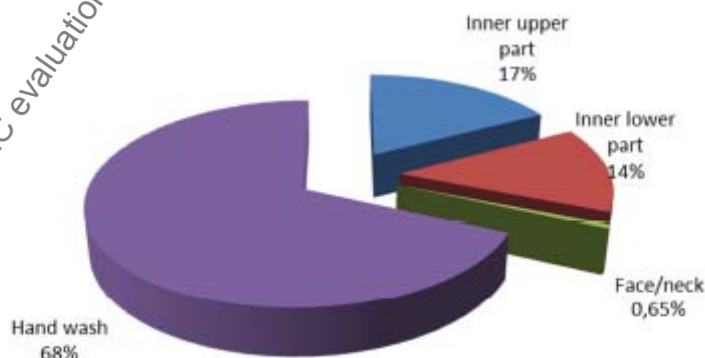
Operators 7, 11, 15, 24, 25, 29 and 30 who used to rinse their gloves after each mixing/loading phase had low residue levels on hands and/or low residue levels in urine.

Figure B.6.14.1.2-1: Distribution of partial potential dermal exposure (arithmetic mean; µg/operator/day). Exposure on protective gloves not measured



When considering the actual dermal exposure, the hands were the most exposed dermal part (68%). The actual dermal exposure (defined as the sum of the residues level measured in/on cotton undergarment, face/neck wipes and hand washes) ranged between 0.142 and 345 µg/kg bw/d. The two highest ADE were due to a combination of high hand and high body residue levels in Operator 8 and high hand residue levels in Operator 18 (see above).

Figure B.6.14.1.2-2: Distribution of actual dermal exposure (arithmetic mean; µg/operator/day)



Gloves were worn during mixing/loading but only on occasions (for repair/maintenance) during application. No level of protection could be calculated for hands as protective gloves were not analysed.

The polyester/cotton coverall afforded a very good level of protection of the operators. Indeed the penetration through these clothes was low (arithmetic mean of 1.8%, calculated as follows: transfer factor for cotton coverall = (undergarment parts) / (undergarment parts + coverall parts)).

The fact to wear rubber boots/hiking-type boots does not seem to have impacted the results.

In the next table were summarized the results of all operators in µg/kg bw/day.

Table B.6.14.1.2-4: Summary of the results of the passive dosimetry

Type of exposure	Exposure (µg amitrole / kg body weight/d)			
	Potential dermal without hands	Actual dermal without hands	Actual hand	Actual dermal
	*	**	#	£
A.M. (Arithmetic Mean)	464	15.4	35.0	51.1
ASD (Arithmetic Standard Deviation)	616	32.8	76.4	97.5
G.M. (Geometric Mean)	203	1.85	4.45	8.36
GSD (Geometric Standard Deviation)	3.9	9.5	9.7	9.2
Min.	29.9	0.0315	0.111	0.142
50th centile	184	1.38	3.55	13.5
75th centile	586	10.9	18.3	27.2
90th centile	1454	46.3	113	151
Max.	2291	121	304	345
No. replicates	20	20	19 [□]	19 [□]

