The evaluation of MRL supplementary information and new MRLs for imazalil in or on various commodities

UK Competent Authority Evaluation Report and Reasoned Opinion

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Summary

Following the MRL review under Article 12 of Assimilated Regulation 396/2005,[1] data gaps were identified and MRL supplementary information requirements were established in accordance with Article 14 of Assimilated Regulation 396/2005. The supplementary information requirements appear as footnotes in the GB MRL Statutory Register. HSE received an application from Certis Europe BV on 24 September 2021 to address the following supplementary information requirement:

- Additional residue trials supporting the GAPs as appropriate on courgettes

On 16 January 2023, HSE received an application from Janssen PMP to address the following supplementary information requirements:

- Full toxicological assessment of metabolite R014821
- Full toxicological assessment of metabolites FK-772 and FK-284
- A study demonstrating the storage stability of imazalil and metabolite R014821 in high acid content commodities
- A study demonstrating the storage stability of imazalil and its metabolite FK-772 in commodities of animal origin

In addition, Janssen PMP submitted an application to set an import tolerance for the active substance imazalil in or on citrus to accommodate an authorisation in the EU. In addition, the application requested the adoption of the CXL for bananas as a GB MRL.

The applicants submitted dossiers in accordance with the data requirements outlined in Assimilated Regulation 544/2011. In accordance with Article 8 of Assimilated Regulation 396/2005, HSE evaluated the dossier in accordance with the uniform principles as outlined in Article 29(6) of Assimilated Regulation 1107/2009.[2]

Based on the assessment HSE prepared an Evaluation Report that included its Reasoned Opinion on the risk to consumers associated with amending the MRLs. HSE also took into account the data assessed for the approval of the active substance and previous Reasoned Opinions.

[1] Assimilated Regulation No 396/2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin

[2] Assimilated Regulation No 1107/2009 concerning the placing of plant protection products on the market

Sufficiently validated analytical methods for the determination of imazalil (and metabolite FK-772) in products of plant and animal origin, in line with the residue definitions for enforcement, are available to support the uses under consideration in the MRL application.

Toxicological reference values (TRVs) were established for the approval of the active substance: an ADI of 0.025 mg/kg bw/day and an ARfD of 0.05 mg/kg bw. Additional toxicological data on metabolites R014821, FK-772 and FK-284 were evaluated; these data have confirmed the original tentative RD-RA for plants and animals. Specific TRVs for metabolite R014821 were set in this assessment: an ADI of 0.014 mg/kg bw/day and an ARfD of 0.03 mg/kg bw.

The metabolism of imazalil in primary crops was investigated in the fruit and root/tuber crop groups following foliar and post-harvest dipping/drenching applications for the approval of the active substance (EFSA, 2010). The crops included in this MRL application belong to the fruit group and are therefore covered by the metabolism studies available.

On the basis of additional data considered in this application, HSE recommends the following residue definition for risk assessment (RD-RA) in plants for post-harvest use:

1. Imazalil (any ratio of constituent isomers)
2. R014821

The residue definition for enforcement (RD-Enf) in plants has been agreed as:

- Imazalil (any ratio of constituent isomers)

For citrus fruits, 64 protected trials on orange and mandarin were submitted to support the MRLs. The post-harvest trials consisted of 34 dripping/drenching (the critical use), 24 low volume spraying, and 6 waxing uses. The trials are supported by validated analytical methods and storage stability data.

For bananas, the applicant requested that the CXL of 3 mg/kg (2019), was considered for adoption as a GB MRL.

For courgette, four additional residue trials have been provided that support the cGAP considered in the MRL review. These trials are sufficient to confirm that the current MRL is sufficiently supported by residue trials. The 4 trials along with 2 trials that were accepted to support the cGAP in the MRL review indicate that the MRL could be lowered from 0.1 mg/kg to 0.09 mg/kg. However, HSE does not recommending lowering the MRL for such a small change. HSE recommends that the current MRL is retained and the footnote associated with the requirement for additional trials is deleted in the GB MRL Statutory Register.

Hydrolysis of imazalil under representative conditions of pasteurisation, baking, brewing, boiling and sterilisation was evaluated for the approval of the active substance. The hydrolysis data demonstrate that imazalil is stable under standard conditions, therefore specific residue definitions for processed commodities are not required. Studies investigating the magnitude of residues on processing were evaluated during the EU Review and the processing factors (Pf) have been used to refine the risk assessment where appropriate.

No consideration of rotational crops was made as the impact of residues in rotational crops on the post-harvest uses under assessment was not required.

The maximum livestock dietary burden exceeded the trigger value for further assessment (0.1 mg/kg DM and AR) for ruminants. Metabolism of imazalil in livestock was considered for the approval of the active substance. The approval assessment concluded that the metabolic pathways in rodents and ruminants are comparable; the findings in ruminants can therefore be extrapolated to pigs.

On the basis of additional data considered in this assessment, HSE recommends the following residue definition for risk assessment (RD-RA) in animals:

- Sum of imazalil, FK-284 and FK-772 (any ratio of constituent isomers), expressed as imazalil.

The residue definition for enforcement (RD-Enf) in animals has been agreed as:

- Sum of imazalil and FK-772 (any ratio of constituent isomers), expressed as imazalil.

Consideration of available feeding studies for ruminants indicate that the MRLs for products of animal origin (POAO) are unlikely to be exceeded and do not need to be amended.

The highest UK NEDI was 35% of the ADI (toddler). The highest PRIMo IEDI was 17% of the ADI (NL toddler). Therefore, no health effects due to chronic exposure are expected from the consumption of commodities treated with imazalil.

For the acute risk assessment for imazalil, data has been provided that supports the default variability factor being replaced by a value of 1.69 specifically for imazalil/ citrus fruits. The highest UK NESTI was 28.6% of the ARfD (oranges/toddler). The highest PRIMo IESTI was 24% of the ARfD (oranges/children). Therefore, no health effects due to acute exposure are expected from the consumption of commodities treated with imazalil.

For R014821, the highest UK NEDI was 7% of the ADI (toddler). The highest PRIMo IEDI was 4% of the ADI (children). Therefore, no health effects due to chronic exposure are expected from the consumption of commodities treated with imazalil.

The highest UK NESTI was 13.9% of the ARfD for R014821 (oranges/infant). The highest PRIMo IESTI was 16% of the ARfD (oranges/children). Therefore, no health effects due to acute exposure are expected from the consumption of commodities treated with imazalil.

HSE concludes that sufficient data have been provided to support the setting of new MRLs for the uses of imazalil on citrus fruits. Additionally, the CXL for banana can be adopted as a GB MRL. These will not result in consumer exposures exceeding the toxicological reference values and therefore harmful effects on human health are not expected. HSE recommends that the MRLs outlined in Table 0.1 are amended in the GB MRL Statutory Register.

Table 0.1 MRLs recommended by HSE

Enforcement residue definition for products of plant origin: Imazalil (any ratio of constituent isomers)

Product code	Product	Existing GB MRL (mg/kg)	New or amended GB MRL (mg/kg)	Comments
0110010	Grapefruits	4.0	7.0	The MRL is sufficiently supported by data. The MRL is derived by extrapolation of residue trials on oranges and mandarins. No health effects are expected. **The MRL will only be adopted once an MRL for the GAP is adopted in the exporting country and proof of authorisation is provided.**
0110020	Oranges	4.0	7.0	
0110030	Lemons	5.0	7.0	
0110040	Limes	5.0	7.0	
0110050	Mandarins	5.0	7.0	
0163020	Bananas	0.01	3.0	The CXL can be adopted as a GB MRL. No health effects are expected.

* Indicates that the MRL is set at the limit of quantification/determination

MRL supplementary information requirements

As a result of this assessment the following MRL supplementary information requirements have been addressed and HSE recommends the associated footnotes are deleted in the GB MRL Statutory Register:

- Full toxicological assessment of metabolite R014821.
- Additional residue trials supporting the GAPs as appropriate on courgettes
- Full toxicological assessment of metabolites FK-772 and FK-284.

The following MRL supplementary information requirements have not been addressed in this assessment and the associated footnotes are retained in the GB MRL Statutory Register:

- Additional residue trials supporting the GAPs as appropriate on melons.
- A study demonstrating the storage stability of imazalil and its metabolite FK-772 in commodities of animal origin
- Information on the storage conditions of the samples of the feeding studies performed with ruminants.

It is noted that the footnotes in relation to products of animal origin combine the data gaps for both the request for toxicological assessment of metabolites, as well as information on storage and storage stability within the same point. As only the toxicity of the metabolites has been addressed within this evaluation, the footnotes will be amended to read:

“The European Food Safety Authority identified some information on the storage stability as unavailable. When re-viewing the MRL, the Commission will take into account the information referred to in the first sentence, if it is submitted by 26 September 2021, or, if that information is not submitted by that date, the lack of it.”

As further information concerning storage stability remains outstanding, the submission deadline remains the same.

Background

Assimilated Regulation 396/2005 outlines the rules for setting MRLs in GB. In accordance with Article 14(3) the competent authority may request at any time that supplementary information be provided to support an MRL. Further data was required to support the MRLs following the MRL review in accordance with Article 12.

On 24 September 2021 HSE accepted an application from Certis Europe BV for the evaluation of supplementary information for the MRL for imazalil/ courgettes.

On 16 January 2023 HSE accepted an application from Janssen PMP for the evaluation of supplementary information related to the toxicological properties of the metabolites R014821, FK-772 and FK-284 and storage stability in high acid commodities. In addition, HSE accepted an application to set an import tolerance based MRL for citrus and adopt the CXL for bananas.

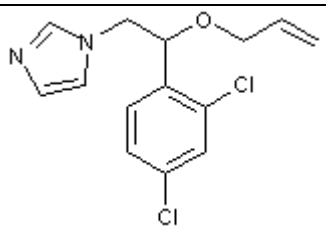
HSE evaluated the information submitted to uniform principles, as defined by Article 8 of Assimilated Regulation 396/2005 and prepared an Evaluation Report. HSE also took into account EFSA Reasoned Opinions and Conclusions on the peer review relating to decisions implemented in GB.

The Evaluation Report is the basis of HSE's Reasoned Opinion.

The active substance and its use pattern

Information on the active substance imazalil is outlined in Table 0.2.

Table 0.2 Information on the active substance

Common name (ISO)	Imazalil
Chemical name (IUPAC)	(*RS*)-1-(β-allyloxy-2,4-dichlorophenylethyl) imidazole or allyl (*RS*)- 1-(2,4-dichlorphenyl)-2-imidazol-1-ylethyl ether
CAS number	35554-44-0
Structural formula	
Molecular formula	$C_{14}H_{14}Cl_2N_2O$
Molecular mass	297.2 g/mol

Imazalil is an approved active substance in GB. An EFSA conclusion is available (EFSA, 2010). The MRLs for imazalil were reviewed under Article 12 of Regulation (EC) No 396/2005 (EFSA, 2017), with an updated review following submission of additional information published the following year (EFSA, 2018). MRLs are established for Imazalil in Part 2 of the GB MRL Statutory Register.

Imazalil is a systemic fungicide with fungicidal action. The fungicidal action involves interference with the ergosterol synthesis which ultimately compromises the membrane integrity of the fungal cells.

Appendix A outlines the GAPs (Good Agricultural Practices) supported in this application.

The Codex MRL (CXL) database is available at the following link:

http://www.fao.org/fao-who-codexalimentarius/codex-texts/dbs/pestres/en/

The following CXLs are available for the proposed uses (Codex Pesticide Residues in food online database).

Table 0.3 CXLs relevant to the proposed uses

Commodity	CXL (mg/kg)	Comments	Year of adoption
Banana	3	Adoption of this CXL as a GB MRL has been considered within this application.	2019
Citrus fruits (group)	15	GB commodities included within this group: Grapefruits, Oranges, Lemons, Limes, Mandarins, Others, Kumquats. The CXL was evaluated by HSE (ER/RO Assessment of Codex MRLs, 2023) and was not adopted due to acute and chronic dietary intake concerns. Alternative data/GAPs for citrus fruits have been considered within this assessment.	2022

Assessment

1 Methods of Analysis

1.1 Methods for enforcement and monitoring of residues in food of plant origin

Analytical methods for the determination of imazalil in products of plant origin, in line with the residue definition for enforcement, were assessed for the approval of the active substance (EFSA, 2010).

The HPLC-MS/MS method DFG S19 was validated in oilseed rape and potatoes (equivalent to high water and high oil content matrices) with an LOQ of 0.01 mg/kg. Acceptable ILV data were provided.

Additional method validations were submitted to address the data gap from the approval assessment for methods of analysis for enforcement in high acid commodities, relevant to this assessment. These methods were submitted previously to support the Article 12 MRL Review and have been fully evaluated at EU level, when the UK was an EU MS.

The method DFG S19 is considered acceptable to monitor residues in citrus (high acid), potato, apple, and banana (high water) and are validated for both imazalil and metabolite R014821. Additional ILV data was also presented and is acceptable.

Extraction efficiency was addressed in a cross-validation study fully evaluated in Section C.1.1.1.4. The data are considered acceptable.

In summary, the crops under consideration in this MRL application (high acid; citrus and high water; banana) are covered by the methods available.

1.2 Methods for enforcement and monitoring of residues in food of animal origin

An enforcement method using HPLC-MS/MS (DFG S19) is available for the determination of imazalil and FK-722 in muscle, fat, liver, kidney and milk with an LOQ of 0.01 mg/kg for each metabolite. This method also has an ILV for imazalil in all commodities, and for FK-722 in meat and liver.

These methods were submitted previously to support the Article 12 MRL Review and have been fully evaluated at EU level, whilst the UK was an EU MS.

As all components of the finalised RD-Enf in products of animal origin have acceptable monitoring methods, this is considered acceptable and no further information is required.

2 Mammalian toxicology

The dietary reference values established for the EU renewal of the active substance imazalil (EFSA Journal 2010; 8(3):1526) are summarised in Table 2.1. The ADI of 0.025 mg/kg bw/d was derived from the NOAEL of 2.5 mg/kg bw/d from the 1-year dog study, and was based on effects in the liver (modifications of biochemical parameters, increase in organ weight). The ARfD of 0.05 mg/kg bw was established from the NOAEL (5 mg/kg bw/d) for maternal and foetal toxicity from the developmental toxicity study in rabbits. An uncertainty factor of 100 (10 for inter-species differences, 10 for intra-species differences) was applied to both NOAELs to derive the ADI and ARfD.

Table 2.1 Overview of the toxicological reference values for imazalil

TRVs	Source	Year	Value	Study relied upon	Safety factor
ADI	EFSA Journal 2010; 8(3):1526	2010	0.025 mg/kg bw/day	1-year dog study	100
ARfD	EFSA Journal 2010; 8(3):1526	2010	0.05 mg/kg bw	Rabbit developmental study	100

During the MRL review under Article 12 of Regulation (EC) No 396/2005 (EFSA, 2017,[3]), EFSA identified data gaps related to the toxicological profile of metabolite R014821 and two other metabolites that were identified in livestock metabolism studies (FK284 and FK772). None of these metabolites were major rat metabolites covered by parent. As part of this evaluation, additional genotoxicity and acute oral toxicity studies conducted on metabolites FK284, FK772 and R014821 and evaluated by the EU RMS (NL, 2018,[4]) were taken into account.

[3] EFSA, 2017. Reasoned opinion on the review of the existing maximum residue levels for imazalil according to Article 12 of Regulation (EC) No 396/2005. *EFSA Journal* 2017, 15(9):4977

[4] NL, 2018. Evaluation report on the modification of MRLs for imazalil in various crops and products of animal origin prepared by the evaluating Member State The Netherlands under Article 8 of Regulation (EC) No 396/2005. March 2015, revised in March 2018 (document was provided to competent authority, but is not publicly available).

It was determined that metabolites FK284, FK772 and R014821 were not acutely toxic by the oral route as the oral LD50 of the three metabolites exceeded 2000 mg/kg bw. As no repeated dose toxicity studies were available, no specific reference values could be derived and it also could not be concluded if the parent's reference values could be applied to the metabolites. In addition, no conclusion could be drawn on the genotoxicity of the three metabolites based on the results of the available genotoxicity studies.

EFSA recommended that data to support the full toxicological assessment of the three metabolites R014821, FK772 and FK284 had to be provided to confirm the residue definition and the tentative existing MRLs.

With this MRL application and in order to address this supplementary information requirement, the applicant has submitted results from a battery of genotoxicity studies on R014821, FK772 and FK284 and a 90-day comparative toxicity study in the rat to compare the toxicity of the three metabolites R014821, FK772 and FK284 with that of the parent imazalil. As the comparative toxicity study did not allow the derivation of NOAELs, an additional 90-day dietary study in the rat was conducted with R014821 in order to derive reference values for this metabolite, which appeared to be slightly more toxic than imazalil. A combined 28-day repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD 422) that had previously been conducted on R014821 was also submitted.

For the purpose of comparing the toxicity profile between the active substance and the metabolites R014821, FK772, and FK284, a summary of the existing information available on the active substance imazalil and the metabolites R014821, FK772, and FK284 taken from the 2009 RAR, the EFSA (2010) Conclusion for imazalil, and the Evaluation Report on Modification of MRLs for imazalil in various crops and products of animal origin (NL, 2017) has been presented below in sections 2.1 – 2.10. Information taken directly from the RAR, the EFSA Conclusion, and the Evaluation Report on Modification of MRLs is *italicised*.

A summary of the new data submitted and evaluated with this application can be found in section 2.8.1, whilst a detailed assessment of these new data has been conducted in Appendix C (section C2 and relevant subsections).

2.1 Absorption, Distribution, Excretion and Metabolism (Toxicokinetics)

From 2009 RAR and EFSA (2010) Conclusion

Absorption: The fraction of imazalil absorbed can be estimated from the amount of total radioactivity excreted via the urine (up to 96 h after dosing) after oral administration in comparison with the amount excreted after intravenous administration. The oral

bioavailability varies between 63 to 117%. Moreover, the fact that the pattern of excretion of imazalil and its metabolites is very similar in urine as in faeces, suggests that most imazalil has been absorbed and metabolized.

Distribution: The only data available are tissue concentrations 96 h after oral dosing. At that time, tissue concentrations of 14C were less than 1% of the total dose administered. The highest concentrations were found in the liver, 0.5%, kidney, 0.03%, content of the large intestine, 0.02%, carcass, 0.4%. This was independent from the route of administration and did not change even after a daily administration for 14 days. The substance does not accumulate at the doses used. Radioactivity levels were approximately 20 times higher in the liver than the corresponding blood levels.

Metabolism: Extensive biotransformation of imazalil occurred in male and female rats after intravenous as well as after oral administration, the metabolic pattern is shown in figure 5.1.2-1 The urinary metabolites, which are most abundant, suggest a process of epoxidation of the allyl moiety, followed by an epoxide hydration. The detection of a minor urinary metabolite suggests that imazalil may also be O-dealkylated.

Similar metabolites were detected in urine and faeces. Moreover, the metabolite profile was largely comparable in all groups, indicating that identical metabolic pathways were involved after exposure to low or high doses, or after single or multiple dosing.

Among the metabolites detected in the 0-24 h urine, the most abundant were: metabolite 3 (5.9-12.6%) and metabolite 4 (4.4-7.7%). Numerous metabolites were detected in faecal methanolic extracts; metabolite 8 accounted for 1.6-3.3% of the total dose.

In another study, in addition to unchanged imazalil, urine contained at least four metabolites, probably formed by dealkylation. Two of these were identified as α-(2,4-dichlorophenyl)-1H-imidazole-1-ethanol and 2,4-dichloromandelic acid.

Generally, higher levels of metabolites were detected in urine and faeces from female rats than in urine from male rats. 24 hr after dosing, the excretion of unchanged imazalil in urine was below the detection limit. In faeces, unchanged imazalil represented 0.6-1% of the administered dose.

Excretion: Radioactivity was rapidly excreted in all dose groups. At 24 hr after a single intravenous administration, + 84% of the administered dose was excreted: 45% in urine and 39% in the faeces of male rats and 55.5% in the urine and 30.5% in the faeces of female rats. Excretion amounted up to + 89% at 96 h after dosing: 42% in faeces and 46% in urine of male rats and 32% in the faeces and 55% in the urine of female rats.

2.2 Acute toxicity

From 2009 RAR and EFSA (2010) Conclusion

With regard to acute toxicity, imazalil has to be classified as harmful by inhalation and via the oral route. Its percutaneous toxicity is low. It is not a skin irritant, but a severe eye irritant. It is not a skin sensitizer.