*: polyester/cotton coverall + undergarment + face/neck wipes

***: actual dermal without hands = undergarment + face/neck wipes

#: actual hands = hand wash

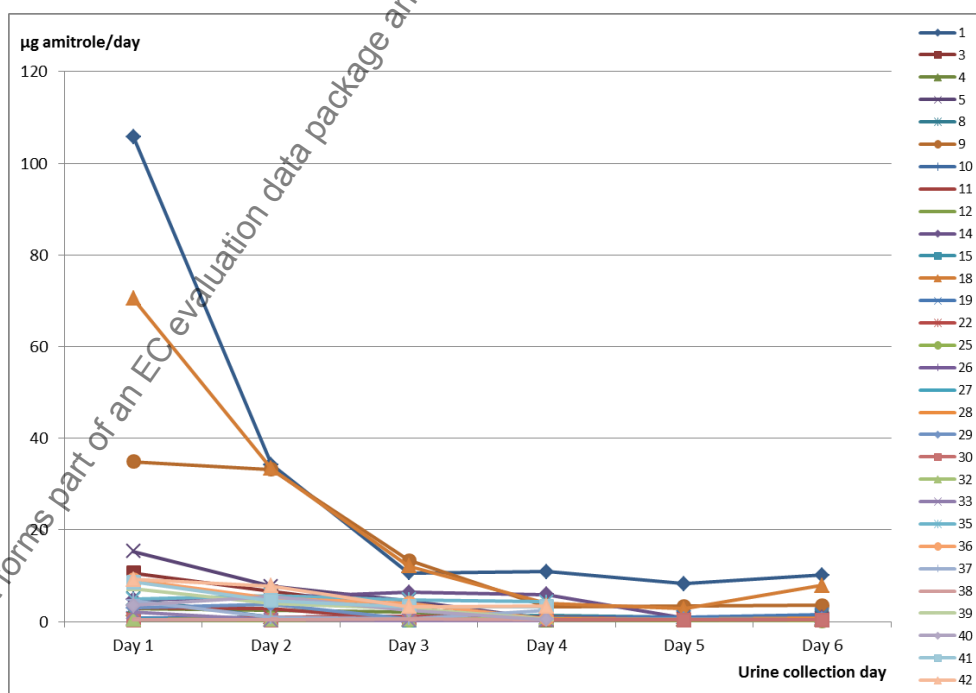
£: actual dermal = actual dermal without hands + actual hands = undergarment + face/neck wipes + hand wash

□: one hand wash specimen was lost at analysis

Biomonitoring in urine:

The highest residue levels were measured on the day of application or on the day after application. The absorbed dose of amitrole was shown to be mostly excreted in 72 hours.

Figure B.6.14.1.2-3: Excretion profile of amitrole in urine



The following tables show total amitrole excretion in urine over the sampling period in operators from the three different sites. Urinary amitrole amounts are corrected by the 60% recovery of the dose excreted unchanged in rat urine as a worst-case. The calculated systemic amitrole content is compared to the AOEL of the active substance (0.001 mg/kg bw/day based on the NOAEL of 0.1 mg/kg bw/day determined in the 90-day rat study using a safety factor of 100).

Table B.6.14.1.2-5: Total amitrole excretion in urine (over 6 days) in operators monitored in Spain (olives) in February/March 2011

Op #	1	3	4	5	8	9	10	11	12
Total amount of amitrole applied (kg)	10.6	11.5	11.1	20.8	15,7	20,8	8,31	11.1	6.92
body weight (Kg)	102	106	104	80	100	91	80	80	69
Application vehicle	with cab	only canopy	no cab	with cab	with cab	w/o cab	with cab	with cab	with cab
ATA urine (µg/d) (total after 5d)	180	21,3	8,4	29,6	10	91,5	3,35	7,71	2,24
ATA urine (µg/kg/d)	1,76	0,20	0,08	0,37	0,10	1,01	0,04	0,10	0,03
systemic ATA (µg/kg/d) = 1,66 urinary ATA*	2,93	0,33	0,13	0,61	0,17	1,67	0,07	0,16	0,05
%AOEL (1µg/kg)	293	33	13	61	17	167	7	16	5

*The results took into consideration the fact that amitrole is excreted at 60% unchanged in the urine.

ATA: amitrole

Table B.6.14.1.2-6: Total amitrole excretion in urine (over 6 days) in operators monitored in France (vineyards) in February/March 2011

Op #	14	15	18	19	22	25	26	27	28	29	30
total amount of amitrole applied (Kg)	11	1.85	5.77	13,8	9.69	9	10.6	15	11.1	24	12
body weight	80	85	70	95	84	62	83	84	83	80	80
Application vehicle	with cab	w/o cab	with cab	with cab	with cab	with cab	with cab	w/o cab	with cab	only canopy	with cab
ATA urine (µg/d)	23,9	2,16	1,1	6,06	4,18	2,39	2,83	3,28	3,49	8,67	2,41
ATA urine (µg/kg/d)	0,30	0,03	1,87	0,06	0,05	0,04	0,03	0,04	0,04	0,11	0,03
systemic ATA = 1.66 ur ATA (µg/kg/d)*	0,50	0,04	3,11	0,11	0,08	0,06	0,06	0,06	0,07	0,18	0,05
%AOEL (1µg/kg)	49,59	4,22	310,66	10,59	8,26	6,40	5,66	6,48	6,98	17,99	5,00

*The results took into consideration the fact that amitrole is excreted at 60% unchanged in the urine.