Table B.6.2.1 : Summary of acute toxicity of imazalil - Scientifically valid studies

Type of test Test species	***Test substance purity***	***Results***	***References***
LD_{50} oral, rat	*technical product, 96.4 % (Makhteshim)*	*664 mg/kg bw*	[redacted] *1990*
LD_{50} oral, rat	*technical product, different imazalil salts, (Janssen Pharmaceutica)*	*m: 343 - 371 mg/kg bw f:227-309 mg/kg bw*	[redacted] *1979*
LD_{50} dermal, rat	*technical product, (96.4 %) (Makhteshim)*	*> 2000 mg/kg*	[redacted] *1990a*
LD_{50} dermal, rabbit	*technical product, (97.6 %) (Janssen Pharmaceutica)*	*> 2000 mg/kg*	[redacted] *et al., 1990*
LC_{50} 4 h smoke, rat	*technical product, (Janssen Pharmaceutica)*	*> 1.073 mg/l air*	[redacted] *et al., 1983*
LC_{50} 4 h dust, rat	*technical product, (96.4%) (Makhteshim)*	*m: 2.88 mg/l air f: 1.84 mg/l air*	[redacted] *1990*
Skin irritation rabbit 4 h	*technical product, (98.8%) (Janssen Pharmaceutica)*	*non irritant*	[redacted] *et al., 1987*
Skin irritation rabbit 4 h	*technical product, (96.4%) (Makhteshim)*	*non irritant*	[redacted] *1990b*
Skin irritation rabbit 4 h	*technical product, (95 %) (Sanachem)*	*non irritant*	[redacted] *1994*
Eye irritation rabbit	*technical product, (96.4 %) (Makhteshim)*	*moderate irritant (reversible)*	[redacted] *, 1990c*
Eye irritation rabbit	*technical product, (97.6 %) (Janssen Pharmaceutica)*	*severe eye irritant*	[redacted] *et al., 1990a*
Skin sensitization guinea pig M&K	*technical product, (98.5 %) (Janssen Pharmaceutica)*	*not a sensitizer*	[redacted] *et al., 1990b*
Skin sensitization guinea pig M&K	*technical product, (96.4 %) (Makhteshim)*	*not a sensitizer*	[redacted] *1990d*

Based on the conclusions on acute toxicity in the 2009 RAR and 2010 EFSA Conclusion, imazalil is classified as Acute Tox. 3, H301, Acute Tox. 4, H332, Eye Dam. 1, H318 in accordance with Regulation 1272/2008 (the CLP Regulation) as it applies in GB. These hazard classes were added to Annex VI of the EU CLP in the 7th ATP and are also included in the GB mandatory classification list.

2.3 Short term toxicity

From 2009 RAR and EFSA (2010) Conclusion

For the renewal of the Annex I inclusion of imazalil no new studies on short-term toxicity in mammals have been submitted. The text below is copied from the original monograph on imazalil written by RMS-Belgium (1996). After repeated oral exposure of rat, mice and dog to imazalil, effects were mainly seen in the liver (modifications of biochemical parameters, increase in organ weight, cell hypertrophy and histopathological changes). These effects were not degenerative and might be interpreted as the result of a chronic stimulation of hepatocytes, due to increased metabolism. In rabbits, no systemic toxic effects were reported after a three week dermal exposure.

The current EU-AOEL is based on the NOAEL of 5 mg/kg bw/day in the 6 months oral study in the rat. The summary of this study in the original monograph on imazalil is very concise. However, a re-evaluation of the study by the current RMS gave no reason to change the conclusion.

Table B.6.3.1: Summary of short term-toxicity of Imazalil - Scientifically valid studies

Type of test, Test species	***Test substance purity***	***Results***			***References***
		NOAEL (mg/kg bw/day)	***LOAEL (mg/kg bw/day)***	***Critical effects***	
21 days, dermal, rabbit	*tech. product, imazalil base, 98.1% (Janssen)*	*160*	*-*	*-*	[redacted] *et al., 1991*
28 days, oral, rat	*tech. product, 98% (Makhteshim)*	*m : 14.2*	*m : 143.1*	*decrease weight of testes*	[redacted] *et al., 1990*
		f : 11.3	*f : 148.5*	*increase weight of liver*	

90 days, oral, rat	*98% (Makhteshim)*	*m : 4*	*m : 18.78*	*liver changes*	[redacted] *et al., 1991*
		f : 4	*f : 20.27*	*liver changes*	
6 months, oral, rat	*tech. product, imazalil base, 98.1 % (Janssen)*	*m : 5.2*	*m : 21.7*	*increase weight of kidneys*	[redacted] *et al., 1983*
		f : 6.2	*f : 25.1*	*increase weight of kidneys and liver*	
1 year, oral, dog	*tech. product, imazalil base, 98.8% (Janssen)*	*2.5*	*20*	*liver (small changes)*	[redacted] *et al., 1989*

2.4 Genotoxicity

From 2009 RAR and EFSA (2010) Conclusion

Imazalil was not genotoxic in a battery of in vitro and in vivo tests, studying different endpoints.

Table B.6.4.1 : Summary of genotoxicity of imazalil

Type of test Cell/Test species	***Test substance purity, company***	***Results***	***References***
Ames test TA1535, TA1538, TA97, TA98 and TA100 +/- S9	*technical product, imazalil base, 98.7% (Janssen Pharmaceutica)*	*not mutagenic*	[redacted] *et al., 1988*
Ames test TA1535, TA1538, TA1537, TA98 and TA100 +/- S9	*technical product, imazalil base, 96.4% (Makhteshim)*	*not mutagenic*	[redacted] *1990*
In vitro chromosomal aberrations in human lymphocytes +/- S9	*technical product, imazalil base, 99.7% (Janssen Pharmaceutica)*	*not clastogenic*	[redacted] *et al., 1990*
CHO/HPRT gene mutation assay +/- S9	*technical product, imazalil base, 98.3% (Janssen Pharmaceutica)*	*not mutagenic*	[redacted] *et al., 1995*
Micronucleus assay in mice, oral single exposure	*technical product, imazalil base, 98.7% Janssen Pharmaceutica*	*no chromosome breaking or spindle poisoning*	[redacted] *et al., 1988a*

Additional information on imazalil genotoxicity reported in the literature:

The data in the table B.6.4.2 were evaluated by the JMPR (Pesticide residues in Food, 1984): original reports (mean results) concerning these studies were supplied by Janssen Pharmaceutica which realised these experiments. These data confirm the absence of mutagenic potential of imazalil. These studies are not reported in detail since adequate reports were available and were included in the foregoing discussion.

Table B.6.4.2 : Additional information on imazalil genotoxicity reported in the literature

Test system	Test object	Doses	Test substance, purity	Results	References
Ames test	*S. typh. TA98, TA100, TA1535, TA1537, TA1538*	*0.5, 5, 10, 20 and 30 µg/plate +/-S9 (mouse liver)*	*imazalil base, tech. grade (R23979) 98.1 % (Janssen Pharmaceutica)*	*negative (control +: 2-AA gave expected response)*	[redacted] *1977 (Janssen Pharmaceutica)*
Micronucleus test	*rat, bone marrow*	*10, 40 and 160 mg/kg bw, i.p.*	*imazalil base, tech. grade 98.1 % (Janssen Pharmaceutica)*	*negative (control +: CP gave expected response)*	[redacted] *1979 (Janssen Pharmaceutica)*
Sex-linked recessive lethal test	*Drosophila melanogaster*	*250 and 1000 ppm for 3 days*	*imazalil base, tech. grade 98.1 % (Janssen Pharmaceutica)*	*negative (control + : procarbazine gave expected increase in recessive lethals*	[redacted] *1982 (Janssen Pharmaceutica)*

Conclusion: imazalil is not genotoxic.

2.5 Long term toxicity

From 2009 RAR and EFSA (2010) Conclusion

In long-term toxicity and carcinogenicity studies, liver and thyroid tumours occurred at high doses (≥ 60 mg/kg bw/day), whose relevance for humans is negative for thyroid and unclear for liver. However, a threshold can be assumed based on the negative genotoxicity results.

After repeated oral exposure of rat, mice and dog to imazalil, effects were mainly seen in the liver (modifications of biochemical parameters, increase in organ weight, cell hypertrophy and histopathological changes). These effects were not degenerative and might be interpreted as the result of a chronic stimulation of hepatocytes, due to increased metabolism.

Imazalil was not reported to have tumorigenic properties or carcinogenic potential in rats, but treatment related histopathological and microscopic liver changes were observed. Increased kidney weight was sometimes reported (18, 24 or 30 months studies realized with 400 or 800 ppm).

In a two year carcinogenicity study in mice, an increased incidence, clinically relevant and statistically significant, of hepatic neoplastic nodules was observed in male mice (200 and 600 ppm) and in female mice (600 ppm).

Increased incidence of some liver changes (p <0.05) in male mice: focal cellular changes at 200 ppm, large vacuoles at 200 and 600 ppm; pigmentation in sinusoidal cells at 600 ppm and swollen sinusoidal cells at 200 ppm nodules and tissue masses. There was also a slight (non-significant) increase in non-neoplastic liver changes in female mice at 600 ppm (small vacuolization, parenchymal cellular swelling, large hepatocytic vacuolization and large sinusoidal vacuoles). No relevant increased incidence of non-hepatic neoplasia was noted up to 600 ppm, both in males and in females.

In dogs, exposed for 2 years at 20 mg/kg bw/d, imazalil produced an increased seric alkaline phosphatase activity, suggesting an impairment of liver function associated to a slight changed cytoplasmic picture of hepatocytes. No pre-neoplastic lesions were observed.

Table B.6.5.1 : Summary of long-term oral toxicity and carcinogenicity of imazalil

Type of test Test species	***Test substance purity***	***Results***			***References***
		NOAEL (mg/kg bw/day)	***LOAEL (mg/kg bw/day)***	***Critical effects***	
18 months, oral, rat	*tech. product, imazalil base, 98.1% (Janssen Pharmaceutica)*	*m: 3.9*	*m: 15.9*	*Liver changes*	*[redacted] et al., 1984*
		f: 5.1	*f: 20.3*		
30 months, oral, rat	*tech. product, imazalil base, 98.1% (Janssen Pharmaceutica)*	*m: 3.6*	*m: 15*	*slight increase liver weight, no tumorigenic potential*	*[redacted] et al., 1985*
		f: 4.7	*f :19.7*		

24 months, oral, mouse	*tech. product, imazalil base, unknown purity (Janssen Pharmaceutica)*	*m: 8.08*	*m: 33.4*	*Liver changes, neoplastic nodules, hepatocytic neoplasms*	[redacted] *et al., 1993*
		f: 9.91	*f: 41.6*		

A harmonised classification for imazalil of Carc.2, H351 has been added to Annex VI of the EU CLP Regulation in the 7th ATP [5] and is also included in the GB mandatory classification list.

2.6 Reproductive toxicity

From 2009 RAR and EFSA (2010) Conclusion

Imazalil is not a reproductive toxicant (the relevant maternal, reproductive and offspring NOAEL are all 20 mg/kg bw/day), and is not a teratogen (the parental and developmental NOAEL is 5 mg/kg bw/day (rabbit study)).

In the rat, reduced litter size and delayed ossification in absence of maternal toxicity was observed. In the rabbit, there was an increased number of resorptions and a reduced number of live pups.

Table B.6.6.1 : Summary of reproductive toxicity and teratogenicity of imazalil

Type of test Test species	***Test substance purity***	***Results***			***References***
		NOAEL (mg/kg bw/day)	***LOAEL (mg/kg bw/day)***	***Critical effects***	
2 generations, oral, rat	*tech. product, imazalil base, 96.4% (Janssen Pharmaceutica)*	*20*	*80*	*increased duration of gestation, effects on pups related to maternal toxicity, no effect on fertility*	[redacted] *et al., 1992*
teratogenicity, oral, rat	*tech. product, imazalil base, 98.1% (Makhteshim)*	*maternal tox: 27*	*maternal tox: 81*	*embryotoxicity but no teratogenic effect; upper dose (81 mg/kg bw/d) not high enough*	[redacted] *, 1991*
		embryo. tox: 9	*embryo. tox: 27*		

[5] *Official Journal of the European Union OJ L 197, 25.7.2015, p. 10–23*

teratogenicity, oral, rat	*tech. product, imazalil sulphate, 99.9 % (Janssen Pharmaceutica)*	*maternal tox : 40*	*maternal tox : 80*	*no teratogenic effect, embryotoxicity at maternal toxic doses*	[redacted] *et al., 1988*
		embryo. tox : 40	*embryo. tox : 80*		
teratogenicity, oral, rabbit	*tech. product, imazalil nitrate, 99 % (Janssen Pharmaceutica)*	*maternal tox : 2.5*	*maternal tox : 5*	*no embryotoxic or teratogenic effect; upper dose was not high enough*	[redacted] *et al., 1985*
		embryo. tox : 5	*embryo. tox : -*		
Developmental toxicity, oral rabbit	*Imazalil sulfate, purity 98.2%, (Janssen Pharmaceutica)*	*maternal: 5*	*maternal: 10*	*No teratogenic effects*	[redacted] *et al. 1992b*
		fetal: 5	*fetal: 10*		

2.7 Neurotoxicity

From 2009 RAR and EFSA (2010) Conclusion

No studies on neurotoxicity have been submitted for the original evaluation for inclusion of imazalil in Annex I. The results of the available repeated dose toxicity studies with imazalil do not indicate neurotoxic effects. Therefore, no new studies are required.

2.8 Further toxicological studies

From Evaluation Report on Modification of MRLs for imazalil in various crops and products of animal origin (NL, 2017)

QSAR evaluations, genotoxicity and acute oral toxicity testing of metabolites FK284, FK772 and R014821 were performed.

The oral LD50 of R014821, FK772 and FK284 in rats was determined to exceed 2000 mg/kg bw.

The results of the in vitro genotoxicity studies are included in the table below.

Table 2.8-1: Summary of the genotoxicity studies

Metabolite	***Ames test***	***Chromosome aberration***	***Mammalian cell gene mutation***
R014821	*Negative*	*Negative*	*+S9: negative* *-S9: equivocal*
FK772	*Negative*	*+S9: negative*	*Negative*

		-S9: equivocal	
FK284	*Negative*	*Negative*	*Negative*

R014821 was negative in the Ames and chromosome aberration tests but was equivocal in the mammalian gene mutation study in the absence of S9. FK772 was negative in the Ames and mammalian cell gene mutation tests but was equivocal in the chromosome aberration study in the absence of S9. No in vivo follow up studies were submitted and therefore for both metabolites it was concluded that genotoxicity could not be excluded.

For metabolite FK284 all three in vitro studies were negative. However, it is noted that an in vitro micronucleus study would have been preferred over an in vitro chromosome aberration study as this study is not able to detect aneugens. Therefore, the issue of aneugencity was not addressed.

QSAR data was also provided by that applicant, but was not accepted by the EU RMS (NL) as only limited information was provided on this QSAR analysis.

Since no conclusion could be drawn on the genotoxicity of the three metabolites and considering that no repeated dose toxicity studies were available, no specific reference values could be derived nor could it be concluded if the metabolites were covered by the parent.

New data

Comparative toxicity study on imazalil and metabolites R014821, FK772 and FK284

As part of this MRL application, the applicant has submitted a 90-day comparative toxicity study in the rat to compare the toxicity of the three metabolites R014821, FK772 and FK284 with that of the parent imazalil.

Under the conditions of this study, the toxicity of metabolite R014821 was slightly higher compared to that of the parent imazalil; the toxicity of FK284 was comparable to that of the parent and the toxicity of FK772 was lower than the toxicity of imazalil.

The comparative toxicity study did not allow the derivation of NOAELs therefore it was proposed by the applicant that the reference values derived for the parent imazalil could be applied to metabolites FK284 and FK772 on the basis that these metabolites showed similar (or less) toxicity than imazalil.

As metabolite R014821 showed slightly higher toxicity compared to the parent imazalil, two additional studies were submitted in order to derive reference values for this metabolite. These comprised of a combined 28-day repeated dose toxicity study with reproduction/developmental toxicity screening (OECD 422) in the rat and a 90-day dietary study in the rat.

Metabolite R014821

Metabolite R014821 is not a major rat metabolite. Four studies have been previously evaluated and found acceptable for R014821, an acute oral toxicity study and a standard package of 3 in vitro mutagenicity tests (Ames, chromosome aberration and mammalian cell gene mutation). Based on the outcome of these studies, the acute oral LD50 of the metabolite was greater than 2000 mg/kg bw and there was no evidence of genotoxicity in the bacterial reverse mutation assay and in the chromosome aberration test, but an equivocal result was observed without S9 in the mammalian cell gene mutation assay. These studies are summarised below:

Table 2.8-2: Previous studies on R014821

Study Type	Species / test system	Result	Reference
Acute oral LD_{50}	Rat	> 2000 mg/kg bw	report no. 25876/1 (2011)
In vitro Ames	*S. typhimurium* 3.16 to 1000 µg/plate	± S9: negative	[redacted] (2010)
In vitro chromosome aberration	Chinese hamster V79 lung cells	± S9: negative	[redacted] (2017)
Mammalian cell gene mutation	CHO K1 Chinese hamster ovary cells, *hprt* locus	-S9: equivocal +S9: negative	[redacted] (2017)

Two additional genotoxicity studies (in vitro micronucleus study and in vivo Comet assay) were submitted in order to fully exclude the genotoxic potential of metabolite R014821.To address the equivocal result in the mammalian cell gene mutation assay, an in vivo Comet assay was provided. In addition, an in vitro micronucleus study was submitted to investigate aneugenicity.

Additional repeat dose studies on R014821 conducted in the rat (a combined 28-day repeated dose toxicity study with reproduction/developmental toxicity screening and a 90-day dietary study) were submitted in order to derive specific reference values for this metabolite. These studies are summarised in Table 2.8-3 below.

Table 2.8-3: New studies submitted on R014821

Study Type	Species / test system	Result	Reference
In vitro micronucleus	Human lymphocytes	± S9: negative	[redacted] 2019 (20192868; AGR 5957)
In vivo Comet	Rat (Wistar Han)	negative	[redacted] 2020a

Study Type	Species / test system	Result	Reference
			(20202368; AGR 5964)
90-day comparative study	Rat (Wistar Han)	slightly higher toxicity compared to imazalil	[redacted] 2021 (20192866; AGR 5959)
Combined 28-day repeated dose toxicity study with reproduction/ developmental toxicity screening, oral gavage	Rat (Wistar Han)	Parental LOAEL: 30 mg/kg bw/d (spleen histopathology) Reproductive and offspring NOAEL: 30 mg/kg bw/d (reduction in implantation sites, fertility index, gestation index and post-implantation survival at 100 mg/kg bw/d)	[redacted] 2018 (517992)
90-day dietary study	Rat (Wistar Han)	NOAEL: 200ppm, equivalent to 14/15 mg/kg bw/d in M/F respectively, based on reduced body weight and effects on liver (increased weight, hypertrophy) at 600 ppm (42/45 mg/kg bw/d)	[redacted] 2022 (20361822; AGR6321)

These new studies have been evaluated and found acceptable by HSE. The following conclusions can be drawn.

Based on a comprehensive genotoxicity package, it can be concluded that R014821 is not genotoxic. With regard to general toxicity, the acute oral LD50 was > 2000 mg/kg bw. Compared to the acute oral LD50 for imazalil of 227 mg/kg bw (EFSA, 2010), R014821 is less acutely toxic than imazalil. In a OECD 422 gavage study in rats, the target organs were the liver, kidney, adrenals and spleen. In addition, there were effects on the oestrus cycle, the fertility index, implantation index, gestation index, litter size, post-implantation survival index and survival of offspring from a dose of 100 mg/kg bw/d. A LOAEL of 30 mg/kg bw/d was identified for parental toxicity and a NOAEL of 30 mg/kg bw/d was identified for reproductive toxicity and offspring toxicity. In a subsequent 90-day dietary study in the rat, the target organs were the liver, the adrenals and the spleen. A NOAEL of 14 mg/kg bw/d was identified based on reductions in body weight and body weight gain in both sexes, haematological changes and histopathological findings in the spleen in females and histopathological findings in the liver and adrenal glands in males at the

LOAEL of 42 mg/kg bw/d. These data indicate that R014821 may be (quantitatively) slightly more toxic than the parent imazalil. However, more importantly, the data show that qualitatively R014821 has a different toxicological profile compared to imazalil, with effects on the haematological system and the spleen, effects on the adrenals and effects on reproduction, not seen with the parent substance. On this basis, metabolite-specific dietary reference values should be derived.

The most appropriate NOAEL for the derivation of the ADI for R014821 is the NOAEL of 14 mg/kg bw/d from the 90-day study. By applying the standard safety factor of 100 for inter- and intra-species variability and an additional factor of 10 for the lack of dog data, cancer studies, multi-generation and developmental studies, an ADI of 0.014 mg/kg bw/d is established.

ADI = 0.014 mg/kg bw/d

For the derivation of the ARfD, the most relevant NOAEL is the reproductive and offspring toxicity NOAEL of 30 mg/kg bw/d from the OECD 422 study. By applying the standard safety factor of 100 for inter- and intra-species variability and an additional factor of 10 for lack of acute neurotoxicity and developmental toxicity studies, an ARfD of 0.03 mg/kg bw is established.

ARfD = 0.03 mg/kg bw

It should be noted that in line with the conclusion that R014821 may be slightly more toxic than imazalil, both of these reference values are lower than those established for imazalil (ADI = 0.025 mg/kg bw/d; ARfD = 0.05 mg/kg bw).

Metabolite FK284

Metabolite FK284 is not a major rat metabolite. Four studies have been previously evaluated and found acceptable for FK284; an acute oral toxicity study and a standard package of 3 in vitro mutagenicity studies (Ames, chromosome aberration and mammalian cell gene mutation). Based on the outcome of these studies, the acute oral LD50 of the metabolite was greater than 2000 mg/kg bw and there was no evidence of genotoxicity in the 3 in vitro tests. These studies are summarised below:

Table 2.8-4: Previous studies on FK284

Study Type	Species / test system	Result	Reference

Acute oral LD_{50}	Rat	> 2000 mg/kg bw	report no. 25985 (2010)
Ames	*S. typhimurium* 31.6 to 5000 µg/plate	± S9: negative	[redacted] (2010)
In vitro chromosome aberration	Chinese hamster V79 lung cells	± S9: negative	[redacted] (2017of)
Mammalian cell gene mutation	CHO K1 Chinese hamster ovary cells *hprt* locus	± S9: negative	[redacted] (2017)

As part of this MRL confirmatory data application, in addition to the 90-day comparative study, the applicant submitted two micronucleus studies (one in vitro and one in vivo) in order to fully exclude the genotoxic potential of the metabolite by addressing the endpoint of aneugenicity. These studies are summarised in Table 2.8-5 below.

Table 2.8-5: New studies submitted on FK284

Study Type	Species / test system	Result	Reference
90-day comparative study	Rat (Wistar Han)	Similar toxicity to imazalil	[redacted] 2021 (20192866; AGR 5959)
In vitro micronucleus study	Human lymphocytes	± S9: positive	[redacted] 2020b (20202367; AGR 5955)
In vivo micronucleus study	Mouse (NMRI)	negative	[redacted] 2020d (20247349; AGR 6184)

Based on a comprehensive genotoxicity package, it can be concluded that FK284 is not genotoxic. With regard to general toxicity, the acute oral LD50 in the rat was > 2000 mg/kg bw. Compared to the acute oral LD50 for imazalil of 227 mg/kg bw (EFSA, 2010), FK284 is less acutely toxic than imazalil. In a comparative 90-day study in the rat, FK284 showed similar toxicity to imazalil. On this basis, the dietary reference values of imazalil (ADI = 0.025 mg/kg bw/d; ARfD = 0.05 mg/kg bw) can be used in the risk assessment of FK284 if required.

Metabolite FK772

Metabolite FK772 is not a major rat metabolite. Four studies have been previously evaluated and found acceptable for FK772; an acute oral toxicity study and a standard battery of 3 in vitro mutagenicity tests (Ames, chromosome aberration and mammalian cell gene mutation). Based on the outcome of these studies, the acute oral LD_{50} of FK772 was greater than 2000 mg/kg bw. FK772 was negative in the Ames test and in the mammalian

cell gene mutation assay; however, it gave an equivocal response in the absence of S9 in the chromosome aberration test. These studies are summarised below:

Table 2.8-6: Previous studies on FK772

Study Type	Species / test system	Result	Reference
Acute oral LD_{50}	**Rat**	**> 2000 mg/kg bw**	**report no. 25983 (2010)**
Ames	*S. typhimurium* 31.6 to 5000 µg/plate	± S9: negative	[redacted] (2012)
In vitro chromosome aberration	Chinese hamster V79 lung cells	+S9: negative -S9: equivocal	[redacted] (2017)
Mammalian cell gene mutation	CHO K1 Chinese hamster ovary cells *hprt* locus	± S9: negative	[redacted] (2017)

As part of this MRL confirmatory data application, in addition to the 90-day comparative study, the applicant submitted two *in vitro* studies (micronucleus study and mammalian gene cell mutation study) in order to fully exclude the genotoxic potential of the metabolite. The in vitro micronucleus test was performed to clarify the equivocal response seen in the chromosome aberration test and to address the endpoint of aneugenicity. It is unclear why a second mammalian cell gene mutation assay was performed as the first gave a negative result. These studies are summarised in Table 2.8-7 below.

Table 2.8-7: New studies submitted on FK772

Study Type	**Species / test system**	**Result**	**Reference**
90-day comparative study	Rat (Wistar Han)	Lower toxicity than imazalil	[redacted] 2021 (20192866; AGR 5959)
In vitro micronucleus	Human lymphocytes	± S9: negative	[redacted] 2020c (20202366; AGR 5956)
In vitro mammalian gene cell mutation	L5178Y mouse lymphoma cells	± S9:negative	[redacted] 2020 (20213109; AGR 6067)

Based on a comprehensive genotoxicity package, it can be concluded that FK772 is not genotoxic. With regard to general toxicity, the acute oral LD50 in the rat was > 2000 mg/kg bw. Compared to the acute oral LD50 for imazalil of 227 mg/kg bw (EFSA, 2010), FK772 is less acutely toxic than imazalil. In a comparative 90-day study in the rat, FK772 showed lower toxicity compared to imazalil. On this basis, the dietary reference values of imazalil

(ADI = 0.025 mg/kg bw/d; ARfD = 0.05 mg/kg bw) can be used in the risk assessment of FK772 if required.

2.9 Medical data

From 2009 RAR and EFSA conclusion (2010)

No data on health effects to workers of manufacturing plants, agricultural workers and consumers are available.

In human volunteers no skin effects after application of different formulations based on imazalil were reported.

Imazalil is used clinically against fungal skin infections.

2.10 Acceptable daily intake (ADI) and acute reference dose (ARfD)

Imazalil

The dietary reference values established for the EU renewal of the active substance imazalil (EFSA Journal 2010; 8(3):1526) are summarised in Table 2.10-1. The ADI of 0.025 mg/kg bw/d was derived from the NOAEL of 2.5 mg/kg bw/d from the 1-year dog study, and was based on effects in the liver (modifications of biochemical parameters, increase in organ weight). The ARfD of 0.05 mg/kg bw was set based on the NOAEL (5 mg/kg bw) for maternal and foetal toxicity from the developmental toxicity study in rabbits. An uncertainty factor of 100 (10 for inter-species differences, 10 for intra-species differences) was applied to both the ADI and ARfD.

Table 2.10-1 Overview of the toxicological reference values for imazalil

TRVs	Source	Year	Value	Study relied upon	Safety factor
ADI	EFSA Journal 2010; 8(3):1526	2010	0.025 mg/kg bw/day	1-year dog study	100
ARfD	EFSA Journal 2010; 8(3):1526	2010	0.05 mg/kg bw	Rabbit developmental study	100

Metabolites

The ADI and ARfD proposals for the 3 metabolites, R014821, FK284 and FK772 are shown in the table below.

Table 2.10-2 Overview of the toxicological reference values for metabolites R014821, FK284 and FK772

TRVs	Source	Year	Value	Study relied upon	Safety factor
R014821					
ADI	New study ([redacted]	2022	0.014 mg/kg bw/d	90-day dietary study, rat	1000
ARfD	New study ([redacted]	2018	0.03 mg/kg bw	28-day gavage OECD 422 study, rat	1000
FK284					
ADI (parent's ADI)	EFSA Journal 2010; 8(3):1526	2010	0.025 mg/kg bw/d	1-year dog study	100
ARfD (parent's ARfD)	EFSA Journal 2010; 8(3):1526	2010	0.05 mg/kg bw	Rabbit developmental study	100
FK772					
ADI (parent's ADI)	EFSA Journal 2010; 8(3):1526	2010	0.025 mg/kg bw/d	1-year dog study	100
ARfD (parent's ARfD)	EFSA Journal 2010; 8(3):1526	2010	0.05 mg/kg bw	Rabbit developmental study	100

3 Residues in Plants

3.1 Nature of residues in primary crops

Metabolism in primary crops was investigated following foliar application (tomatoes), post-harvest application (oranges, apples, potatoes) and seed treatment (wheat and potatoes) (EFSA Conclusion, 2010).

The following summary is presented by EFSA (EFSA Conclusion, 2010):

"In all studies, imazalil was found to be the major constituent of the residues, accounting for 89-99% TRR in orange and apples (dipping), ca. 80% TRR in tomato (foliar application), 17-24% TRR in wheat forage and straw (seed treatment). The residue definition for monitoring was therefore limited to imazalil. The experts discussed if the metabolite R014821 should be included in the residue definition for risk assessment since, following post harvest application, it was observed in significant proportions in the apple metabolism study (11% TRR after a 7 month storage period). The degradation from parent to R014821 during fruit storage was confirmed by the residue trials performed in Spain, where the ratio R014821/imazalil increased slowly from 0.05 one week after treatment to 0.26 after 12 weeks. Considering that it was not possible to conclude on its toxicological relevance, having regard to the significant proportion of R014821 that is observed when the length of storage is increasing, and in view of future representative uses, the experts were of the opinion that the residue definition for risk assessment should be sum of imazalil and R014821 expressed as imazalil. It was however concluded that R014821 is of no concern for the supported uses as it was not observed in significant levels in tomato and cereals and its transfer in citrus pulp is limited."

A summary of the available primary crop metabolism data is presented in the EFSA RO, 2017 and copied below:

Table 3.1-1 Summary of the primary plant metabolism studies

Crop groups	Crop(s)	Applications	DAT [(a)] (days)
Fruit crops	Orange	Post-harvest dipping, 0.05 kg/hL	2 to 12 hours
	Apple	Post-harvest dipping, 0.05 kg/hL	Up to 7 months

	Tomato	3 x 300 g/ha foliar, BBCH 79-87 3 x 1500 g/ha foliar, BBCH 79-89	1 1
Cereals / grass crops	Wheat forage & straw	Seed treatment, 0.49 kg/tonnes	After growing under normal conditions. Forage: 42 DAT Grain: 150 DAT
Root/tuber	Potato	Seed treatment 15 g/tonnes & 75 g/tonnes	After growing under normal conditions
		Post-harvest treatment 15 g/tonnes	0 ,14, 29, 91, 188 DAT
Fruit	Apple	Foliar, 2 x 500 g a.s./ha, BBCH 69 & 71	63

(a) DAT where identification/characterisation of the residues has been investigated (*interim samplings with information limited to TRR levels only, can be omitted*)

The data gap concerning the toxicity and subsequent inclusion of metabolite R014821 in the residue definition has been addressed within the scope of this MRL evaluation.

Current residue definitions

The residue definition for enforcement (RD-Enf) in plants has been agreed as:

- Imazalil (any ratio of constituent isomers)

The residue definition for risk assessment (RD-RA) in plants for foliar uses and seed treatments has been agreed as:

- Imazalil (any ratio of constituent isomers)

EFSA (2018) recommended that any new trials should include the analysis for metabolite R014821, even though it was not included in the RD-RA for seed and foliar uses. As metabolite R014821 is not included in the RD-RA for foliar and seed treatments, and given imazalil is stable under high temperature hydrolysis, then there is no need for the analysis to include R014821 specifically for foliar or seed treatment uses.

- The RD-RA for post-harvest uses has not been agreed and was left open in the EU MRL review (EFSA 2018). Full toxicological assessment of metabolite R014821

Re-consideration of the residue definitions

In relation to the RD-Enf, imazalil is clearly the most prominent residue in all metabolism studies, and further evidenced by the magnitude of residues studies presented within this application. Sufficient analytical methods are available to enforce this residue definition and therefore HSE confirms that the RD-Enf remains as imazalil (any ratio of constituent isomers) for all crops and following all treatment regimes.

For the RD-RA for post-harvest uses, additional consideration should be made towards the potential inclusion of metabolite R014821. This metabolite was tentatively included in the RD-RA during approval (EFSA, 2010) and also considered during the MRL Review (EFSA, 2017). In the updated EU MRL review (EFSA, 2018) the RD-RA for post-harvest uses was left open. A lack of information on its genotoxicity and its general toxicological profile prevented any firm conclusions.

As sufficient information on the toxicity of this metabolite is now available, HSE can conclude on the RD-RA for post-harvest uses.

It is clear from consideration of the metabolism studies and residue trials presented in this evaluation that levels of R014821 increase during storage of the post-harvest treated commodity. While the ratio of the metabolite to imazalil measured within these trials does not exceed more than 13:87, the levels do not plateau before the trial ends. For this reason it must be concluded that the potential residues could increase beyond this ratio, and it is not clear how much imazalil could convert to R014821.

Additional data provided during the Member State consultation following the MRL review,[6] provided monitoring data from citrus fruits purchased in the EU indicating that the ratio may increase to exceed 50:50. While not directly relied on within this assessment, this data does confirm that residues of R014821 may increase beyond the levels analysed in the metabolism and residue trial studies presented.

Consideration should also be made of the toxicity profile of the R014821. The detailed toxicological consideration of the metabolite is outlined in Section 2. The metabolite is not

[6] Report on the MS Consultation for EFSA-Q-2008-00562 (Imazalil), 2017, available at https://open.efsa.europa.eu/study-inventory/EFSA-Q-2008-562.

genotoxic and a summary of the NOAEL and toxicological reference values is outlined below:

Table 3.1-2 Summary of the chronic toxicological data of imazalil and R014821

Component	NOAEL (mg/kg bw/d)	Safety factor	ADI (mg/kg bw/d)
Imazalil	2.5	100	0.025
R014821	14	1000	0.014

Table 3.1-3 Summary of the acute toxicological data of imazalil and R014821

Component	NOAEL (mg/kg bw)	Safety factor	ARfD (mg/kg bw)
Imazalil	5	100	0.05
R014821	30	1000	0.03

Based on the toxicological profile of metabolite R014821 and its occurrence in post-harvest fruits, HSE recommends that it should be included in the RD-RA for post-harvest applications. Imazalil and R014821 should be determined separately as different toxicological end-points have been established for each analyte. A combined risk assessment is not required as the toxicological profile of each analyte are qualitatively different.

Recommended residue definitions:

For post-harvest uses on fruit crops only, HSE recommends the following RD-RA:

1. Imazalil (any ratio of constituent isomers)
2. R014821

As additional data on R014821 is not required for processed commodities at this time, this RD is also relevant to processed commodities. See section 3.4 for further information.

3.2 Magnitude of residues in primary crops

Residue trials

The uses notified in the MRL application are outlined in Appendix A

0110000 Citrus fruits

There are several post-harvest application methods for use on citrus fruits that are authorised in the EU:

Dipping/drenching: 1 x 0.05 kg a.s./hL with a 0 day WHP

Low volume spray: 1 x 0.15 kg a.s./hL with a 0 day WHP.

Waxing: 1 x 0.2 kg a.s./hL with a 0 day WHP.

As the uses are post-harvest, then the term PHI (pre-harvest interval) is not appropriate. The term WHP (withholding period) is used throughout to clarify that samples have been stored post application prior to being sampled for analysis. Following the post-harvest application, the citrus fruits are stored (WHP) at ambient temperature. The samples taken at different WHP are then stored frozen prior to analysis.

For foliar treatments, the minimum PHI should be 1 day. Although a WHP of 0 days for a post-harvest use is less of a concern, as this is a GAP authorised in another jurisdiction, the WHP will be > 0 days when placed on the GB market. As the metabolite R014821 can increase with the increase in the WHP, this has been taken into account in the risk assessment.

The dipping/drenching application leads to the most critical residues and is considered to be the critical GAP in this assessment on which the MRL will be set. Residue trials data from the other application types has been presented as supporting information.

In total 64 trials have been submitted, or referenced from previous evaluations, to support the GAPs:

- 34 x drenching/dipping
- 24 x low volume spraying
- 16 x waxing

For the dipping/drenching treatment a number of the residue trials have been evaluated previously as part of the EFSA conclusion, 2010. These trials did not analyse for metabolite R014821. Additional trials were submitted to support this GAP in the MRL Review (EFSA 2017 and 2018). The full evaluation of these trials has been presented in Section C.3.1.2.

The trials were conducted on oranges and mandarins and of these 34 trials:

- 8 of the trials are not valid for the determination of R014821 as samples were stored frozen, prior to analysis, for longer than the 8 months for which acceptable storage stability data is available.
- 2 of the trials (SX11014/1 and SX11014/2) took samples outside of the acceptable WHP (31 days in the trials).

It is noted that there are several replicate trials (trials conducted on the same day in the same location). For these trials, as the trial design is slightly different (varying formulation type and crop variety) the highest residue has been selected for both whole fruit and pulp, as well as for the RD-Enf and RD-RA. Selected values have been underlined within the tables listed in Section C.3.1.2.

In total 22 trials support the GAP for dip/drench application, ten from EFSA Conclusion (2010), and twelve submitted later. The later twelve have been presented in Section C.3.1.2.

Trials using a waxing or low volume spray application have not been used to set MRLs as they demonstrate less critical residues in the RAC. The studies containing these trials have been presented in Section C.3.1.2 for completeness.

Consideration of R014821

R014821 is included in the RD-RA for post-harvest uses based on the evidence that residues will increase upon storage post-application. As the residues are to be considered separately, a sufficient number of trials are required that analyse for this metabolite in order to complete a separate risk assessment.

Residue trials supporting this use analyse for R014821, but these samples have been taken primarily straight after treatment when residues of the metabolite will be at their lowest. While in principle the data requirements for number of trials have been met, (minimum of four orange trials and four mandarin trials to extrapolate to citrus fruit group), according to OECD 509, trials should be designed to reflect pesticide use patterns that lead to the highest possible residues. For this reason, two trials on orange and two trials on mandarin have been selected, as these are the only trials that analysed samples at later WHP than 0 days (the WHP in the trials were 61-86 days) and represent the most realistic residues expected after longer withholding periods. While combining these trials is not standard procedure, it is considered in this scenario that this approach would provide more robust values on which to base the consumer risk assessment.

Table 3.2-1 Summary of R014821 residues

Application Type	Residues of R014821 (mg/kg) †	STMR	HR	Comments

	Whole fruit	Pulp			
Dipping/drenching at a max. concentration of 0.045 to 0.05 kg a.s./hL.	<0.01, <0.01, 0.013, 0.02, 0.024, 0.024, 0.027, 0.077, 0.084, 0.127	<0.01, <0.01, <0.01, <0.01, <0.01, <0.01, <0.01, 0.014, 0.017, 0.018	0.016	0.018	Residues underlined were sampled at later with-holding periods (61 or 86 days) and used for risk assessment. Other values were sampled after a 0 day WHP and therefore do not represent the most critical residue situation for this metabolite.
Waxing at a max concentration of 0.2 kg a.s./hL	2 x <0.01, 0.019, 0.022, 0.032, 0.037	5 x <0.01, 0.019	-	-	Application type produces less critical residues, values presented for information only
Low volume spraying at a concentration of 0.15 kg a.s./hL	6 x <0.01	6 x <0.01	-	-	

† Combined orange and mandarin trials

Storage stability

The use being considered is a post-harvest treatment of citrus. After the post-harvest application of imazalil, the treated citrus fruits are stored (withholding period) at ambient temperature. The citrus fruits sampled at different WHP are then stored frozen, following

standard practices, prior to analysis. The storage data outlined below is in relation to the freezer storage of the samples prior to analysis.

Storage stability of residues of the active substance imazalil was already addressed during the renewal process. Residues of imazalil were stable for at least 6 months when stored at ≤ -18ºC in tomato (water containing material), cereal grains (dry matrix (EU-Guidance), starch containing material (OECD)) and straw. Specific storage stability studies for citrus were not available. However, tomato was considered representative for citrus as the pH of tomato matrices is quite low (approximately 3-4). The use in citrus was supported by the deep frozen storage stability study in tomato along with a storage stability study in apples. Storage stability of R014821 was not demonstrated during the peer review.

New storage stability studies were evaluated by NL and presented in EFSA, 2018. These evaluations have also been presented in Section C.3.6.1 for information.

Imazalil and metabolite R014821 were shown to be stable in oranges and its processed fractions (dried peel, chopped peel, oil, molasses, and juice) for a period of up to 8 months, when stored under frozen conditions (-20°C).

Imazalil and R014821 residues were stable for at least 9 and 6 months, respectively, when stored at ≤-18°C in potato.

Imazalil and R014821 residues were stable for 12 months when stored at -20ºC in apple and apple processing fractions, and when stored at ≤ -18ºC in citrus processing fractions marmalade and essential oil.

Storage stability periods for R014821 were exceeded in a number of trials submitted by the applicant. Data from these trials are not considered for deriving end-points on the magnitude of residues used within the consumer risk assessment.

Table 3.2-2: Proven storage stability in relation to the crops under consideration (EFSA 2018):

Crops under consideration	**OECD Category**	**Imazalil**	**R014821**
Citrus and processed fractions (chopped peel and juice)	high acid content	8 months	8 months
Citrus oil	High oil content	8 months	8 months

Citrus dried peel	Dry	8 months	8 months
Citrus molasses	Miscellaneous	8 months	8 months
Apple, pear	High water content	12 months	12 months
Tomato	High water content	6 months	--

Analytical methods

The analytical methods used to support the residue trials were also evaluated during the EU review (NL, 2015 and EFSA 2017 & 2018). All methods were validated satisfactorily according to SANCO 3029/99 Rev. 4.

Extraction efficiency has been satisfactorily addressed in a study evaluated by HSE within the scope of this assessment. This evaluation can be found in Section C.1.1.4.