ATA: amitrole

Table B.6.14.1.2-7: Total amitrole excretion in urine (over 4 days) in operators monitored in France (orchards) in November 2011

Op #	32	33	35	36	37	38	39	40	41	42
total amount of amitrole applied (Kg)	13,8	15,6	11,5	5,77	4,8	6,9	11,3	11,9	12	11,5
body weight	89	69	71	84	68	84	79	73	65	74
Application vehicle	with cab (rear window opened)	with cab	with cab	w/o cab	opened cab	with cab	w/o cab	w/o cab	w/ocab	with cab
ATA urine (µg/d)	2,24	2,88	19,6	18,9	8,5	1,79	14,5	12	19,8	23,6
ATA urine (µg/kg/d)	0,03	0,04	0,28	0,23	0,13	0,02	0,18	0,16	0,30	0,32
systemic ATA = 1.66 ur ATA (µg/kg/d)*	0,04	0,07	0,46	0,37	0,21	0,04	0,30	0,27	0,51	0,53
%AOEL (1µg/kg)	4,18	6,93	45,83	37,35	20,75	3,54	30,47	27,29	50,57	52,94

*The results took into consideration the fact that amitrole is excreted at 60% unchanged in the urine.

ATA: amitrole

Operators 1, 9 and 18 showed higher systemic exposures than other operators. Those high systemic exposures could be explained by the following facts:

- **Operator 1** had very high urine volume on each sampling day (from 3 300 to 4 350 mL). He might be diabetic even if he did not mention this during the health questionnaire. There was no incident reported for this operator during the working day during which he was monitored. No further contamination (after the day of amitrole application) could be completely excluded as the internal exposure (biomonitoring) and the dermal exposure (whole-body dosimetry) were not consistent for this operator.

- **Operator 9:** further to a misunderstanding this operator had urinated just before the beginning of the first application phase without asking for hand wash by the study team. An indirect exposure of the urine from hands cannot be excluded. Nevertheless, there were other operators who did not wash their hands before urination on the day of application but with no obvious consequence on the level of exposure.

- **Operator 18:** experienced an incident when he opened the formulation container and he could have received droplets of concentrated product on his bare hands. As his hands were not rinsed just after this incident and as he used to smoke while driving the tractor during application, the hand wash specimen taken *ca.* one hour later (before he went to the toilet) could collect a part of this incidental exposure but there was time left meanwhile for dermal penetration and oral absorption.

These incidents were considered as usual and all operators were considered to work according to usual practices (even if not always in accordance with the recommended good agricultural practices). The residue levels measured for these operators should not be excluded from calculations.

Some of the tractors mounted boom sprayers were equipped with cab or canopy. This does not seem to have influence the results.

Extrapolation of the results to estimate the operator exposure to amitrole in CA2735:

The preparation used in this study, CA2735 is the same as the representative preparation for this current amitrole renewal. Application technique with a tractor mounted boom sprayer was the same as recommended for CA2735. Use rate was also approximately the same: 5.8 L/ha for olives (6 L/ha recommended) and 12 and 12.9 L/ha respectively for vineyards and orchards (13 L/ha recommended). Working time (arithmetic mean of 277 minutes) and treated surface (9.8 ha in olive groves, 4.2 and 3.6 ha in vineyards and orchards respectively) is also considered to be representative of a typical work day. In fact, it could be taken into account that this herbicidal preparation is applied on the soil under the row. Due to this band application it is commonly admitted that the surface which is actually treated represents 1/3 of the total surface of a vineyards or orchards; the treated surfaces used in the field study are thus considered to be representative.