MRL

The OECD calculator indicates that the rounded MRL should be 8 mg/kg. However, this is based on CF x 3 x mean, which has been used as the RSD (relative standard deviation) for the residue trials data is < 50 %. However, for this post-harvest treatment on citrus, less variability in the residue levels is expected. It is therefore not appropriate to base the MRL on the outcome of the CF x 3 x mean. The MRL should be based on the mean + 4SD which gives a rounded MRL of 7 mg/kg. It is noted that the exporting jurisdiction has set the MRL on the same RD-Enf as GB at 7 mg/kg.

Extrapolation

According to SANCO 7525/VI/95, Rev. 10.3 extrapolation to the whole group of citrus fruits requires four trials on oranges and four trials on mandarins when the application is post-harvest. A sufficient number of trials on oranges and mandarins are available to support this extrapolation.

Variability factor

Following the treatment of a crop, the concentration of a pesticide residue can vary between individual units of any food commodity as a result of a variety of factors (EFSA, 2005). In the acute risk assessment default variability factors (Vf) are used to reflect the small sample sizes analysed within standard residue trials and the fact the pesticide residue may vary from unit to unit.

The acute risk assessment can be refined using a variability factor calculated for a specific active substance/ commodity use pattern. In order to refine the default Vf of 7 (5 for grapefruits in PRIMo), the applicant supplied a study on oranges performed in 2019 which has been fully evaluated in Section C.3.7. This study analysed 122 units (whole fruit).

The Vf is calculated with the following formula:

$$Vf = \frac{97.5th\ percentile}{mean}$$

The Hamilton method (2004) uses cumulative probability contributions to estimate the percentiles and is recommended by the JMPR and OECD 509. The variability factor produced using this method is 1.69.

As outlined in OECD 509, at least 119 single units should be sampled and analysed separately, and data generated from more than one trial would normally be preferable to capture wider variation to that than could be expected from analysing units from one trial. The study included 122 units (whole oranges) but the data is from only one trial. As the use under consideration is a post-harvest use, then the variability from unit to unit will be lower, which is reflected in the specific Vf calculated of 1.69, compared to the default value of 7 (or 5). Therefore, data from one trial is acceptable in this case.

It is noted that the specific Vf calculated is for whole oranges, and investigating the variability of the whole fruit vs edible portion has not been considered. Owing to no available guidance in this area, a further consideration is not required at this time. It is deemed appropriate to extrapolate the Vf calculated for oranges to all other citrus fruits, particularly given the use is a post-harvest treatment which as stated above is likely to have less unit to unit variability compared to pre-harvest treatments. The data are for treated oranges at a WHP of 0 days. This is in line with the GAP and is acceptable for imazalil. For R014821, residues may increase at longer WHP, so the data have not been used to calculate a Vf. The default Vf has been used in the acute risk assessment for the metabolite.

0163020 Bananas (CXL consideration)

The current CXL for bananas is 3 mg/kg which is higher than the current GB MRL of 0.01* mg/kg. The JMPR Report (JMPR, 2018) is available and contains residue values derived from trials conducted on banana in Central America in the 1990/91 growing seasons, as well as decline trials performed in Europe. While the JMPR did not include metabolite R014821 in the RD-RA, the residue trials outlined in the JMPR evaluation (2018) did include the analysis of this metabolite, and therefore it is possible to calculate inputs for the consumer risk assessment using the RD-RA proposed by HSE.

For all appropriate trials, the metabolite R014821 was measured at <0.05 mg/kg for both whole fruit and pulp. The residues and STMR/HR are presented in the table below.

Commodity	Crop fraction	Imazalil (mg/kg) (RD-RA 1)	R014821 (mg/kg) (RD-RA 2)	STMR	HR
Banana	Whole fruit	0.22, 0.31, 0.39, 0.58, 0.61, 0.71, 1.01, 1.12, 1.30, 1.34, 1.54, 1.68, 1.69	All <0.05 (13)	-	-
	Pulp	8 x 0.05, 0.06, 0.07, 0.1	All <0.05 (11)	**1) 0.05** **2) 0.05**	**1) 0.1** **2) 0.05**

0232030 Courgettes

The GAP supporting the MRL in force is 75 g a.s./ha x 4, with a RTI of 7 days and a PHI of 1 day. In the MRL review 4 trials were available to support this GAP and an MRL of 0.1 mg/kg. However, only two trials were compliant with the GAP and in a further two trials, 3 applications instead of 4 applications were made. The following supplementary information requirement was set to retain the MRL:

- Additional residue trials supporting the GAP as appropriate on courgettes

Four new residues trials, conducted on protected cucumber has been submitted to support the GAP considered in the MRL review. The extrapolation of residues trials data from cucumbers to courgette is acceptable.

A full analysis of the trials can be found in section C.3.1.2.

The application rate in the trials was 4 x 75 g a.s./ha with a RTI of 5 days and a PHI of 1 day. The deviation from a RTI of 7 days to 5 days is outside the 25 % deviation. However, it is noted that the two trials accepted in the MRL review as being conducted at the GAP, also had a RTI of 5 days. Therefore, no further consideration is required in the framework of this assessment. The trials were conducted in accordance with the relevant guidance documents (OECD 509), and no significant deviations were noted in the trials.

Storage stability

The samples were stored at ≤ -18°C for a maximum period of 117 days. The MRL review confirmed that for imazalil data for high water commodities is available for apples (raw and

processed) for 12 months of storage and tomatoes for 6 months of storage. Data for R014821 is only available in apples. These data are sufficient to support the new trials.

The metabolite R014821 is only relevant to post harvest uses, and therefore has not been considered further.

Analytical methods

Analysis for residues of imazalil and metabolite R014821 was performed using an LC-MS/MS method which was regarded as fully validated for the residue trials for cucumbers accepted for the MRL review. The procedural recovery data for the new trials were acceptable.

Previous residue trials from the MRL review

In the MRL review, two residue trials were conducted at the GAP of 4 x 75 g a.s./ha and accepted as supporting the GAP. For completeness these trials are summarised in section C.3.1.2.

Summary

There are 6 trials that support the cGAP (4 x 75 g a.s./ha, RTI of 5-7 days, PHI 1 day) considered for the MRL review. Courgettes are regarded as a tall crop and the spray concentration can impact the residue levels. As this was not considered within the MRL review, no further consideration to this issue has been made in the framework of this assessment. Future amendments to the MRL may require a consideration of the spray concentration on the residue levels.

The residue trials included the analysis of imazalil and the metabolite R014821. This metabolite is not relevant to the use on courgettes as it is relevant to post-harvest uses only, noting in all the trials the metabolite was < 0.01 mg/kg.

As courgettes are a minor crop in GB, then 4 trials would be sufficient. The data support an MRL of 0.09 mg/kg. The current MRL is 0.1 mg/kg. HSE recommends that the current MRL is retained rather than lowering the MRL by one MRL class.

HSE recommends that the footnote outlining the data gap for additional residue trials for courgette is deleted in the GB MRL Statutory Register.

Table 3.2-3 Overview of the available residue trials data

Commodity	Region/ Indoor (a)	Residue levels (mg/kg) observed in the trials representative for the intended GAPs (b)	Recommendations/ comments (OECD calculations)	MRL proposals (mg/kg)	HR (mg/kg) (c)	STMR (mg/kg) (d)
Citrus – dripping/drenching (1 x 0.05 kg/hL) Combined EFSA, 2010 and additional trials Orange and mandarin trials combined	Indoor	**RD-Enf (whole fruit only):** 1.14, 1.30, 1.36, 1.51, 1.83, 2.06, 2.09, 2.19, 2.26, 2.46, 2.55, 2.58, 2.59, 2.66, 2.69, 2.72, 2.81, 2.89, 3.12, 3.34, 4.84, 4.95 **RD-Enf (pulp):** 0.021, 0.032, 0.135, 0.147, 0.183, 0.189, 0.210, 0.370, 0.395, 0.430, 0.689, 0.698	**RD-Enf: whole fruit MRL_{OECD} unrounded** Mean + 4SD: 6.391 CF x 3 x mean: 7.628 The OECD calculator has proposed the rounded MRL should be 8 mg/kg. This is based on the unrounded value of CF x 3 x mean. The MRL is proposed on this basis as	**7**	RD-Enf (whole fruit): 4.95 RD-Enf (pulp): 0.698 RD-RA (whole fruit): 1) 4.95 2) 0.127	RD-Enf (whole fruit): 2.57 RD-Enf (pulp): 0.20 RD-RA (whole fruit): 1) 2.57 2) 0.081
		RD-RA (whole fruit)[(e)]: 1) Imazalil 1.14, 1.30, 1.36, 1.51, 1.83, 2.06, 2.09, 2.19, 2.26, 2.46,	the RSD for the dataset is < 50 %. However, for a post-harvest use a lower RSD is expected. The		RD-RA (pulp): 1) 0.698 2) 0.018	RD-RA (pulp): 1) 0.20 2) 0.016

Commodity	Region/ Indoor (a)	Residue levels (mg/kg) observed in the trials representative for the intended GAPs (b)	Recommendations/ comments (OECD calculations)	MRL proposals (mg/kg)	HR (mg/kg) (c)	STMR (mg/kg) (d)
		2.55, 2.58, 2.59, 2.66, 2.69, 2.72, 2.81, 2.89, 3.12, 3.34, 4.84, 4.95 2) R014821 <0.01, <0.01, 0.01, 0.013, 0.02, 0.02, 0.024, 0.024, 0.027, 0.077, 0.084, 0.127 **RD-RA (pulp)[(e)]:** 1) Imazalil 0.021, 0.032, 0.135, 0.147, 0.183, 0.189, 0.210, 0.370, 0.395, 0.430, 0.689, 0.698 2) R014821 <0.01, <0.01, <0.01, <0.01, <0.01, 0.014, 0.017, 0.018	MRL should be based on the mean + 4SDs: **MRL_{OECD} unrounded/ unrounded: 6.391/7**			

Commodity	**Region/ Indoor (a)**	**Residue levels (mg/kg) observed in the trials representative for the intended GAPs (b)**	**Recommendations/ comments (OECD calculations)**	**MRL proposals (mg/kg)**	**HR (mg/kg) (c)**	**STMR (mg/kg) (d)**
Citrus – waxing 1 x 0.2 kg/hL EFSA, 2010 and additional trials combined. Orange and mandarin trials combined	Indoor	**RD-Enf (whole fruit):** 0.20, 0.30, 0.39, 0.40, 0.49, 0.52, 0.56, 0.61, 0.62, 0.62, 0.76, 0.77, 0.91, 0.94, 0.97, 1.14 **RD-Enf (pulp):** 4 x <0.01, 0.013, 0.039 **RD-RA (whole fruit)[(e)]:** 1) 0.20, 0.30, 0.39, 0.40, 0.49, 0.52, 0.56, 0.61, 0.62, 0.62, 0.76, 0.77, 0.91, 0.94, 0.97, 1.14 2) 2 x <0.01, 0.019, 0.022, 0.032, 0.037	**RD-Enf: whole fruit** Mean + 4SD: 1.684 CF x 3 x mean: 1.913 **MRL_{OECD} unrounded/rounded: 1.684/2**	**2**	RD-Enf (whole fruit): 1.14 RD-Enf (pulp): 0.039 RD-RA (whole fruit): 1) 1.14 2) 0.037 RD-RA (pulp): 1) 0.039 2) 0.019	RD-Enf (whole fruit): 0.62 RD-Enf (pulp): <0.01 RD-RA (whole fruit): 1) 0.62 2) 0.021 RD-RA (pulp): 1) <0.01 2) <0.01

Commodity	Region/ Indoor (a)	Residue levels (mg/kg) observed in the trials representative for the intended GAPs (b)	Recommendations/ comments (OECD calculations)	MRL proposals (mg/kg)	HR (mg/kg) (c)	STMR (mg/kg) (d)
		RD-RA (pulp)[(e)]: 1) 4 x <0.01, 0.013, 0.039 2) 5 x <0.01, 0.019				
Citrus – Low volume spray Orange and mandarin trials combined	Indoor	**RD-Enf (whole fruit only):** 0.55, 0.64, 0.87, 1.05, 1.08, 1.16, 2.07, 2.40 **RD-Enf (pulp):** <0.01, 0.016, 0.019, 0.022, 0.046, 0.048, 0.05, 0.974 **RD-RA (whole fruit):** 1) 0.55, 0.64, 0.87, 1.05, 1.08, 1.16, 2.07, 2.40	**RD-Enf: whole fruit** Mean + 4SD: 3.878 CF x 3 x mean: 3.683 **MRL_{OECD} unrounded/rounded: 3.683/4**	**4**	RD-Enf (whole fruit): 2.4 RD-Enf (pulp): 0.974 RD-RA (whole fruit): 1) 2.4 2) <0.01	RD-Enf (whole fruit): 1.07 RD-Enf (pulp): 0.034 RD-RA (whole fruit): 1) 1.07 2) <0.01

Commodity	Region/ Indoor (a)	Residue levels (mg/kg) observed in the trials representative for the intended GAPs (b)	Recommendations/ comments (OECD calculations)	MRL proposals (mg/kg)	HR (mg/kg) (c)	STMR (mg/kg) (d)
		2) 6 x <0.01 **RD-RA (pulp):** 1) <0.01, 0.016, 0.019, 0.022, 0.046, 0.048, 0.05, 0.974 2) 6 x <0.01			RD-RA (pulp): 1) 0.974 2) <0.01	RD-RA (pulp): 1) 0.034 2) <0.01
Cucumbers → Courgettes (at 4 x 0.075 kg/ha)	Indoor/glass house.	<u>New trials:</u> RD-Enf = RD-RA: 3x<0.01; 0.02 <u>Netherlands, 2015</u> RD-Enf = RD-RA: 0.02; 0.05	**RD-Enf:** **MRL_{OECD} unrounded/rounded:** **0.082/0.09**	**0.09§**	0.05	0.015

§ The current GB MRL of 0.1 mg/kg is recommended to be retained. See text for justification.

(a) GB or the country for an import tolerance and whether the GAP is for an outdoor use or an indoor/protected/glasshouse use.

(b) Residue levels in trials conducted according to GAPs reported in ascending order. When residue definitions for enforcement and risk assessment differ, **Enf/RA** differentiate data expressed according to the residue definition for **Enf**orcement and **R**isk **A**ssessment.

(c) **HR**: Highest residue, according to the residue for risk assessment, (within brackets when expressed according to the residue definition for enforcement: HR_{Enf})

(d) **STMR**: Supervised Trials Median Residue according to the residue definition risk assessment (within brackets when expressed according to the residue definition for enforcement: $STMR_{Enf}$)

(e) Where values are underlined, these are the residues of R017821 in samples taken following longer WHPs, and represent the more critical and more realistic residues expected after longer storage periods

Note:

The residue definition for enforcement (RD-Enf) in plants has been agreed as:

- Imazalil (any ratio of constituent isomers)

The residue definition for risk assessment (RD-RA) in plants has been agreed as:

Post-harvest

1. Imazalil (any ratio of constituent isomers)
2. R014821

For foliar treatment and seed treatment

- Imazalil (any ratio of constituent isomers)

3.3 Conversion factors for risk assessment for products of plant origin

For foliar and seed treatments, the RD-RA and RD-Enf are the same and therefore no conversion factors are required.

For post-harvest uses, as the residue definitions for enforcement (RD-Enf) and risk assessment (RD-RA) are different, conversion factors (CFs) for enforcement to risk assessment should be calculated. Typically, conversion factors are used to estimate the RD-RA as a multiplication of the RD-Enf, to account for the inclusion of additional analytes (metabolites) in the RD-RA compared to the RD-Enf. In this specific case, R014821 is not summed with parent and is determined and assessed separately. For this reason, the term conversion factor is not considered appropriate and instead 'formation factor' is used. This formation factor is a measure of the level of R014821 present when compared to the level of imazalil.

The formation factor is variable, as it is dependent on the withholding period, and therefore the formation fraction for different scenarios has been calculated. In all cases the formation factors have been calculated for the whole fruit, reflecting the commodity analysed for the RD-Enf and the fact this will give a worst case residue level for the metabolite, compared to its level in pulp.

Tables 3.3-1 and 3.3-2 are a summary of the formation fractions calculated for a WHP of 0 days and at the longest WHP respectively.

Table 3.3-1 Formation factors for imazalil to R014821 for a WHP of 0 days

Imazalil residue (mg/kg)	R014821 residue (mg/kg)	Formation factor
2.26	0.012	0.005
3.34	0.02	0.006
2.81	0.01	0.004
2.46	0.01	0.004
2.66	0.018	0.007
1.77	0.01	0.006
2.09	0.01	0.005
1.56	0.01	0.006
2.12	0.019	0.009
2.76	0.014	0.005
1.39	0.01	0.007
1.84	0.01	0.005
1.13	0.01	0.009

2.72	0.027	0.010
1.39	0.01	0.007
2.58	0.01	0.004
1.48	0.01	0.007
2.55	0.01	0.004
2.17	0.01	0.005
2.69	0.01	0.004
2.37	0.02	0.008
2.44	0.016	0.007
1.89	0.01	0.005
2.14	0.013	0.006
0.604	0.01	0.017
1.308	0.011	0.008
0.544	0.01	0.018
1.016	0.01	0.010
1.056	0.01	0.009
0.628	0.01	0.016
1.092	0.026	0.024
0.444	0.016	0.036
0.972	0.024	0.025
0.636	0.024	0.038
	Median:	**0.007**

Table 3.3-2 Formation factors for imazalil to R014821 at the longest WHP

Trial (Trial number)	WHP (days)	Imazalil residue (mg/kg)	R014821 residue (mg/kg)	Formation factor
1 (S11-03184-02)	61	1.03	0.077	0.075
2 (S11-03184-06)	85	0.564	0.084	0.149
3 (S11-03184-01)	86	1.34	0.127	0.095
4 (S11-03184-05)	86	0.908	<0.01	<0.011
			Median:	**0.085**

The residue trials demonstrate that imazalil residue will decline with longer WHP, whereas the residue levels of R014821 will increase with the WHP. To reflect this, formation factors

have been calculated considering the imazalil residue at a WHP of 0 days and the highest residue of R014821 found. These formation fractions are summarised in Table 3.3-3.

Table 3.3-3 Formation factors for imazalil to R014821 (worst case)

Trial (Trial number)	Imazalil residue (PHI 0) (mg/kg)	WHP (days) giving HR of R014821	R014821 residue (Longest WHP) (mg/kg)	Formation factor
1 (S11-03184-02)	2.26	61	0.077	0.034
2 (S11-03184-06)	2.81	85	0.084	0.030
3 (S11-03184-01)	2.66	86	0.127	0.048
4 (S11-03184-05)	2.09	86	<0.01	<0.005
			Median:	**0.032**

3.4 Effect of industrial processing and/or household preparation

3.4.1 Nature of the residues in processed commodities

Hydrolysis of imazalil under representative conditions of pasteurisation, baking, brewing, boiling and sterilization was evaluated after the peer review, in the framework of the confirmatory data process (EFSA Article 12 RO, 2017).

A summary of the available hydrolysis data copied from EFSA Article 12 RO, 2017 and copied below:

"*Studies were conducted with imazalil, simulating representative hydrolytic conditions for pasteurisation (20 min at 90°C, pH 4), boiling/brewing/baking (60 min at 100°C, pH 5) and sterilisation (20 min at 120°C, pH 6). Although this study was not conducted with radiolabelled material, the test compound was found at an amount of 94–99% after any kind of hydrolysis. Therefore, it was concluded that processing by pasteurisation, baking/brewing/boiling and sterilisation is not expected to have a significant impact on the composition of residues in matrices of plant origin.*"

3.4.2 Magnitude of the residues in processed commodities

The magnitude of residues, for imazalil, on the processing of oranges was considered in the MRL review. Robust processing factors were derived for processed commodities of orange juice (0.08), dry pomace (4.2), wet pomace (2) and marmalade (0.27). For dry pomace, the Pf of 4.2 has been used in the livestock dietary burden calculation (see section 4).

In addition, the distribution of residues in the whole fruit and pulp of oranges was considered. A peeling factor of 0.07 was determined for imazalil.

Consideration of R014821

The nature and magnitude of residues on processing for R014821 has not been investigated. The chronic exposures for all citrus are < 10 % of the ADI and therefore in accordance with the data requirements outlined in Assimilated Regulation 544/2011, a further consideration is not required.

The distribution of residues in the whole citrus fruit and peel can be determined for the longer WHP, and the data are summarized in Table 3.4-1. In the majority of cases, residues of R014821 were < 0.01 mg/kg in the pulp at a WHP of 0 days and peeling factors have not been determined.

Table 3.4-1 Peeling factors for R014821

Trial (Trial number)	WHP (days)	R014821 residue in whole fruit (mg/kg)	R014821 residue in pulp (mg/kg)	Peeling factor
1 (S11-03184-02)	61	0.077	0.017	0.22
2 (S11-03184-06)	85	0.084	0.018	0.21
3 (S11-03184-01)	86	0.127	0.014	0.11
4 (S11-03184-05)	86	<0.01	<0.01	N/A
			Median:	0.21

Peeling factor = Residue level in pulp
Residue in whole fruit

3.5 Rotational crops

The uses on citrus and banana are post-harvest uses, and therefore residues in rotational crops are not relevant. The use on courgettes is being considered with respect to the MRL supplementary information requirements only, and no data gaps were identified with respect to residues in rotational crops.

4 Residues in livestock

As the crops under consideration are fed to animals, consideration of residues in products of animal origin are required.

4.1 Dietary burden

The dietary burden calculation has been undertaken using the OECD feeding tables (OECD guidance document on overview of residue chemistry studies Series 64/32) and the EFSA Animal Model 2017.

The following assumptions have been made:

- The highest likely inclusion rate of all crops which may have been treated has been used with the proviso that the aggregate does not exceed 100% diet.
- All produce eaten which may have been treated, has been treated and contains residues at the STMR/HR found in the trials considered to support the GAP.
- There is no loss of residue during transport, storage, preparation of feed prior to consumption.
- Default processing factors as set out in the EFSA Animal Model 2017 have been used unless other specific processing factors are available or residues <LOQ are expected.

Input values are summarised in Table 4.1-1 and the estimated animal intakes are summarised in Table 4.1-2.

Table 4.1-1 Input values for the dietary burden calculation
Risk assessment residue definition: 1) Imazalil (any ratio of constituent isomers)

Feed commodity	Median dietary burden		Maximum dietary burden	
	Input (mg/kg)	Comment	Input (mg/kg)	Comment
Citrus (dried pulp)	10.79	STMR – whole fruit (RD-RA) X PF (4.2)	10.79	STMR – whole fruit (RD-RA) X PF (4.2)
Potato, culls	0.01*	STMR (EFSA, 2017)	0.01*	STMR (EFSA, 2017)
Potato, process waste	0.01*		0.01*	
Potato, dried pulp	0.01*		0.01*	

Feed commodity	Median dietary burden		Maximum dietary burden	
	Input (mg/kg)	Comment	Input (mg/kg)	Comment
Brewer's grain, dried	0.01*		0.01*	
Wheat, distiller's grain (dry)	0.01*		0.01*	
Wheat gluten, meal	0.01*		0.01*	
Wheat, milled by-products	0.01*		0.01*	
Small grain cereals, grain	0.01*		0.01*	
Small grain cereals, straw	0.01*		0.01*	

Table 4.1-2 Estimated animal intakes

Animal	Median burden (mg/kg bw)	Maximum burden (mg/kg bw)	>0.1 mg /kg DM (Y/N)	Maximum burden (mg/kg DM)	Highest contributing commodity[(a)]	Previous assessment (Max. burden: mg/kg DM)
Cattle (all diets)	0.092	0.092	Y	2.39	Citrus dried pulp	1.96
Cattle (dairy only)	0.092	0.092	Y	2.39	Citrus dried pulp	1.96
Sheep (all diets)	0.002	0.002	N	0.05	Potato processed waste	0.05
Sheep (ewe only)	0.002	0.002	N	0.05	Potato processed waste	0.05
Swine (all diets)	0.042	0.042	Y	1.81	Citrus dried pulp	1.48
Poultry (all diets)	0.001	0.001	N	0.02	Potato culls	0.02
Poultry (layer only)	0.001	0.001	N	0.02	Potato culls	0.02

(a) Considering the maximum dietary animal burden

Based on the dietary burden calculations consideration of the likely residues in food of animal origin is required as the trigger of 0.1 mg/kg DM and AR is exceeded for ruminants and swine. On this basis further consideration of the residues in POAO is required.

Consideration of R014821

As metabolite R014821 is to be considered separately, a dietary burden has been calculated based on residues found in the trials on citrus (multiplied by default processing factor for dried pulp). Additional inputs for potato (and processed potato fractions) has been included as this is a potential post-harvest use. It is noted that as predicted residues in potato are <LOQ, no processing factor has been applied. A summary of the input values is provided below:

Table 4.1-3 Estimated animal intakes
Risk assessment residue definition: 2) R014821

Feed commodity	**Median dietary burden**		**Maximum dietary burden**	
	Input (mg/kg)	**Comment**	**Input (mg/kg)**	**Comment**
Citrus (dried pulp)	0.16	STMR – whole fruit (RD-RA) X PF (10)	0.16	STMR – whole fruit (RD-RA) X PF (10)
Potato, culls	0.01*	STMR (EFSA, 2017)	0.01*	STMR (EFSA, 2017)
Potato, process waste	0.01*		0.01*	
Potato, dried pulp	0.01*		0.01*	

Table 4.1-4 Estimated animal intakes

Animal	Median burden (mg/kg bw)	Maximum burden (mg/kg bw)	>0.1 mg /kg DM (Y/N)	Maximum burden (mg/kg DM)	Highest contributing commodity[(a)]
Cattle (all diets)	0.002	0.002	N	0.05	Citrus dried pulp
Cattle (dairy only)	0.002	0.002	N	0.05	Citrus dried pulp
Sheep (all diets)	0.002	0.002	N	0.05	Potato processed waste
Sheep (ewe only)	0.002	0.002	N	0.05	Potato processed waste
Swine (all diets)	0.001	0.001	N	0.05	Citrus dried pulp
Poultry (all diets)	0.001	0.001	N	0.01	Potato culls
Poultry (layer only)	0.000	0.000	N	0.01	Potato culls

(a) Considering the maximum dietary animal burden

Residues of R014821 are less than 0.1 mg/kg (DM and AR) in the diets of livestock and therefore residues in POAO are not expected. A further consideration is not required at this time.

4.2 Nature of residues (Animal metabolism studies)

Metabolism in livestock was investigated in lactating goats (ruminants) and laying hens (poultry) for the approval of imazalil (EFSA, 2010). Metabolism in pig and/or rat was also considered for approval of the active.

The following summary is presented by EFSA (EFSA Conclusion, 2010):

"*The metabolism of imazalil was investigated in lactating goats and laying hens (Belgium, 1996). The summary of the study with laying hens initially provided during the two peer reviews was not sufficient to conclude on a metabolic pathway in poultry as the identification of the metabolites was limited. Although further detailed results were provided by the RMS in its evaluation report (Netherlands, 2015), EFSA is of the opinion that an additional study would be needed to fully depict the metabolic pathway in poultry.*

The available metabolism studies showed imazalil to be extensively metabolised in livestock. In goat tissues, the parent compound represents less than 6% of the TRR and is not detected at all in milk. Two metabolites, FK-772 (goat kidney and muscle) and FK-284 (goat muscle), were found in higher proportions than the parent compound. These metabolites are the only degradation products representing more than 10% of the TRR in goat tissues (15–21% TRR) and they were also present in low proportion in milk (3–6% TRR).

In poultry, imazalil was only detected in eggs and fat, representing 8% and 11% of TRR, respectively. The metabolite FK-772 was only retrieved in liver, where it accounted for less than 9% of the TRR. One metabolite (FK-858) was found in proportion higher than 10% of the TRR in eggs and hen muscle *(11–15% TRR)* but corresponding to quite low levels in these matrices (0.02–0.09 mg *eq/kg). In both ruminants and poultry, the remaining radioactivity consists of several minor metabolites, all remaining in very low proportions.*

Based on the above results, imazalil may not be a sufficient marker in livestock commodities. The peer review under the renewal procedure concluded that metabolites FK-772 and/or FK-284 should be taken into account for enforcement purpose in ruminant matrices (EFSA, 2010). Assuming that these compounds share a similar toxicity as the parent compound and considering that metabolite FK-772 was a better marker for enforcement compared to FK-284, a residue definition for enforcement was previously and provisionally defined as the 'sum of imazalil and its metabolite FK-772 (any ratio of constituent isomers), expressed as imazalil'. For risk assessment, the proposal was extended to the sum of imazalil and all identified/characterised metabolites (EFSA, 2017). These proposals were tentative pending the full assessment of the toxicological properties for metabolites FK-772 and/or FK-284. In the framework of a MRL application, the applicant provided additional toxicological studies to address these data gaps (Netherlands, 2015). The additional data submitted were found to be insufficient to

conclude whether the toxicological reference values derived for parent imazalil would be appropriate for these metabolites (EFSA, 2018). Consequently, a decision on the residue definitions for enforcement and risk assessment cannot be derived for livestock commodities. Thus, the tentative residue definitions for enforcement and risk assessment previously proposed are suspended. In addition, it was also noted that the available metabolism studies do not investigate the possible impact of the livestock metabolism on the isomer ratio of imazalil and its metabolites."

A summary of the available livestock metabolism data is presented in the EFSA Conclusion, 2010 and copied below:

Table 4.2-1 Summary of the available animal metabolism studies

Animal	Dose rate (mg/kg bw/day	Duration	Comment
Laying hen	4.6	10 days	It is noted in EFSA that the poultry study was not deemed sufficient and a data gap was set for an additional metabolism study on poultry. As the dietary burden is only exceeded for ruminants, this data gap has not been considered within this assessment.
Lactating goat	10	3 days	

Current residue definitions

As outlined in the updated EU MRL review (EFSA 2018), there were no proposals for the residue definitions for livestock. MRLs were established for products of animal origin on the basis of the following RD-Enf:

RD-Enf: Sum of imazalil and metabolite FK-772 (any ratio of constituent isomers) expressed as imazalil

This RD-Enf was retained in the GB MRL Statutory Register.

In terms of the RD-RA, the following is noted from EFSA 2018:

It is noted that metabolites FK-772 and FK-284 can be found in significant levels in livestock tissues and products. Due to the equivocal results for genotoxicity for metabolite FK-772, the lack of a repeated dose study for metabolite FK-772 and FK-284 and the lack of information on the impact livestock of metabolism on the isomer ratio of imazalil, the previously derived residue definitions have been suspended, pending submission of

further data as specified in EFSAs reasoned opinion published in June 2018 (EFSA, 2018).

The following MRL supplementary information was established, and has been assessed in this application:

- Full toxicological assessment of metabolites FK-772 and FK-284

Re-consideration of residue definitions

Following submission of additional data concerning the relative toxicity of metabolites FK-772 and FK-284, it is possible to conclude on the RD-Enf and RD-RA for animal products.

For enforcement, imazalil is extensively metabolised in livestock and is not present in significant levels in any matrix, and was not detected at all in milk. Therefore, an additional marker is required to allow for enforcement of MRLs across all product of animal origin. The only metabolite present in all matrices (except fat) in the ruminant metabolism study was FK-772 and therefore this would be a suitable marker to include with parent in the RD-Enf. It is noted that as the residue is not fat-soluble, a marker not being available in fat is not of concern.

The current RD-Enf in the GB MRL Statutory Register of the sum of imazalil and metabolite FK-772 (any ratio of constituent isomers) expressed as imazalil can therefore be confirmed.

According to the Evaluation Report produced by NL (2015) and EFSA (2017), sufficient methods are available to determine imazalil and FK-772 in products of ruminant origin. As demonstrated by the calculated dietary burden from all currently authorised uses, residues in poultry are negligible and therefore an enforcement method in poultry matrices is not required at this time.

For risk assessment, in addition to imazalil and FK-772, only the potential inclusion of the metabolite FK-284 needs to be considered, at this time, based on the specific MRL supplementary information requirement that was set (see above).

It has been established within the toxicological evaluation that FK-284 and FK-772 are not genotoxic and that they are of a lower toxicity than imazalil; HSE concludes that the metabolites are covered by the toxicological reference values established for imazalil.

The following table provides a summary of the identified metabolites of concern in each ruminant commodity:

Table 4.2-2 Summary of identified metabolites (%TRR)

Matrix	Parent	FK 284	FK 772
Milk	-	6.4	5.5
Liver	5.9	2.1	4.1
Kidney	3.6	-	15
Muscle	2.9	15.2	21.2
Fat	5.7	9.0	-

Overall, neither FK-284 or FK-772 should be excluded from the RD-RA as both metabolites are present in varying ruminant matrices and are the only metabolites present above 10% of the TRR.

Livestock feeding studies evaluated during renewal (RAR, 2009) indicate variable but broadly comparable patterns of residues, and do demonstrate that all three components are present in all matrices. This is not in line with the metabolism study which indicated that no parent would be detected in milk, no FK-284 would be present in kidney, and no FK-772 would be present in fat. With this established, this provides further reassurance that all three components should be included in the RD-RA.

It is noted that there are two MRL supplementary requirements relating to the livestock feeding studies (also see section 7);

- A study demonstrating the storage stability of imazalil and its metabolite FK-772 in commodities of animal origin.
- Information on the storage conditions of the samples of the feeding studies performed with ruminants.

No data have been considered in this assessment to address these two requirements. Whilst, the dietary burden has increased, as a result of information laid out within this assessment, no amendments to the MRLs for products of animal origin are required. Therefore, the two requirements relating to the feeding study do not need to be considered further in this assessment, and will be considered separately. However, to support new MRLs for products of animal origin, these two requirements will need to be fully addressed. It should also be further noted that in the MRL review (EFSA, 2017), the following was stated by EFSA:

Although imazalil and its metabolite FK-772 are expected to be sufficient residue markers in animal matrices, it is highlighted that the remaining identified and characterised radioactivity still represents an important part of the TRR (30–60% TRR in ruminants; information not available for poultry).For risk assessment purpose, it was therefore proposed to consider the sum of imazalil and all identified/characterised metabolites observed in the goat metabolism study (EFSA, 2010). CFs from enforcement to risk assessment were therefore tentatively derived on the basis of the goat metabolism study: 3 in muscle, 11 in fat, 4 in liver, 3 in kidney and 12 in milk

Additional data may therefore be required to reconsider the RD-RA if new MRLs are to be established.

Conclusion

HSE recommends the following residue definitions for livestock:

RD-Enf (ruminants): Sum of imazalil and metabolite FK-772 (any ratio of constituent isomers) expressed as imazalil

RD-RA (ruminants): Sum of imazalil, FK-284 and FK-772 (any ratio of constituent isomers), expressed as imazalil.

The molecular weight conversions are as follows:

$MW_{imazalil}$: 297.2 g/mol

MW_{FK-772}: 363.2 g/mol Conversion to imazalil: 0.82

MW_{FK-284}: 289.1 g/mol Conversion to imazalil: 1.03

These residue definitions are only relevant for ruminants and the current MRLs established in the GB MRL Statutory Register, noting the additional data that may be required if new MRLs are to be established. Residues are not expected in products of poultry origin, so specific residue definitions do not need to be established at this time. It is important to note that in the MRL review, it was concluded that the poultry metabolism study was not sufficient to elucidate the metabolic pathway in poultry and a new study may be required.

4.3 Magnitude of residues

As the proposed MRLs include animal feedstuffs and the dietary burden is ≥0.1 mg/kg (AR and DM) consideration of feeding studies for ruminants is required.

A livestock feeding study was undertaken in lactating cows (ruminants) and laying hens for the renewal of imazalil (NL, 2009a).

Lactating cows were dosed with imazalil, at a rate of 45.4, 136 and 454 mg/kg DM (which equates to 1.65, 4.95 and 16.5 mg/kg bw/day). The study duration was 28 days. Residues of imazalil and metabolites FK-772 and FK-284 were analysed for.

The following summary is presented by EFSA (EFSA Conclusion, 2018):

"*The magnitude of imazalil residues in livestock was investigated in one study performed with lactating cows and one study performed with laying hens, both assessed during the European peer review (Netherlands, 2009a). Depending on the dietary burden that might be calculated in the future and pending a final conclusion regarding the residue definitions for livestock commodities, these studies may be used to derive MRL and risk assessment values. It is however noted that some deficiencies related to these studies were highlighted in the previous EFSA opinion (EFSA, 2017).*"

It is noted that the deficiencies referred to by EFSA relate to the poultry livestock studies and are therefore not relevant to this assessment.

Feeding studies have been used to calculate chronic, acute and MRL values for animal commodities as set out in 7.2.4.1.

The MRL proposals do not exceed the current GB MRLs based on the proposed uses therefore no new MRLs need to be set for POAO.

Table 4.3-1 MRL, STMR and HR proposals for cattle

RD-Enf: Sum of imazalil and metabolite FK-772 (any ratio of constituent isomers) expressed as imazalil
RD-RA: Sum of imazalil, FK-284 and FK-772 (any ratio of constituent isomers), expressed as imazalil

Closest feeding level[a] **1.65 mg/kg bw** **18 N rate (all diets)** **18 N rate (dairy only)**	**Mean residues at the closest feeding level (mg/kg)**	**Highest residues at the closest feeding level (mg/kg)**	**Estimated STMR-Enf[b] at 1N[c] (mg/kg)**	**Estimated HR-Enf at 1N[c] (mg/kg)**	**MRL proposal (mg/kg)**	**STMR (mg/kg)**	**HR (mg/kg)**
Muscle	0.01	0.02	0.00	0.00	**< 0.02**	0.00	0.00
Fat	0.01	0.01	0.00	0.00	**< 0.02**	0.00	0.00
Liver	0.44	0.55	0.02	0.03	**0.03**	0.03	0.04
Kidney	0.08	0.09	0.00	0.01	**< 0.02**	0.01	0.01
Milk[d]	0.02	0.03[e]	0.00	0.00	**< 0.02**	0.01	n/a

Table 4.3-2 MRL, STMR and HR proposals for sheep

RD-Enf: Sum of imazalil and metabolite FK-772 (any ratio of constituent isomers) expressed as imazalil
RD-RA: Sum of imazalil, FK-284 and FK-772 (any ratio of constituent isomers), expressed as imazalil

Closest feeding level[a] **xxx mg/kg bw** **xx N rate (all diets)** **xx N rate (dairy only)**	**Mean residues at the closest feeding level (mg/kg)**	**Highest residues at the closest feeding level (mg/kg)**	**Estimated STMR-Enf[b] at 1N[c] (mg/kg)**	**Estimated HR-Enf at 1N[c] (mg/kg)**	**MRL proposal (mg/kg)**	**STMR (mg/kg)**	**HR (mg/kg)**

Muscle	0.01	0.02	0.00	0.00	**<0.02**	0.00	0.00
Fat	0.01	0.01	0.00	0.00	**<0.02**	0.00	0.00
Liver	0.44	0.55	0.00	0.00	**<0.02**	0.00	0.00
Kidney	0.08	0.09	0.00	0.00	**<0.02**	0.00	0.00
Milk[(e)]	0.02	0.03 [(e)]	0.00	0.00	**<0.02**	0.00	n/a

Table 4.3-3 MRL, STMR and HR proposals for pigs

RD-Enf: Sum of imazalil and metabolite FK-772 (any ratio of constituent isomers) expressed as imazalil
RD-RA: Sum of imazalil, FK-284 and FK-772 (any ratio of constituent isomers), expressed as imazalil

Closest feeding level[(a)] xxx mg/kg bw xx N rate (all diets)	**Mean residues at the closest feeding level (mg/kg)**	**Highest residues at the closest feeding level (mg/kg)**	**Estimated STMR-Enf[(b)] at 1N[(c)] (mg/kg)**	**Estimated HR-Enf at 1N[(c)] (mg/kg)**	**MRL proposal (mg/kg)**	**STMR (mg/kg)**	**HR (mg/kg)**
Muscle	0.01	0.02	0.00	0.00	**<0.02**	0.00	0.00
Fat	0.01	0.01	0.00	0.00	**<0.02**	0.00	0.00
Liver	0.44	0.55	0.01	0.01	**<0.02**	0.02	0.02
Kidney	0.08	0.09	0.00	0.00	**<0.02**	0.00	0.00

(a) Closest feeding level and N dose rate related to the maximum dietary burden (see Table 4.2-1)

(b) Residues may have been derived by the application of a transfer factor, by interpolation or by linear regression.

(c) Median dietary burden considered to derive the STMR values at 1N dose rate.

(d) For milk, HR and STMR derived from the mean residue level observed at the relevant feeding level (FAO, 2009). The HR is used as the basis of the MRL. The STMR is used in both the acute and chronic consumer risk assessments.

(e) Mean residue at the feeding level closest to the maximum dietary burden

(f) Where values for STMR or HR are below the default combined LOQ of 0.03 mg/kg, a value of 0.03 mg/kg has been used in the dietary risk assessment

4.4 Conversion factor for risk assessment for products of animal origin

As the residue definitions for enforcement (RD-Enf) and risk assessment (RD-RA) are different, conversion factors (CFs) for enforcement to risk assessment have been calculated.

These calculations were performed based on the feeding studies presented in the RAR (Netherlands, 2009) which analysed for imazalil, FK-772 and FK-284.

Table 4.4 Conversion factors derived from the livestock feeding studies - Lactating cow

N rate levels	**Level 1 (17.9N)**			**Level 2 (53.8N)**			**Level 3 (179.2N)**		
	RD-Enf (mg/kg)	**RD-RA (mg/kg)**	**CF**	**RD-Enf (mg/kg)**	**RD-RA (mg/kg)**	**CF**	**RD-Enf (mg/kg)**	**RD-RA (mg/kg)**	**CF**
Muscle	0.014	0.025	1.8	0.016	0.0459	2.87	0.04	0.137	3.4
Fat	0.01	0.013	1.31	0.022	0.033	1.52	0.163	0.216	1.32
Liver	0.440	0.600	1.36	2.426	2.772	1.14	9.838	10.951	1.11
Kidney	0.077	0.091	1.19	0.389	0.445	1.14	1.732	2.428	1.40
Milk	0.018	0.029	1.63	0.017	0.040	2.33	0.026	0.095	3.65

NB: Median value has been selected for all commodity types.