For these reasons, results of the study can be used to assess the systemic exposure to amitrole when applying CA2735.

- Passive dosimetry:

The results obtained by analysis of passive dosimetry specimens are not considered adequate to conclude on the risk to operators for the following reasons:

- Results are expressed per working day; therefore, no separate results are available for mixing/loading and for application. The dermal absorption values to be used in risk assessments are 1% for the undiluted preparation and 10% for the spray dilution (see Section B.6.12). As a worst-case, the dermal absorption of 10% would be used in this study to determine systemic exposures, resulting in an over-estimation of the operator exposure.

- No data are available for orchards application. Indeed, no passive dosimetry was planned and done for operators monitored in orchards.

- As the residues deposited on the protective gloves were not analysed, only a 'partial' potential dermal exposure can be calculated.
- Inhalation exposure was not measured.
- The biomonitoring part of this study, where exposure levels were determined by means of measurement of urinary excretion of amitrole, is considered to give more reliable results.

- Biomonitoring:

In olive groves, the amount of amitrole measured over 6 days ranged from 0.03 to 1.76 µg/kg bw/day without correction and from 0.05 to 2.93 µg/kg bw/day after the correction for the 60% recovery of unchanged amitrole in urine.

In vineyards, the amount of amitrole measured over 6 days ranged from 0.02 to 1.87 µg/kg bw/day without correction and from 0.04 to 3.12 µg/kg bw/day after the correction for the 60% recovery of unchanged amitrole in urine.

In orchards, the amount of amitrole measured over 4 days ranged from 0.04 to 0.53 µg/kg bw/day without correction and from 0.04 to 0.53 µg/kg bw/day after the correction for the 60% recovery of unchanged amitrole in urine.

As actual exposure was determined in a biomonitoring study and as a low number of subjects were included in each group, it is considered that all individual measured systemic amitrole should be less than the AOEL of amitrole to consider the risk as acceptable (the accepted AOEL of amitrole is 1 µg/kg bw/day).

Table B.6.14.1.2-8: Systemic exposure to amitrole determined in the biomonitoring study

	Systemic amitrole (µg/kg bw/d)		
	Olive	Vineyards	Orchards
Min	0.05	0.04	0.04
Max	2.93	3.12	0.53
Number of subjects	9	11	10
Number of subjects with systemic exposure > 100% AOEL	2	1	0

Two operators in **olive groves** and one operator in **vineyards** showed exposure greater than the AOEL of amitrole. Therefore, the risk to operators is not considered acceptable in olive groves and in vineyards.

None of the operators showed exposure greater than the AOEL of amitrole in **orchards**.

Nevertheless, one operator (Operator 34) was excluded from the study after completion of his work day and before analyses of his collected specimens. This operator experienced big tank leakage problems which implied transfer of the spray solution into big containers, repair and then re-filling of the spray solution from the containers. As this incident can be considered as possible to occur, the exclusion of this operator is not considered adequate and it is considered that specimens should have been analysed. It cannot be excluded that exposure of this operator, who worked according to usual practices, would be greater than the AOEL.

Moreover, the polyester/cotton coverall afforded a very high protection factor in this study (98.2%). This level of protection is very high compared to usual protection factor of 90-95%, as used in the validated European models. It would have been more relevant to use a 5-10% penetration factor for the assessment.

Based on available information, it is considered that the risk to operators is not acceptable in orchards.

Therefore it can be concluded from the field study that tractor mounted boom sprayer application of CA2735 in vineyards, orchards and olive groves will result in an unacceptable risk to operators.

To be noted: The applicant gave its opinion on the RMS assessment in December 2012 and summarized it as follows:

“30 subjects were monitored (biomonitoring): 10 in olives and 20 in grapes/orchards. These Subjects were shown to belong to an homogeneous population (Geometric standard deviation analysis and variance analysis). Moreover even if the GAPs were different between olives and grapes/orchards because the treated surfaces for olives were more important, the handled quantities were comparable. The exposure to amitrole results from these handled quantities. For these reasons, the 30 subjects have to be considered all together in the risk assessment.