5 Residues in honey

No consideration of residues in honey is required, as the application is to ‘old’ data requirements set out under Assimilated Regulation 544/2011.

6 MRLs for products not covered in Sections 3 and 4

This section is to cover MRLs that can be extrapolated to other products included in Part 1 of the GB MRL Statutory Register but are not covered in Sections 3 and 4 of this ER/RO. Examples include MRLs for edible offals (other than liver and kidney) or MRLs to cover products derived from other species such as goat or equine.

No MRL extrapolations are recommended on the basis of this assessment.

7 Consumer risk assessment

7.1 Dietary Exposure

Consumer intake calculations have been performed using the UK chronic and acute dietary exposure models and the EFSA PRIMo.

The chronic risk assessments have been estimated based on the uses of imazalil considered in this application, the uses considered in previous assessments supporting existing GB MRLs (EFSA, 2017) and, for the remaining commodities, the existing MRLs established in the GB MRL Statutory Register.

The acute risk assessments have only been estimated for the uses of imazalil considered in this application.

The RD-RA is 1) Imazalil (any ratio of constituent isomers) and 2) R014821; therefore independent exposure assessments have been undertaken. A combined risk assessment is not required.

Imazalil

UK NEDIs and NESTIs

The UK NEDIs and NESTIs for the active substance and commodities listed in Table 7.1-1 have been calculated for ten consumer groups using the UK chronic and acute models (versions 1.1 and 1.2 respectively) as detailed in Regulatory Update 21/2005. The following assumptions have been made:

- For the NESTIs, upper range of normal (97.5th percentile) consumption of each individual crop which may have been treated.
- For the NEDIs, the 'Rees-Day' approach is taken which sums the two highest 97.5th percentile intakes and the mean intakes for all remaining commodities.
- All produce eaten which may have been treated has been treated and contains residues at the levels given in Table 7.1-1.
- There is no loss of residue during transport, storage or processing of foods prior to consumption.

Additionally, a specific variability factor of 1.69 for citrus fruits based on a unit to unit variability study, evaluated in section C.3.7, has been used in the acute risk assessment. This specific Vf for imazalil/citrus fruits has been used in place of the default value for the estimation of the NESTI. In the UK model, the default Vf for citrus is 7.

The input values for the UK consumer risk assessment are given in Table 7.1-1. For citrus fruits, a peeling factor has been applied to the whole fruit value in order to more accurate reflect the residue in the consumed portion.

Table 7.1-1 Input values for the UK consumer risk assessment

RD-RA (plant commodities): 1) Imazalil (any ratio of constituent isomers)

RD-RA (animal commodities): Sum of imazalil, FK-284 and FK-772 (any ratio of constituent isomers), expressed as imazalil‡

Commodity	**Chronic risk assessment**		**Acute risk assessment**	
	Input (mg/kg)	**Comment**	**Input (mg/kg)**	**Comment**
Citrus fruits	**0.18**	**$STMR_{RA}$ (whole fruit x PF) (current assessment)**	**0.35**	**HR_{RA} (whole fruit x PF) (current assessment)**
Bananas	**0.05**	**Calculated $STMR_{RA}$ (pulp) (JMPR assessment)**	**0.1**	**Calculated HR_{RA} (pulp) (JMPR assessment)**
Courgettes	**0.015**	**STMR (current assessment)**	**0.05**	**HR (current assessment)**
Strawberries	2.0	CXL x CF (EFSA, 2017)	Acute risk assessment undertaken only for the uses proposed as part of the current assessment	
Blackberries	0.94	HR x CF (EFSA, 2017)		
Raspberries	0.94	HR x CF (EFSA, 2017)		

Commodity	Chronic risk assessment		Acute risk assessment	
	Input (mg/kg)	Comment	Input (mg/kg)	Comment
Potatoes	0.01*	STMR x CF (fall-back) (EFSA, 2017)		
Tomatoes	0.09	STMR x CF (EFSA, 2017)		
Sweet pepper/bell peppers	0.05	MRL x CF (EFSA, 2017)		
Cucumbers/Gherkins	0.5	CXL x CF (EFSA, 2017)		
Melons	0.24	MRL x CF x PF (0.12) (EFSA, 2017)		
Small grain cereals	0.01	STMR x CF (EFSA, 2017)		
All other plant commodities	0.01*	Default LOQ GB MRL†		
All mammalian commodities except bovine liver	0.03*	LOQ (more critical than calculated level, see Section 4.3)	0.03*	LOQ (more critical than calculated level, see Section 4.3)
Liver (bovine)	0.03	STMR calculated from all uses	0.04	HR calculated from all uses

* indicates residues at the LOQ

†GB MRL Statutory Register and Regulation (EU) No 856/2020

‡ $MW_{imazalil}$: 297.2 g/mol

MW_{FK-772}: 363.2 g/mol Conversion to imazalil: 0.82

MW_{FK-284}: 289.1 g/mol Conversion to imazalil: 1.03

In bold uses proposed as part of the current assessment.

Model outputs for the UK chronic and acute models (versions 1.1 and 1.2 respectively) are presented in Appendix B.

PRIMo

The PRIMo IEDIs and IESTIs for the active substance and commodities listed in Table 7.1-2 have been calculated using PRIMo revision 3.1 – Pesticide Residues Intake Model.

The following assumptions have been made:

- All produce eaten which may have been treated, has been treated and contains residues at the levels as given in Table 7.1-2.
- There is no loss of residue during transport, storage or processing of foods prior to consumption.

Additionally, a specific variability factor of 1.69 for citrus fruits based on a unit to unit variability study, evaluated in section C.3.7, has been used in the acute risk assessment. This specific Vf for imazalil/ citrus fruits has been used in place of the default value for the estimation of the IESTIs. In the PRIMo the default Vf for grapefruits is 5 and 7 for all other citrus fruits.

The input values for the PRIMo risk assessment are given in Table 7.1-2.

Table 7.1-2 Input values for the PRIMo consumer risk assessment
RD-RA (plant commodities): 1) Imazalil (any ratio of constituent isomers)

RD-RA (animal commodities): Sum of imazalil, FK-284 and FK-772 (any ratio of constituent isomers), expressed as imazalil‡

Commodity	**Chronic risk assessment**		**Acute risk assessment**	
	Input (mg/kg)	**Comment**	**Input (mg/kg)**	**Comment**
Citrus fruits	**0.18**	**$STMR_{RA}$ (whole fruit x PF) (current assessment)**	**0.35**	**HR_{RA} (whole fruit x PF) (current assessment)**

Commodity	**Chronic risk assessment**		**Acute risk assessment**	
	Input (mg/kg)	**Comment**	**Input (mg/kg)**	**Comment**
Bananas	**0.05**	**Calculated $STMR_{RA}$ (pulp) (JMPR assessment)**	**0.1**	**Calculated HR_{RA} (pulp) (JMPR assessment)**
Courgettes	**0.015**	**STMR (current assessment)**	**0.05**	**HR (current assessment)**
Strawberries	2.0	CXL (EFSA, 2017)	Acute risk assessment undertaken only for the uses proposed as part of the current assessment	
Blackberries	0.94	HR (EFSA, 2017)		
Raspberries	0.94	HR (EFSA, 2017)		
Persimmons	1.6	HR (EFSA, 2017)		
Potatoes	0.01*	STMR (fall-back) (EFSA, 2017)		
Tomatoes	0.09	STMR (EFSA, 2017)		
Sweet pepper/bell peppers	0.05	MRL (EFSA, 2017)		
Cucumbers/Gherkins	0.5	CXL (EFSA, 2017)		

Commodity	Chronic risk assessment		Acute risk assessment	
	Input (mg/kg)	Comment	Input (mg/kg)	Comment
Melons	0.24	MRL x PF (0.12) (EFSA, 2017)		
Small grain cereals	0.01	STMR (EFSA, 2017)		
All other plant commodities	0.01*	Default LOQ GB MRL†		
All animal commodities except bovine liver	0.03*	LOQ (more critical than calculated level, see Section 4.3)	0.03*	LOQ (more critical than calculated level, see Section 4.3)
Liver (bovine)	0.03	STMR calculated from all uses (this assessment)	0.4	HR calculated from all uses

* indicates residues at the LOQ

†GB MRL Statutory Register and Regulation (EU) No 856/2020

‡ $MW_{imazalil}$: 297.2 g/mol

MW_{FK-772}: 363.2 g/mol Conversion to imazalil: 0.82

MW_{FK-284}: 289.1 g/mol Conversion to imazalil: 1.03

In bold uses proposed as part of the current assessment.

Model outputs for EFSA PRIMo revision 3.1 are presented in Appendix B.

Conclusions

The highest UK NEDI was 35% of the ADI (toddler). The highest PRIMo IEDI was 17% of the ADI (NL toddler). Therefore, no health effects due to chronic exposure are expected from the consumption of commodities treated with imazalil.

The highest UK NESTI was 28.6% of the ARfD (oranges/toddler). The highest PRIMo IESTI was 24% of the ARfD (oranges/children). Therefore, no health effects due to acute exposure are expected from the consumption of commodities treated with imazalil.

R014821

Metabolite R014821 is only relevant to post harvest treatments. There are no post-harvest treatments authorised in the UK. As a result of this assessment and the MRL review, the following crops need to be considered in the consumer risk assessment for R014821:

- Citrus fruits
- Banana
- Melon

The GAP has a 0 day WHP. This gives the critical imazalil residue. However, as outlined in section 3, R014821 may increase on storage. Therefore, the STMR and HR for the consumer risk assessment have been estimated from the following residues determined in pulp taken at WHP of 61 to 86 days:

<0.01, 0.014, 0.017 and 0.018 mg/kg

This gives an STMR of 0.016 mg/kg and a HR of 0.018 mg/kg for use in the consumer risk assessment.

The default Vf have been used to calculate the NESTIs.

Input values are summarised below:

Table 7.1-1 Input values for R014821 for the UK consumer and PRIMo risk assessments

Risk assessment residue definition (plants): 2) R014821

Commodity	Chronic risk assessment		Acute risk assessment	
	Input (mg/kg)	Comment	Input (mg/kg)	Comment
Citrus fruits	**0.016**	**STMR$_{RA}$ (pulp) (current assessment)**	**0.018**	**HR$_{RA}$ (pulp) (current assessment)**
Bananas	**0.05**	**STMR$_{RA}$ (pulp) (JMPR, 2018)**	**0.05**	**HR$_{RA}$ (pulp) (JMPR, 2018)**
Melons	0.008	0.24 (Residue of imazalil: EFSA, 2017) x formation fraction (0.032)§	Acute risk assessment undertaken only for the uses proposed as part of the current assessment	

§ The residue level of R014821 has been estimated based on the imazalil residue in melon pulp (EFSA 2017) and the formation factor of 0.032 estimated for citrus. There is uncertainty in using this formation factor and hence in the residue level of R014821 estimated in melon. The MRL supplementary information requirement for additional residue trials, including the analysis of R014821, for melon remains outstanding.

The highest UK NEDI was 7% of the ADI for R014821 (toddler). The highest PRIMo IEDI was 4% of the ADI (children). Therefore, no health effects due to chronic exposure are expected from the consumption of commodities treated with imazalil.

The highest UK NESTI was 13.9% of the ARfD for R014821 (oranges/infant). The highest PRIMo IESTI was 16% of the ARfD (oranges/children). Therefore, no health effects due to acute exposure are expected from the consumption of commodities treated with imazalil.

7.2 Other routes of exposure

N/A

8 The conclusion of the competent authority

Sufficiently validated analytical methods for the determination of imazalil (and metabolite FK-772) in products of plant and animal origin, in line with the residue definitions for enforcement, are available to support the uses under consideration in the MRL application.

Toxicological reference values (TRVs) were established for the approval of the active substance: an ADI of 0.025 mg/kg bw/day and an ARfD of 0.05 mg/kg bw. Additional toxicological data on metabolites R014821, FK-772 and FK-284 were evaluated; these data have confirmed the validity of the original tentative RD-RA for plants and animals. Specific TRVs for R014821 were set in this assessment: an ADI of 0.014 mg/kg bw/day and an ARfD of 0.03 mg/kg bw.

The metabolism of imazalil in primary crops was investigated in the fruit and root/tuber crop groups following foliar and post-harvest dipping/drenching applications for the approval of the active substance (EFSA, 2010). The crops included in this MRL application belong to the fruit group and are, therefore, covered by the metabolism studies available.

On the basis of additional data considered in this application, HSE recommends the following residue definition for risk assessment (RD-RA) in plants for post-harvest use:

1. Imazalil (any ratio of constituent isomers)
2. R014821

The residue definition for enforcement (RD-Enf) in plants has been agreed as:

- Imazalil (any ratio of constituent isomers)

For citrus fruits, 64 protected trials on orange and mandarin were submitted to support the MRLs. The post-harvest trials consisted of 34 dripping/drenching (the critical use), 24 low volume spraying, and 6 waxing uses. The trials are supported by validated analytical methods and storage stability data.

For bananas, the applicant requested that the CXL of 3 mg/kg (2019), was considered for adoption as a GB MRL.

For courgette, four additional residue trials have been provided that support the cGAP considered in the MRL review. These trials are sufficient to confirm that the current MRL is sufficiently supported by residue trials. The 4 trials along with 2 trials that were accepted to support the cGAP in the MRL review indicate that the MRL could be lowered from 0.1

mg/kg to 0.09 mg/kg. However, HSE does not recommending lowering the MRL for such a small change. HSE recommends that the current MRL is retained and the footnote associated with the requirement for additional trials is deleted in the GB MRL Statutory Register.

Hydrolysis of imazalil under representative conditions of pasteurisation, baking, brewing, boiling and sterilisation was evaluated for the approval of the active substance. The hydrolysis data demonstrate that imazalil is stable under standard conditions, therefore specific residue definitions for processed commodities are not required. Studies investigating the magnitude of residues on processing were evaluated during the EU Review and the processing factors (Pf) have been used to refine the risk assessment where appropriate.

No consideration of rotational crops was made as the impact of residues in rotational crops on the post-harvest uses under assessment was not required.

The maximum livestock dietary burden exceeded the trigger value for further assessment (0.1 mg/kg DM and AR) for ruminants. Metabolism of imazalil in livestock was considered for the approval of the active substance. The approval assessment concluded that the metabolic pathways in rodents and ruminants are comparable; the findings in ruminants can therefore be extrapolated to pigs.

On the basis of additional data considered in this assessment, HSE recommends the following residue definition for risk assessment (RD-RA) in animals:

- Sum of imazalil, FK-284 and FK-772 (any ratio of constituent isomers), expressed as imazalil.

The residue definition for enforcement (RD-Enf) in animals has been agreed as:

- Sum of imazalil and FK-772 (any ratio of constituent isomers), expressed as imazalil.

Consideration of available feeding studies for ruminants indicate that the MRLs for products of animal origin (POAO) are unlikely to be exceeded and do not need to be amended.

The highest UK NEDI was 35% of the ADI (toddler). The highest PRIMo IEDI was 17% of the ADI (NL toddler). Therefore, no health effects due to chronic exposure are expected from the consumption of commodities treated with imazalil.

The highest UK NESTI was 28.6% of the ARfD (oranges/toddler). The highest PRIMo IESTI was 24% of the ARfD (oranges/children). Therefore, no health effects due to acute exposure are expected from the consumption of commodities treated with imazalil.

For the acute risk assessment for imazalil, data has been provided that supports the default variability factor being replaced by a value of 1.69 specifically for imazalil/ citrus fruits. The highest UK NESTI was 28.6% of the ARfD (oranges/toddler). The highest PRIMo IESTI was 24% of the ARfD (oranges/children). Therefore, no health effects due to acute exposure are expected from the consumption of commodities treated with imazalil.

For R014821, the highest UK NEDI was 7% of the ADI for R014821 (toddler). The highest PRIMo IEDI was 4% of the ADI (children). Therefore, no health effects due to chronic exposure are expected from the consumption of commodities treated with imazalil.

The highest UK NESTI was 13.9% of the ARfD for R014821 (oranges/infant). The highest PRIMo IESTI was 16% of the ARfD (oranges/children). Therefore, no health effects due to acute exposure are expected from the consumption of commodities treated with imazalil.

HSE concludes that sufficient data have been provided to support the setting of new MRLs for the uses of imazalil on citrus fruits. Additionally, the CXL for banana can be adopted as a GB MRL. These will not result in consumer exposures exceeding the toxicological reference values and therefore harmful effects on human health are not expected. HSE recommends that the MRLs outlined in Table 0.1 are amended in the GB MRL Statutory Register.

Table 8.1 MRLs recommended by HSE

Enforcement residue definition for products of plant origin: Imazalil (any ratio of constituent isomers)

Product code	Product	Existing GB MRL (mg/kg)	New or amended GB MRL (mg/kg)	Comments
0110010	Grapefruits	4.0	7.0	The MRL is sufficiently supported by data. The MRL is derived by extrapolation of residue trials on oranges and mandarins. No health effects are expected. **The MRL will only be adopted once an MRL for the GAP is adopted in the exporting country and proof of authorisation is provided.**
0110020	Oranges	4.0	7.0	
0110030	Lemons	5.0	7.0	
0110040	Limes	5.0	7.0	
0110050	Mandarins	5.0	7.0	

Product code	Product	Existing GB MRL (mg/kg)	New or amended GB MRL (mg/kg)	Comments
0163020	Bananas	0.01	3.0	The CXL can be adopted as a GB MRL. No health effects are expected.

* Indicates that the MRL is set at the limit of quantification/determination

References

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The Netherlands, 2009. Draft Assessment Report (DAR) on the active substance imazalil, prepared by the rapporteur Member State The Netherlands in the framework of framework of Regulation (EC) No 1107/2009, May 2009

The Netherlands, 2018. Evaluation Report for the modification of MRLs for imazalil in various crops and products of animal origin, March 2018

HSE, 2023. Evaluation Report and Reasoned Opinion on the Assessment of Codex MRLs (CXLs), December 2023.

Appendix A – GAPs notified in the MRL application

Crop and/or situation (a)	GB or Country For Import Tolerance	Product name	F,G or I (b)	Pests or Group of pests controlled (c)	Preparation		Application				Application rate per treatment			WHP (days) (m)	Remarks
					Type (d-f)	Conc. a.s. (i)	method kind (f-h)	range of growth stages & season (j)	number min-max (k)	Interval between application (min)	kg a.s /hL min-max (l)	Water (L/ha) min-max	kg a.s./ha min-max (l)		
Citrus	EU	NA	I	*Penicillium digitatum* *Penicillium italicum* *Diplodia spp.* *Phomopsis spp.*	EC	500	Dipping / drenching	Ripe citrus fruit; during post-harvest	1	-	0.05	-	-	0	cGAP for MRL setting
Citrus	EU	NA	I	*Penicillium digitatum* *Penicillium italicum* *Diplodia spp.* *Phomopsis spp.*	EC	500	Low volume spray	Ripe citrus fruit; during post-harvest	1	-	0.15	-	-	0	
Citrus	EU	NA	I	*Penicillium digitatum* *Penicillium italicum* *Diplodia spp.* *Phomopsis spp.*	EC	500	Wax	Ripe citrus fruit; during post-harvest	1	-	0.2	-	-	0	

WHP: Withholding period

(a) For crops, the GB and Codex classifications (both) should be taken into account; where relevant, the use situation should be described (e.g. fumigation of a structure)	(i) g/kg or g/L. Normally the rate should be given for the active substance (according to ISO) and not for the variant in order to compare the rate for same active substances used in different

(b) State if the use is outdoor, field use (F) or glasshouse (G) or indoor use (I). (c) *e.g.* biting and sucking insects, soil borne insects, foliar fungi, weeds (d) *e.g.* wettable powder (WP), emulsifiable concentrate (EC), granule (GR) (e) CropLife International Technical Monograph no 2, 6th Edition. Revised May 2008. Catalogue of pesticide (f) All abbreviations used must be explained (g) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench (h) Kind, *e.g.* overall, broadcast, aerial spraying, row, individual plant, between the plant- type of equipment used must be indicated	variants (e.g. fluroxypyr). In certain cases, where only one variant is synthesised, it is more appropriate to give the rate for the variant (e.g. benthiavalicarb-isopropyl). (j) Growth stage range from first to last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application (k) Indicate the minimum and maximum number of applications possible under practical conditions of use (l) The values should be given in g or kg whatever gives the more manageable number (e.g. 200 kg/ha instead of 200 000 g/ha or 12.5 g/ha instead of 0.0125 kg/ha (m) PHI - minimum pre-harvest interval

Appendix B – UK models and Pesticide Residues Intake Model (PRIMo)

Imazalil (RD-RA 1)

Table B.1 Chronic risk assessment undertaken using UK model version 1.1

Active substance: **ADI:** 0.025 mg/kg bw/day **Source:**

			TOTAL INTAKE based on 97.5th percentile									
			ADULT	INFANT	TODDLER	4-6 YEARS	7-10 YEARS	11-14 YEARS	15-18 YEARS	VEGETARIAN	ELDERLY (OWN HOME)	ELDERLY (RESIDENTIAL)
	mg/kg bw/day		0.00238	0.00804	0.00879	0.00613	0.00508	0.00344	0.00274	0.00334	0.00339	0.00209
	% of ADI		10%	32%	35%	25%	20%	14%	11%	13%	14%	8%
Commodity	STMR (mg/kg)	P	COMMODITY INTAKES (mg/kg bw/day)									
Grapefruit	0.18		0.00035	0.00029	0.00105	0.00095	0.00207	0.00038	0.00026	0.00042	0.00043	0.00035
Lemons	0.18		0.00002	0.00012	0.00006	0.00002	0.00002	0.00001	0.00003	0.00004	0.00006	0.00001
Limes	0.18		0.00005	L/C	0.00029	0.00004	0.00003	0.00003	0.00004	0.00005	0.00006	0.00010
Mandarins	0.18		0.00025	L/C	0.00115	0.00065	0.00053	0.00027	0.00030	0.00021	0.00028	0.00012
Oranges	0.18		0.00069	0.00193	0.00295	0.00202	0.00150	0.00144	0.00119	0.00082	0.00064	0.00049
Almonds	0.01		0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
Brazil nuts	0.01		0.00000	L/C	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	L/C
Cashew nuts	0.01		0.00000	L/C	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000

Chestnuts	0.01		0.00000	L/C	L/C	L/C	L/C	L/C	0.00000	0.00000	0.00000	L/C
Coconuts	0.01		0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
Hazelnuts	0.01		0.00000	L/C	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
Pecan nuts	0.01		0.00000	L/C	0.00000	L/C	0.00000	0.00000	L/C	0.00000	0.00000	L/C
Pistachios	0.01		0.00000	L/C	0.00000	L/C	0.00000	L/C	L/C	0.00000	L/C	L/C
Walnuts	0.01		0.00000	L/C	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
Peanuts	0.01		0.00000	0.00001	0.00001	0.00001	0.00001	0.00001	0.00000	0.00000	0.00000	0.00000
Apples	0.01		0.00003	0.00008	0.00015	0.00009	0.00008	0.00004	0.00004	0.00003	0.00002	0.00001
Pears	0.01		0.00001	0.00003	0.00007	0.00004	0.00002	0.00002	0.00001	0.00002	0.00002	0.00001
Apricots	0.01		0.00000	0.00001	0.00001	0.00001	0.00000	0.00000	0.00000	0.00001	0.00000	0.00000
Peaches	0.01		0.00001	0.00001	0.00003	0.00002	0.00001	0.00001	0.00000	0.00001	0.00001	0.00000
Plums	0.01		0.00001	0.00000	0.00002	0.00001	0.00001	0.00000	0.00000	0.00001	0.00001	0.00000
Cherries	0.01		0.00000	0.00001	0.00001	0.00002	0.00001	0.00001	0.00001	0.00001	0.00000	0.00000
Table grapes	0.01		0.00001	0.00002	0.00005	0.00002	0.00003	0.00001	0.00001	0.00002	0.00001	0.00000
Wine grapes	0.01		0.00010	0.00001	0.00001	0.00001	0.00000	0.00001	0.00004	0.00010	0.00007	0.00001
Strawberries	2		0.00118	0.00384	0.00399	0.00269	0.00179	0.00137	0.00101	0.00194	0.00205	0.00102
Blackberries	0.94		0.00011	L/C	0.00183	0.00006	0.00031	0.00024	0.00009	0.00029	0.00025	0.00004
Loganberries	0.01		0.00000	0.00001	0.00001	0.00000	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000
Raspberries	0.94		0.00039	L/C	0.00224	0.00061	0.00095	0.00025	0.00006	0.00038	0.00078	0.00035
Gooseberries	0.01		0.00000	0.00001	0.00001	0.00000	0.00000	0.00000	0.00000	0.00001	0.00001	0.00000
Blackcurrants	0.01		0.00001	0.00001	0.00002	0.00002	0.00001	0.00001	0.00001	0.00000	0.00001	0.00000
Red currants	0.01		0.00000	L/C	0.00001	L/C	0.00000	0.00000	0.00000	0.00000	0.00000	L/C
White currants	0.01		L/C	L/C	L/C	L/C	L/C	L/C	L/C	L/C	L/C	L/C
Avocados	0.01		0.00001	L/C	0.00001	L/C	L/C	L/C	0.00000	0.00001	0.00001	L/C
Bananas	0.05		0.00010	0.00035	0.00033	0.00022	0.00017	0.00010	0.00007	0.00011	0.00008	0.00009
Dates	0.01		0.00000	L/C	0.00000	0.00000	0.00000	0.00000	L/C	0.00000	0.00001	0.00000

Figs	0.01		0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00001	0.00000	0.00000
Kiwi fruit	0.01		0.00001	L/C	0.00002	0.00002	0.00001	0.00001	0.00002	0.00001	0.00001	0.00000
Lychees	0.01		0.00000	L/C	L/C	L/C	L/C	L/C	L/C	L/C	L/C	L/C
Mangoes	0.01		0.00001	L/C	0.00002	0.00001	0.00002	0.00001	0.00004	0.00001	0.00000	L/C
Olives	0.01		0.00000	L/C	0.00001	0.00001	L/C	0.00000	L/C	0.00000	0.00000	L/C
Passion fruit	0.01		0.00000	L/C	L/C	L/C	L/C	L/C	L/C	0.00000	L/C	L/C
Pineapples	0.01		0.00001	0.00005	0.00005	0.00007	0.00002	0.00001	0.00001	0.00001	0.00001	0.00001
Pomegranates	0.01		0.00001	0.00001	0.00001	0.00000	0.00000	0.00001	0.00000	0.00001	0.00001	0.00001
Beetroot	0.01		0.00000	L/C	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
Carrots	0.01		0.00001	0.00004	0.00002	0.00002	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001
Celeriac	0.01		0.00000	L/C	L/C	0.00000	0.00000	L/C	L/C	L/C	L/C	L/C
Horseradish	0.01		0.00000	L/C	L/C	L/C	L/C	L/C	L/C	L/C	0.00000	L/C
Jerusalem artichokes	0.01		0.00000	L/C	L/C	L/C	L/C	L/C	L/C	L/C	L/C	L/C
Parsnips	0.01		0.00000	0.00001	0.00001	0.00001	0.00000	0.00000	0.00000	0.00000	0.00001	0.00000
Radishes	0.01		0.00000	L/C	0.00001	L/C	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
Salsify	0.01		L/C	L/C	L/C	L/C	L/C	L/C	L/C	L/C	L/C	L/C
Swedes	0.01		0.00000	0.00003	0.00002	0.00001	0.00001	0.00001	0.00000	0.00000	0.00001	0.00000
Turnips	0.01		0.00000	L/C	0.00001	0.00001	0.00001	0.00001	0.00000	0.00000	0.00001	0.00000
Yam	0.01		0.00003	L/C	L/C	L/C	L/C	L/C	L/C	L/C	L/C	L/C
Garlic	0.01		0.00000	L/C	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	L/C
Onions	0.01		0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00000
Spring onions	0.01		0.00000	L/C	0.00000	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
Tomatoes	0.09		0.00012	0.00016	0.00024	0.00017	0.00017	0.00010	0.00012	0.00016	0.00013	0.00012
Peppers	0.05		0.00002	L/C	0.00004	0.00002	0.00003	0.00002	0.00001	0.00003	0.00003	0.00001
Aubergines	0.01		0.00000	L/C	0.00002	0.00001	0.00000	0.00001	0.00000	0.00001	0.00000	L/C
Marrows	0.01		0.00001	L/C	0.00002	0.00000	0.00001	0.00001	0.00000	0.00001	0.00001	0.00001

Cucumbers	0.5		0.00020	0.00011	0.00121	0.00077	0.00052	0.00026	0.00023	0.00027	0.00024	0.00009
Gourd	0.01		0.00001	L/C	L/C	L/C	L/C	0.00000	L/C	0.00000	L/C	L/C
Courgettes	0.015		0.00001	0.00002	0.00004	0.00002	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001
Melons	0.24		0.00059	0.00073	0.00126	0.00085	0.00073	0.00052	0.00066	0.00061	0.00072	0.00024
Sweet corn	0.01		0.00001	0.00001	0.00002	0.00001	0.00001	0.00000	0.00001	0.00001	0.00001	0.00000
Broccoli	0.01		0.00001	0.00001	0.00002	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00000
Cauliflower	0.01		0.00001	0.00003	0.00002	0.00002	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001
Brussels sprouts	0.01		0.00001	0.00002	0.00002	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00000
Head cabbage	0.01		0.00001	0.00002	0.00002	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001
Chinese cabbage	0.01		0.00000	L/C	L/C	L/C	L/C	L/C	L/C	0.00001	0.00000	L/C
Kohl Rabi	0.01		L/C	L/C	L/C	L/C	L/C	L/C	L/C	L/C	L/C	L/C
Cress	0.01		0.00000	L/C	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
Lettuce	0.01		0.00001	0.00000	0.00001	0.00001	0.00001	0.00000	0.00000	0.00001	0.00001	0.00000
Spinach	0.01		0.00001	0.00001	0.00002	0.00001	0.00001	0.00001	0.00000	0.00001	0.00001	0.00000
Watercress	0.01		0.00000	L/C	L/C	0.00000	0.00000	0.00000	L/C	0.00000	0.00000	L/C
Chicory	0.01		0.00000	L/C	L/C	L/C	L/C	L/C	L/C	0.00000	L/C	L/C
Parsley	0.02		0.00000	L/C	0.00000	L/C	0.00000	0.00000	0.00000	0.00000	0.00000	0.00001
Beans with pods	0.01		0.00001	0.00001	0.00002	0.00001	0.00001	0.00000	0.00001	0.00000	0.00001	0.00000
Runner Beans	0.01		0.00001	L/C	0.00001	0.00000	0.00001	0.00001	0.00000	0.00002	0.00001	0.00001
Beans without pods	0.01		0.00000	0.00001	0.00002	0.00000	0.00001	0.00000	0.00000	0.00001	0.00001	0.00001
Peas with pods	0.01		0.00000	L/C	0.00000	0.00001	0.00000	0.00000	0.00000	0.00000	0.00001	L/C
Peas without pods	0.01		0.00001	0.00002	0.00002	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001
Beansprouts	0.01		0.00000	L/C	0.00001	0.00001	0.00001	0.00000	0.00000	0.00000	0.00001	0.00000
Asparagus	0.01		0.00000	L/C	L/C	L/C	L/C	L/C	0.00000	0.00001	0.00000	L/C
Bamboo shoots	0.01		0.00000	L/C	0.00000	L/C	0.00000	0.00000	0.00000	0.00000	0.00000	L/C
Celery	0.01		0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000

Fennel	0.01		0.00000	L/C	L/C	L/C	L/C	L/C	L/C	L/C	L/C	L/C
Globe artichokes	0.01		0.00000	L/C	L/C	L/C	L/C	L/C	L/C	0.00000	L/C	L/C
Leeks	0.01		0.00000	L/C	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00001	0.00000
Rhubarb	0.01		0.00000	0.00001	0.00001	0.00000	0.00001	0.00000	0.00000	0.00000	0.00001	0.00000
Cultivated mushrooms	0.01		0.00000	0.00000	0.00001	0.00000	0.00000	0.00000	0.00000	0.00001	0.00000	0.00000
Beans	0.01		0.00002	0.00006	0.00005	0.00003	0.00003	0.00002	0.00002	0.00002	0.00001	0.00001
Lentils	0.01		0.00001	0.00001	0.00002	0.00002	0.00001	0.00001	0.00001	0.00001	0.00001	0.00000
dried Peas	0.01		0.00001	L/C	0.00002	0.00000	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001
Oilseeds	0.01		0.00003	0.00006	0.00007	0.00007	0.00006	0.00004	0.00004	0.00005	0.00003	0.00004
Potatoes	0.01		0.00003	0.00011	0.00009	0.00008	0.00007	0.00005	0.00005	0.00004	0.00003	0.00003
Tea (dried leaves)	0.05		0.00001	0.00004	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001
Hops (dried 0.25% of beer)	0.05		0.00000	L/C	L/C	L/C	L/C	0.00000	0.00000	0.00000	0.00000	0.00000
Oats	0.01		0.00000	0.00002	0.00001	0.00001	0.00000	0.00000	0.00001	0.00001	0.00001	0.00001
Barley	0.01		0.00000	L/C	0.00000	0.00000	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000
Millet	0.01		L/C	L/C	0.00000	L/C	L/C	L/C	L/C	L/C	L/C	L/C
Buckwheat	0.01		L/C	L/C	L/C	L/C	L/C	L/C	L/C	L/C	L/C	L/C
Maize	0.01		0.00000	0.00005	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
Wheat	0.01		0.00004	0.00003	0.00008	0.00009	0.00007	0.00005	0.00004	0.00004	0.00003	0.00003
Rice	0.01		0.00002	0.00003	0.00005	0.00004	0.00005	0.00004	0.00003	0.00002	0.00001	0.00000
Rye	0.01		0.00001	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00001	0.00000	0.00000
Poultry	0.02		0.00003	0.00003	0.00006	0.00006	0.00004	0.00003	0.00003	0.00003	0.00003	0.00002
Meat fat	0.03		0.00001	0.00001	0.00002	0.00001	0.00001	0.00001	0.00001	0.00000	0.00001	0.00000
Meat excl. poultry & offal	0.03		0.00006	0.00012	0.00012	0.00010	0.00009	0.00006	0.00006	0.00001	0.00005	0.00005
All types of kidney	0.03		0.00001	0.00001	0.00004	0.00001	0.00001	0.00001	0.00001	L/C	0.00001	0.00001
All types of Liver	0.03		0.00001	0.00007	0.00007	0.00001	0.00001	0.00002	0.00001	L/C	0.00002	0.00001
Other types of offal	0.03		0.00002	0.00005	0.00007	0.00003	0.00003	0.00003	0.00001	0.00000	0.00002	0.00002

Eggs	0.02		0.00002	0.00009	0.00007	0.00005	0.00003	0.00003	0.00002	0.00002	0.00002	0.00003
Milk	0.03		0.00025	0.00293	0.00167	0.00088	0.00055	0.00035	0.00028	0.00029	0.00026	0.00035
Total all foods	0.01		0.00022	0.00125	0.00079	0.00056	0.00040	0.00029	0.00025	0.00026	0.00021	0.00022
Sugar beet	0.01		0.00014	0.00033	0.00056	0.00034	0.00031	0.00020	0.00019	0.00012	0.00011	0.00015

* 0.00000 corresponds to <0.000005 mg/kg bw/day (any value ≥0.000005 is rounded to 0.00001

L/C Low consumption (<0.1 g/day) or low number of consumers (<4)

Table B.2 Chronic risk assessment undertaken using PRIMo revision 3.1

Normal mode

Chronic risk assessment: JMPR methodology (IEDI/TMDI)

No of diets exceeding the ADI : ---

	Calculated exposure (% of ADI)	MS Diet	Expsoure (µg/kg bw per day)	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities	Exposure resulting from: MRLs set at the LOQ (in % of ADI)	Exposure resulting from: commodities not under assessment (in % of ADI)
TMDI/NEDI/IEDI calculation (based on average food consumption)	17%	NL toddler	4.20	7%	Milk: Cattle	3%	Strawberries	2%	Oranges		11%
	14%	DE child	3.42	4%	Strawberries	3%	Oranges	2%	Milk: Cattle		6%
	11%	NL child	2.69	3%	Milk: Cattle	3%	Strawberries	1%	Oranges		5%
	10%	FR child 3 15 yr	2.45	3%	Milk: Cattle	2%	Oranges	2%	Strawberries		6%
	9%	UK infant	2.19	5%	Milk: Cattle	2%	Strawberries	0.9%	Oranges		6%
	8%	FR toddler 2 3 yr	2.06	4%	Milk: Cattle	1%	Strawberries	1%	Oranges		6%
	8%	DK child	2.05	3%	Cucumbers	2%	Milk: Cattle	1%	Strawberries		2%
	8%	UK toddler	1.95	2%	Milk: Cattle	2%	Strawberries	1%	Oranges		5%
	7%	FI 3 yr	1.78	3%	Strawberries	2%	Cucumbers	0.7%	Raspberries (red and yellow)		0.7%
	7%	SE general	1.70	1%	Milk: Cattle	1%	Strawberries	0.6%	Cucumbers		3%
	7%	IE adult	1.65	1%	Strawberries	0.8%	Melons	0.8%	Oranges		3%
	7%	GEMS/Food G06	1.63	1%	Tomatoes	1%	Cucumbers	0.7%	Oranges		2%
	6%	DE women 14-50 yr	1.54	1%	Milk: Cattle	1%	Oranges	1.0%	Strawberries		3%
	6%	ES child	1.42	2%	Oranges	1%	Milk: Cattle	0.6%	Strawberries		4%
	6%	DE general	1.41	1%	Milk: Cattle	1%	Oranges	0.9%	Strawberries		3%
	6%	GEMS/Food G10	1.40	0.9%	Strawberries	0.8%	Oranges	0.7%	Milk: Cattle		2%
	5%	FI 6 yr	1.34	2%	Strawberries	1%	Cucumbers	0.5%	Raspberries (red and yellow)		0.5%
	5%	GEMS/Food G11	1.27	0.9%	Milk: Cattle	0.8%	Strawberries	0.5%	Oranges		2%
	5%	GEMS/Food G07	1.27	1%	Oranges	0.8%	Milk: Cattle	0.6%	Strawberries		3%
	5%	GEMS/Food G08	1.25	0.8%	Strawberries	0.7%	Milk: Cattle	0.4%	Tomatoes		2%
	5%	GEMS/Food G15	1.17	0.8%	Milk: Cattle	0.5%	Oranges	0.4%	Strawberries		2%
	5%	RO general	1.13	1%	Milk: Cattle	0.7%	Tomatoes	0.5%	Cucumbers		2%
	4%	FR infant	1.09	2%	Milk: Cattle	1%	Strawberries	0.2%	Oranges		2%
	4%	NL general	1.06	1%	Milk: Cattle	0.7%	Oranges	0.7%	Strawberries		2%
	4%	FI adult	1.00	1%	Coffee beans	1%	Strawberries	0.7%	Cucumbers		0.5%
	4%	ES adult	0.88	0.9%	Oranges	0.6%	Milk: Cattle	0.5%	Strawberries		2%
	3%	FR adult	0.83	1.0%	Strawberries	0.5%	Milk: Cattle	0.4%	Oranges		1%
	3%	DK adult	0.72	0.6%	Milk: Cattle	0.6%	Strawberries	0.4%	Cucumbers		1%
	3%	IT toddler	0.70	1.0%	Strawberries	0.5%	Tomatoes	0.3%	Oranges		0.6%
	3%	UK vegetarian	0.69	0.6%	Oranges	0.6%	Strawberries	0.4%	Milk: Cattle		1%
	2%	LT adult	0.62	0.8%	Cucumbers	0.5%	Milk: Cattle	0.3%	Strawberries		0.7%
	2%	PT general	0.54	0.4%	Oranges	0.3%	Tomatoes	0.3%	Strawberries		0.6%
	2%	UK adult	0.53	0.4%	Oranges	0.4%	Strawberries	0.4%	Milk: Cattle		1%
	2%	IT adult	0.47	0.4%	Strawberries	0.4%	Tomatoes	0.3%	Oranges		0.5%
	1%	PL general	0.28	0.3%	Tomatoes	0.2%	Strawberries	0.1%	Cucumbers		0.1%
	1%	IE child	0.25	0.4%	Milk: Cattle	0.2%	Strawberries	0.1%	Oranges		0.6%

Conclusion:
The estimated long-term dietary intake (TMDI/NEDI/IEDI) was below the ADI.
The long-term intake of residues of Imazalil is unlikely to present a public health concern.