Furthermore because:

- the number of subjects is high enough
- The results follow a log Normal distribution
- the toxicological profile of amitrole results from repeated administration and the thyroid effects were shown to be reversible when the exposure of short duration which is the case for amitrole
- the margin of safety before thyroid effects would be observed in Human are very important (the exposure duration to amitrole of professional operators would be 20 days per year maximum and the levels at which operators are exposed is 10 000 lower than the levels that could lead to thyroid effects in Human)

and in accordance with the guidance document for conducting field operator exposure studies and EFSA scientific opinion regarding the operator, worker, bystander and resident exposure risk assessment, 2010, the use of the 75th percentile for the risk assessment is justified.

When the 75th percentile of the exposures of the 30 subjects is considered the exposures were shown to be 44% of the AOEL so the operator risk assessment is acceptable whatever is the crop.”

Nevertheless, even if all operators are considered all together, it remains operators that have systemic exposure higher than 100% of the AOEL of amitrole, which, in the RMS opinion, is not judged acceptable in the case of a biomonitoring study that directly measures the systemic exposure of operators in urine.

B.6.14.2. Bystander exposure

B.6.14.2.1. Estimation of bystander exposure

A bystander may be exposed to spray drift during the tractor-mounted applications. Bystander exposure is estimated according to the parameters outlined in the following Table B.6.14.2.1-1, the results of the exposure calculations are presented in Table B.6.14.2.1-2.

As mentioned by the applicant in the GAPs, applications in **orchards, vineyards and olive groves** will be done under the row or on localized areas. It was thus considered that only one third of the total crop surface is really treated. Therefore only 1/3 of the total dose (0.993 kg a.s./ha) were actually applied on the soil.

As the applicant did not provide any data on bystander exposure during applications on **non cropped areas**, this exposure will not be estimated.

Table B.6.14.2.1-1: Parameters applied for the assessment of bystander exposure

Parameters and units		Orchards, vineyards, olive groves
	Application rate (g a.i./ha):	993
	Min. application volume (L/ha)	300
AR	Maximum Application rate (mg/m ²)	99.3
C	Maximum Spray concentration (mg a.i./mL)	3.31
DA	Dermal absorption of diluted formulation (%)	10
D	Drift at a distance of 7 m (% of applied dose)	0.41%
BSE	Exposed body surface (m ²)	1

BW	Body weight (kg)	60
IE	Inhalation exposure (mL/hr) (90 th percentile)#	0.03
T	Duration of exposure (hr)	0.083

based on 0.03 mL spray/m³ (90th percentile for field crops) and a breathing rate of 1 m³/hr

Table B.6.14.2.1-2: Estimated bystander exposure

Parameters	Orchards, vineyards, olive groves
Dermal exposure (mg/person/day) = AR x D x BSE	0.40713
Dermal absorbed dose (mg/person/day) = dermal exposure x DA	0.04071
Inhalation exposure (mg/person/day) = IE x T x C	0.00824
Total systemic exposure (mg/person/day) = dermal absorbed dose + inhalation exposure	0.04895
Total systemic exposure (mg/kg bw/day) = total systemic exposure/BW	0.00082
% of AOEL	82%

In **orchards, vineyards and olive groves**, taking into account the application of the preparation under the row, exposure of a bystander amounts to 82% of the AOEL of amitrole. The risk to bystander exposed to CA2735 is thus acceptable, only for applications performed under the row.

Conclusions:

The risk to a bystander incidentally exposed to CA2735 is acceptable in orchards, vineyards and olive groves.

In non crop areas the bystander exposure cannot be finalised due to the absence of data provided by the applicant. It should be addressed at national level.

B.6.14.2.2. Measurement of bystander exposure

Not performed.

B.6.14.3. Worker reentry exposure

B.6.14.3.1. Estimation of worker exposure

Re-entry following an herbicide application is not usually necessary. The only scenario for re-entry is likely to be for scouting purposes. This activity is considered to be of rather short duration, i.e. 2 hours per day.

Re-entry exposure was estimated using parameters from the EUROPOEM II database.

The notifier assumes a transfer coefficient of 1000 cm²/person/h proposed by the EPA (1998) for mowing and maintaining turfgrass. Indeed CA2735 is an herbicide applied directly onto the soil and the worker re-entering the field and performing inspection activities is not supposed to come into contact with the applied product

which would have dried on the soil. The RMS is of the opinion that a transfer coefficient of 5000 cm²/person/h, based on data for low crops in EUROPOEM II, may be more appropriate for such tasks.

As mentioned by the applicant in the GAPs, applications in **orchards, vineyards and olive groves** will be done under the row or on localized areas. It was thus considered that only one third of the total crop surface is really treated. Therefore only 1/3 of the total dose (0.993 kg a.s./ha) were actually applied on the soil.

As the applicant did not provide any data on worker exposure during applications on **non cropped areas**, this exposure will not be estimated.

Table B.6.14.3.1-1: Parameters applied for the assessment of worker exposure

Parameters and units		Orchards, vineyards, olive groves
DFR	Dislodgeable Foliar Residues (µg/cm ² /kg a.i./ha) (default value)	3
AR	Application rate (kg a.i./ha)	0.993
TC	Transfer coefficient (cm ² / person/h)	5000
T	Task duration (h)	2
P	Penetration through skin factor	1 without PPE* 0.1 with PPE**
BW	Body weight (kg)	60
DA	Dermal absorption of undiluted formulation (%)	1%

* It is assumed that the worker wears short-sleeved shirt and knickers.

** It is assumed that the worker wears long-sleeved shirt and trousers.

The potential dermal exposure of a worker is calculated by the following approach:

$$D \text{ (mg/person/d)} = 0.001 \times DFR \text{ (}\mu\text{g/cm}^2\text{/kg a.i./ha)} \times AR \text{ (kg a.i./ha)} \times TC \text{ (cm}^2\text{/person/h)} \times T \text{ (h/day)} \times P$$

As the potential inhalation exposure is considered to be negligible (outdoor applications), the worker total systemic exposure is calculated as follows:

$$\text{Total systemic exposure (mg/kg bw/d)} = \frac{D \text{ (mg/person/d)} \times DA \text{ (\%)}}{BW \text{ (kg)}}$$

Table B.6.14.3.1-2: Estimated worker exposure

Parameters	Orchards, vineyards, olive groves
Without PPE: short-sleeved shirt and knickers	
Dermal exposure (mg/person/day) = $D = 0.001 \times DFR \times AR \times TC \times T \times P$	29.79
Total systemic exposure (mg/kg bw/day) = $(D \times DA) / BW$	0.00497
% of AOEL	497%
With PPE: long-sleeved shirt and trousers	

Dermal exposure (mg/person/day) = $D = 0.001 \times \text{DFR} \times \text{AR} \times \text{TC} \times \text{T} \times \text{P}$	2.979
Total systemic exposure (mg/kg bw/day) = $(D \times \text{DA}) / \text{BW}$	0.0005
% of AOEL	50%

Worker exposure is estimated to be >100% of the AOEL of amitrole if the worker does not wear PPE. If the worker wears PPE, worker exposure amounts to 50 % of the established AOEL. In **orchards, vineyards and olive groves**, the risk to worker exposed to CA2735 is thus acceptable with PPE and only for applications performed under the row.

Conclusion:

It is concluded that there is no unacceptable risk anticipated from amitrole for the worker wearing PPE (long-sleeved shirt and trousers), when re-entering vineyards, orchards and olive groves treated with CA2735.

In absence of data provided by the applicant, the risk to workers re-entering non crop areas after application of CA2735 cannot be finalised and should be addressed at Member State level.

B.6.14.3.2. Measurement of worker exposure

Not performed.

Overall conclusion on risk to operators, bystanders and workers:

- **Non-crop uses:**

In absence of data provided by the applicant, the risk to operator, bystander and worker cannot be finalised for **non crop areas** and should be addressed at national level, in each Member State.

- **Vineyards, orchards and olive groves:**

- **Operator:** The risk to operators using a **tractor-mounted/trailed boom sprayer** is not acceptable in vineyards, orchards and olive groves. Uses of a **lance** or a **hand-held sprayer** are not considered by the applicant and should thus be dealt at Member State level.
- **Bystander:** The risk to a bystander incidentally exposed to CA2735 is acceptable in orchards, vineyards and olive groves.
- **Worker:** There is no unacceptable risk anticipated from amitrole for the worker wearing PPE (long-sleeved shirt and trousers), when re-entering vineyards, orchards and olive groves treated with CA2735.

As a conclusion, none of the intended uses are considered to lead to an acceptable risk for the operator, the bystander and the worker.

B.6.15. REFERENCES RELIED ON**B.6.15.1. Active substance: amitrole**

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
IIA 5.2.4	[REDACTED]	2001	Acute dermal irritation in rabbits. [REDACTED] GLP Not published	Y	NUFARM
IIA 5.2.5	[REDACTED]	2002	Acute eye irritation study with rinse in the rabbit. [REDACTED] GLP Not published	Y	NUFARM
IIA 5.4.1	Wallner, B.	2009	Reverse mutation assay using bacteria (Salmonella typhimurium and Escherichia coli) with amitrole technical. BSL study n° 092153 GLP Not published	Y	NUFARM
IIA 5.4.2	Oppong-Nketiah, M.	2009	In vitro mammalian chromosome aberration test in human lymphocytes with amitrole technical. BSL Bioservice study n° 092154 GLP Not published	Y	NUFARM
KIIA 5.6.11	[REDACTED]	2000	Amitrole. Study of maternotoxicity in the pregnant female rabbit following oral administration (gavage). [REDACTED] GLP Not published	Y	NUFARM
IIA 5.11	Anonymous	2012	Amitrole: why it should not be considered as an endocrine disruptor in Human? Nufarm's Position Paper Not GLP Not published	N	NUFARM

B.6.15.2. Plant protection product: CA2735

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
IIIA 7.1.1	[REDACTED]	2004a	CA2210 - Acute oral toxicity study in the rat: Acute toxic class method. [REDACTED] GLP Not published	N	NUFARM
IIIA 7.1.5	[REDACTED]	2004b	CA2210 - Acute eye irritation study in the rabbit. [REDACTED] GLP Not published	N	NUFARM
IIIA 7.1.6	[REDACTED]	2004b	CA2210 - Skin sensitization study in the guinea pigs (Magnusson and Kligman maximization test method). [REDACTED] GLP Not published	N	NUFARM
IIIA 7.3.2	Redondi, S.	2010	Justification of biomonitoring to measure the operator exposure to aminotriazole. Not GLP Not published	N	NUFARM
IIIA 7.3.3/01	Wilson, A.J.	1999	Amitrole ammonium thiocyanate. Pesticide exposure study to mixers/loaders and applicators during typical applications of Weedazol TL in France in 1999. Vol 182. Report n° CPF/054 GLP Not published	N but Still protected in FR	NUFARM
IIIA 7.3.3 /02	Thouvenin, I.	2011	Determination of external and systemic exposure (using biomonitoring in urine) of operators to amitrole during downward application. Cehtra/Eurofins ADME Report n° ChR-11-8256 GLP Not published	Y	NUFARM
IIIA 7.6.2/01	Gelis, C	2007	Comparison of the <i>in vitro</i> percutaneous absorption of Weedazol TL old (CA0123) versus new (CA2210) generation. ADME report n° NUF/WEE/06003R GLP Not published	Y	NUFARM
IIIA 7.6.2/02	Gelis, C	2010	In vitro skin penetration of amitrole ADME report n° NUF/AMI/10001R Not GLP Not published	Y	NUFARM