Table B.3 Acute risk assessment undertaken using UK model version 1.2

Acute Intakes (97.5th percentiles)

			adult	infant	toddler	4-6 year old child	7-10 year old child

commodity	HR	P	NESTI	%ARfD	NESTI	%ARfD	NESTI	%ARfD	NESTI	%ARfD	NESTI	%ARfD
Grapefruit	0.35		0.00203	4.1	0.00303	6.1	0.00691	13.8	0.00589	11.8	0.00976	19.5
Lemons	0.35		0.00031	0.6	0.00163	3.3	0.00073	1.5	0.00043	0.9	0.00028	0.6
Limes	0.35		0.00063	1.3	0.00000	0.0	0.00170	3.4	0.00070	1.4	0.00066	1.3
Mandarins	0.35		0.00143	2.9	0.00170	3.4	0.00666	13.3	0.00426	8.5	0.00399	8.0
Oranges	0.35		0.00402	8.0	0.01224	24.5	0.01428	28.6	0.01061	21.2	0.00789	15.8
Bananas	0.10		0.00122	2.4	0.00836	16.7	0.00561	11.2	0.00427	8.5	0.00296	5.9
Courgettes	0.05		0.00055	1.1	0.00159	3.2	0.00232	4.6	0.00200	4.0	0.00129	2.6
Meat fat	0.03		0.00002	0.0	0.00006	0.1	0.00006	0.1	0.00006	0.1	0.00004	0.1
Meat excl.poultry & offal	0.03		0.00015	0.3	0.00035	0.7	0.00030	0.6	0.00027	0.5	0.00023	0.5
All types of kidney	0.03		0.00005	0.1	0.00007	0.1	0.00011	0.2	0.00007	0.1	0.00005	0.1
All types of liver	0.04		0.00011	0.2	0.00032	0.6	0.00027	0.5	0.00007	0.1	0.00010	0.2
Other types of offal	0.03		0.00009	0.2	0.00022	0.4	0.00021	0.4	0.00017	0.3	0.00016	0.3
Milk	0.03		0.00039	0.8	0.00373	7.5	0.00220	4.4	0.00140	2.8	0.00089	1.8

			11-14 year old child		15-18 year old child		vegetarian		Elderly - own home		Elderly - residential	
commodity	HR	P	NESTI	%ARfD	NESTI	%ARfD	NESTI	%ARfD	NESTI	%ARfD	NESTI	%ARfD
Grapefruit	0.35		0.00277	5.5	0.00245	4.9	0.00259	5.2	0.00223	4.5	0.00154	3.1
Lemons	0.35		0.00025	0.5	0.00036	0.7	0.00044	0.9	0.00042	0.8	0.00014	0.3
Limes	0.35		0.00062	1.2	0.00059	1.2	0.00017	0.3	0.00036	0.7	0.00060	1.2
Mandarins	0.35		0.00241	4.8	0.00210	4.2	0.00192	3.8	0.00192	3.8	0.00067	1.3
Oranges	0.35		0.00651	13.0	0.00607	12.1	0.00450	9.0	0.00243	4.9	0.00221	4.4
Bananas	0.10		0.00173	3.5	0.00147	2.9	0.00142	2.8	0.00113	2.3	0.00127	2.5
Courgettes	0.05		0.00057	1.1	0.00048	1.0	0.00067	1.3	0.00057	1.1	0.00063	1.3
Meat fat	0.03		0.00003	0.1	0.00003	0.1	0.00001	0.0	0.00001	0.0	0.00001	0.0
Meat excl.poultry & offal	0.03		0.00017	0.3	0.00017	0.3	0.00008	0.2	0.00011	0.2	0.00010	0.2
All types of kidney	0.03		0.00004	0.1	0.00006	0.1	0.00000	0.0	0.00005	0.1	0.00004	0.1
All types of liver	0.04		0.00017	0.3	0.00008	0.2	0.00000	0.0	0.00009	0.2	0.00008	0.2
Other types of offal	0.03		0.00014	0.3	0.00007	0.1	0.00003	0.1	0.00007	0.1	0.00007	0.1

Milk	0.03		0.00062	1.2	0.00053	1.1	0.00045	0.9	0.00033	0.7	0.00043	0.9

Table B.4 Acute risk assessment undertaken using PRIMo revision 3.1

Show results of IESTI calculation only for crops with GAPs under assessment

Results for children
No. of commodities for which ARfD/ADI is exceeded (IESTI): ---

IESTI

Highest % of ARfD/ADI	Commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
24%	Oranges	0 / 0.35	12
19%	Bananas	0 / 0.1	9.7
19%	Grapefruits	0 / 0.35	9.3
12%	Mandarins	0 / 0.35	6.2
8%	Lemons	0 / 0.35	3.8
7%	Milk: Cattle	0 / 0.03	3.7
5%	Courgettes	0 / 0.05	2.3
3%	Limes	0 / 0.35	1.7
1%	Milk: Goat	0 / 0.03	0.73
0.7%	Swine: Muscle/meat	0 / 0.03	0.36
0.6%	Bovine: Liver	0 / 0.04	0.32
0.4%	Bovine: Edible offals (other	0 / 0.03	0.22
0.4%	Bovine: Muscle/meat	0 / 0.03	0.22
0.4%	Other farmed animals:	0 / 0.03	0.21
0.4%	Equine: Muscle/meat	0 / 0.03	0.18

Expand/collapse list

Results for adults
No. of commodities for which ARfD/ADI is exceeded (IESTI): ---

IESTI

Highest % of ARfD/ADI	Commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
12%	Oranges	0 / 0.35	6.1
8%	Mandarins	0 / 0.35	4.1
6%	Grapefruits	0 / 0.35	3.1
4%	Bananas	0 / 0.1	2.1
2%	Courgettes	0 / 0.05	1.2
2%	Milk: Cattle	0 / 0.03	1.2
2%	Lemons	0 / 0.35	0.76
1%	Limes	0 / 0.35	0.60
1%	Milk: Goat	0 / 0.03	0.55
0.9%	Milk: Sheep	0 / 0.03	0.45
0.3%	Bovine: Muscle	0 / 0.03	0.17
0.3%	Other farmed animals:	0 / 0.03	0.17
0.3%	Bovine: Liver	0 / 0.04	0.16
0.3%	Swine: Muscle/meat	0 / 0.03	0.15
0.3%	Equine: Muscle/meat	0 / 0.03	0.14

Total number of commodities exceeding the ARfD/ADI in children and adult diets (IESTI calculation)

Metabolite R014821 (RD-RA 2)

Table B.1 Chronic risk assessment undertaken using UK model version 1.1

Active substance: **ADI:** 0.014 mg/kg bw/day **Source:**

	TOTAL INTAKE based on 97.5th percentile									
	ADULT	INFANT	TODDLER	4-6 YEARS	7-10 YEARS	11-14 YEARS	15-18 YEARS	VEGETARIAN	ELDERLY (OWN HOME)	ELDERLY (RESIDENTIAL)
mg/kg bw/day	0.00027	0.00074	0.00096	0.00063	0.00058	0.00036	0.00033	0.00026	0.00021	0.00026
% of ADI	2%	5%	7%	5%	4%	3%	2%	2%	2%	2%

	STMR	P	COMMODITY INTAKES									
Commodity	**(mg/kg)**		**(mg/kg bw/day)**									
Grapefruit	0.016		0.00003	0.00003	0.00009	0.00008	0.00018	0.00003	0.00002	0.00004	0.00004	0.00003
Lemons	0.016		0.00000	0.00001	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00001	0.00000
Limes	0.016		0.00000	L/C	0.00003	0.00000	0.00000	0.00000	0.00000	0.00000	0.00001	0.00001
Mandarins	0.016		0.00002	L/C	0.00010	0.00006	0.00005	0.00002	0.00003	0.00002	0.00003	0.00001
Oranges	0.016		0.00006	0.00017	0.00026	0.00018	0.00013	0.00013	0.00011	0.00007	0.00006	0.00004
Bananas	0.05		0.00010	0.00035	0.00033	0.00022	0.00017	0.00010	0.00007	0.00011	0.00008	0.00009
Melons	0.008		0.00002	0.00002	0.00004	0.00003	0.00002	0.00002	0.00002	0.00002	0.00002	0.00001
Potatoes	0.01		0.00003	0.00011	0.00009	0.00008	0.00007	0.00005	0.00005	0.00004	0.00003	0.00003
Milk	0		0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
Sugar beet	0.01		0.00014	0.00033	0.00056	0.00034	0.00031	0.00020	0.00019	0.00012	0.00011	0.00015

* 0.00000 corresponds to <0.000005 mg/kg bw/day (any value ≥0.000005 is rounded to 0.00001

L/C Low consumption (<0.1 g/day) or low number of consumers (<4)

Table B.2 Chronic risk assessment undertaken using PRIMo revision 3.1

Normal mode

Chronic risk assessment: JMPR methodology (IEDI/TMDI)

No of diets exceeding the ADI : ---

	Calculated exposure (% of ADI)	MS Diet	Expsoure (µg/kg bw per day)	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities	Exposure resulting from: MRLs set at the LOQ (in % of ADI)	Exposure resulting from: commodities not under assessment (in % of ADI)
TMDI/NEDI/IEDI calculation (based on average food consumption)	4%	NL toddler	0.62	2%	Bananas	2%	Sugar beet roots	0.3%	Potatoes		
	4%	NL child	0.59	3%	Sugar beet roots	0.7%	Bananas	0.2%	Potatoes		
	2%	DE women 14-50 yr	0.29	2%	Sugar beet roots	0.2%	Oranges	0.1%	Bananas		
	2%	FR child 3 15 yr	0.29	1%	Sugar beet roots	0.4%	Oranges	0.2%	Bananas		
	2%	UK toddler	0.29	1%	Sugar beet roots	0.4%	Bananas	0.2%	Potatoes		
	2%	DE general	0.27	2%	Sugar beet roots	0.2%	Oranges	0.1%	Bananas		
	2%	FR toddler 2 3 yr	0.23	1%	Sugar beet roots	0.2%	Bananas	0.2%	Oranges		
	1%	NL general	0.20	1%	Sugar beet roots	0.2%	Potatoes	0.1%	Oranges		
	1%	UK infant	0.20	0.5%	Bananas	0.5%	Sugar beet roots	0.2%	Potatoes		
	1%	DE child	0.18	0.6%	Bananas	0.5%	Oranges	0.2%	Potatoes		
	1%	SE general	0.15	0.6%	Bananas	0.3%	Potatoes	0.1%	Oranges		
	1%	GEMS/Food G06	0.14	0.5%	Sugar beet roots	0.1%	Potatoes	0.1%	Oranges		
	0.9%	FI 3 yr	0.12	0.5%	Bananas	0.3%	Potatoes	0.0%	Mandarins		
	0.9%	RO general	0.12	0.5%	Sugar beet roots	0.3%	Potatoes	0.1%	Bananas		
	0.8%	ES child	0.12	0.4%	Bananas	0.2%	Oranges	0.1%	Potatoes		
	0.8%	IE adult	0.11	0.3%	Bananas	0.2%	Potatoes	0.1%	Oranges		
	0.7%	FR infant	0.10	0.5%	Sugar beet roots	0.1%	Potatoes	0.1%	Bananas		
	0.6%	GEMS/Food G07	0.09	0.3%	Potatoes	0.2%	Oranges	0.1%	Bananas		
	0.6%	DK child	0.09	0.4%	Bananas	0.2%	Potatoes	0.0%	Oranges		
	0.6%	FI 6 yr	0.09	0.3%	Potatoes	0.3%	Bananas	0.0%	Mandarins		
	0.6%	PT general	0.08	0.4%	Potatoes	0.1%	Bananas	0.1%	Oranges		
	0.6%	GEMS/Food G11	0.08	0.3%	Potatoes	0.1%	Bananas	0.1%	Oranges		
	0.5%	GEMS/Food G08	0.08	0.3%	Potatoes	0.1%	Bananas	0.1%	Oranges		
	0.5%	UK vegetarian	0.08	0.2%	Sugar beet roots	0.1%	Bananas	0.1%	Oranges		
	0.5%	GEMS/Food G10	0.08	0.2%	Potatoes	0.1%	Bananas	0.1%	Oranges		
	0.5%	GEMS/Food G15	0.07	0.3%	Potatoes	0.1%	Bananas	0.1%	Oranges		
	0.5%	UK adult	0.07	0.2%	Sugar beet roots	0.1%	Bananas	0.1%	Potatoes		
	0.5%	FR adult	0.07	0.3%	Sugar beet roots	0.1%	Oranges	0.1%	Bananas		
	0.4%	ES adult	0.06	0.1%	Oranges	0.1%	Bananas	0.1%	Potatoes		
	0.3%	IT toddler	0.05	0.2%	Bananas	0.1%	Potatoes	0.1%	Oranges		
	0.3%	PL general	0.05	0.2%	Potatoes	0.1%	Bananas	0.0%	Lemons		
	0.3%	DK adult	0.04	0.2%	Bananas	0.1%	Potatoes	0.0%	Oranges		
	0.3%	LT adult	0.04	0.2%	Potatoes	0.0%	Bananas	0.0%	Oranges		
	0.3%	FI adult	0.04	0.1%	Bananas	0.1%	Potatoes	0.0%	Oranges		
	0.2%	IT adult	0.03	0.1%	Bananas	0.0%	Potatoes	0.0%	Oranges		
	0.1%	IE child	0.02	0.1%	Bananas	0.0%	Potatoes	0.0%	Oranges		

Conclusion:
The estimated long-term dietary intake (TMDI/NEDI/IEDI) was below the ADI.
The long-term intake of residues of is unlikely to present a public health concern.

Table B.3 Acute risk assessment undertaken using UK model version 1.2

Goto Inputs

Acute Intakes (97.5th percentiles)

			adult		infant		toddler		4-6 year old child		7-10 year old child	
commodity	HR	P	NESTI	%ARfD	NESTI	%ARfD	NESTI	%ARfD	NESTI	%ARfD	NESTI	%ARfD
Grapefruit	0.02		0.00031	1.0	0.00064	2.1	0.00141	4.7	0.00105	3.5	0.00100	3.3
Lemons	0.02		0.00007	0.2	0.00035	1.2	0.00015	0.5	0.00009	0.3	0.00006	0.2
Limes	0.02		0.00012	0.4	0.00000	0.0	0.00036	1.2	0.00015	0.5	0.00014	0.5
Mandarins	0.02		0.00020	0.7	0.00036	1.2	0.00100	3.3	0.00069	2.3	0.00051	1.7
Oranges	0.02		0.00041	1.4	0.00239	8.0	0.00179	6.0	0.00129	4.3	0.00090	3.0
Bananas	0.05		0.00061	2.0	0.00418	13.9	0.00280	9.3	0.00214	7.1	0.00148	4.9

			11-14 year old child		15-18 year old child		vegetarian		Elderly - own home		Elderly - residential	
commodity	HR	P	NESTI	%ARfD	NESTI	%ARfD	NESTI	%ARfD	NESTI	%ARfD	NESTI	%ARfD
Grapefruit	0.02		0.00046	1.5	0.00037	1.2	0.00036	1.2	0.00033	1.1	0.00033	1.1
Lemons	0.02		0.00005	0.2	0.00008	0.3	0.00009	0.3	0.00009	0.3	0.00003	0.1
Limes	0.02		0.00013	0.4	0.00013	0.4	0.00004	0.1	0.00008	0.3	0.00013	0.4
Mandarins	0.02		0.00032	1.1	0.00026	0.9	0.00024	0.8	0.00023	0.8	0.00014	0.5
Oranges	0.02		0.00065	2.2	0.00055	1.8	0.00046	1.5	0.00034	1.1	0.00036	1.2
Bananas	0.05		0.00087	2.9	0.00074	2.5	0.00071	2.4	0.00056	1.9	0.00064	2.1

Pesticide
ARfD 0.030 mg/Kg bw/day
Source
* 0.00000 corresponds to <0.000005 mg/kg bw/day (any value ≥0.000005 is rounded to 0.00001

Table B.4 Acute risk assessment undertaken using PRIMo revision 3.1

Show results for all crops

Results for children				**Results for adults**			
No. of commodities for which ARfD/ADI is exceeded (IESTI):			---	No. of commodities for which ARfD/ADI is exceeded (IESTI):			---
IESTI				**IESTI**			
Highest % of ARfD/ADI	Commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)	Highest % of ARfD/ADI	Commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
16%	Bananas	0 / 0.05	4.9	4%	Bananas	0 / 0.05	1.1
8%	Oranges	0 / 0.02	2.4	2%	Oranges	0 / 0.02	0.55
5%	Grapefruits	0 / 0.02	1.4	1%	Mandarins	0 / 0.02	0.32
4%	Mandarins	0 / 0.02	1.1	1%	Grapefruits	0 / 0.02	0.32
2%	Lemons	0 / 0.02	0.62	0.5%	Lemons	0 / 0.02	0.16
1%	Limes	0 / 0.02	0.36	0.4%	Limes	0 / 0.02	0.13
Expand/collapse list							
Total number of commodities exceeding the ARfD/ADI in children and adult diets (IESTI calculation)							

Results for children		**Results for adults**	
No of processed commodities for which ARfD/ADI is exceeded (IESTI):	---	No of processed commodities for which ARfD/ADI is exceeded (IESTI):	---

Appendix C - Detailed evaluation of the additional studies relied on

C.1 Methods of analysis

C.1.1 Methods for enforcement and monitoring of residues in food and feed of plant origin

C.1.1.1 Method validation

Not required.

C.1.1.2 Independent laboratory validation

Not required.

C.1.1.3 Confirmatory method (if necessary)

Not required.

C.1.1.4 Extraction efficiency

Reference:	Cross validation of analytical methodology for Imazalil and its metabolite R014821 in mandarin samples. [redacted] 2020, Report no. AGR6053, 358-20
GLP:	Yes
Acceptability of the method:	Acceptable

Principle of the method:

The objective of this study was to carry out a cross validation of analytical methodology for Imazalil and its metabolite R014821, by comparing the amounts of residues extracted from samples with incurred residues using the solvent system of the monitoring method and the solvent system of the metabolism studies. Residues obtained by applying these procedures have been compared with those obtained when the extraction solvent of the metabolism studies is used instead of the solvent used in the monitoring method.

A cross validation of analytical methodology for Imazalil and its metabolite R014821 has been carried out. Amounts of residues extracted from mandarin pulp and peel samples, in the case of Imazalil, and from mandarin peel, in the case of R014821, with incurred residues using the solvent system of the monitoring method and the solvent system of the metabolism studies have been compared.

Imazalil and R014821 residues have been determined by applying analytical methodology based on Liquid Chromatography coupled to Mass Spectrometry in tandem (LC-MS/MS) previously validated in the LARP.

The monitoring methods for Imazalil and R014821 are based on sample extraction with acetonitrile in presence of the content of a QuEChERS Extraction Kit (magnesium sulphate, sodium chloride, sodium hydrogencitrate sesquihydrate and sodium citrate). The extract was diluted 10-fold, in the case of pulp, or 20-fold, in the case of peel, and injected on the LC-MS/MS system. Additional dilutions have been made when necessary depending on the amount of residues. Mass spectrometry detection was carried out using tandem mass spectrometry with positive ionization in electrospray interface, registering the mass transitions:

• Imazalil: m/z 297>159 (quantification) and m/z 297>69 (confirmation).

• R014821: m/z 257>69 (quantification) and m/z 257>125 (confirmation).

Imazalil and R014821 quantification was carried out by means of matrix matched external calibration curves.

The amount of residues extracted from samples has been compared with the residues obtained when several organic solvents (n-heptane/isoamyalcohol (95:5, v:v), and chloroform) at different pH values have been used as extraction solvents.

Results:

Table C.1.1.4-1 Imazalil residues in samples using metabolism and monitoring methods

Matrix	Sample	Imazalil (metabolism method[a]) (mg/kg)	Imazalil (monitoring method[b]) (mg/kg)	Difference (%)
Mandarin pulp	358/4	0.007*	0.006*	14*
	358/6	0.033	0.025	24
Mandarin peel	358/3	17.1	14.2	17
	358/5	18.6	17.2	8

A: mixture of organic solvents

B: acetonitrile

*estimated values

Table C.1.1.4-2 R014821 residues in samples using metabolism and monitoring methods

Matrix	Sample	R014821 (metabolism method[a]) (mg/kg)	R014821 (monitoring method[b]) (mg/kg)	Difference (%)
Mandarin peel	358/3	0.421	0.294	30
	358/5	0.181	0.129	29

A: mixture of organic solvents

B: acetonitrile

Conclusion:

Results obtained demonstrate that the extraction efficiency can be considered sufficient for Imazalil in mandarin pulp and for Imazalil and R014821 in mandarin peel samples, as residues obtained by applying the methodology validated for the determination of residues differs by no more than 30% compared to the results obtained with the solvent of the

metabolism studies. 70% extraction of a method is sufficient to establish acceptable extraction efficiency in most cases, and as the reduction is 30% (maximum in R014821 samples) this is considered to be acceptable.

C.1.2 Methods for enforcement and monitoring of residues in food and feed of animal origin

Not required.

C.1.3 Methods for risk assessment for residues in food and feed of plant origin

C.1.3.1 Method validation (Variability study)

Reference:	Determination of variability factor of residues of imazalil and its metabolite R014821 following a post-harvest application by drencher of Fecundal 500 EC (Imazalil 50% w/v) on oranges (citrus sinensis) in southern Europe 2019, [redacted] 2020b, report No. 168SRES19R04, AGR 6003, SRES19-616-168FR (Analytical phase code: 344-19)
GLP:	Yes
Acceptability of the method:	Acceptable

Principle of the method

To homogenised samples (5 g) 5 mL water is added in a 50 mL polypropylene tube and sample is left in contact with the water for at least 5 min. Extraction solvent (20 mL acetonitrile) is added and sample was shaken manually for 1 min following addition of QuEChERS Extraction Kit (4 g magnesium sulphate, 1 g sodium chloride, 0.5 g sodium hydrogencitrate sesquihydrate and 1 g sodium citrate) and shaking in the rotatory agitator for 30 min. After centrifugation, an aliquot of 0.1 mL of the supernatant is diluted 10-fold with water and analysed using LC-MS/MS system (Phenomenex Kinetex XB-C18 (5 µm, 50 x 2.1 mm) column) assessing two mass transition for Imazalil (m/z 297 to m/z 159 and m/z 297 to m/z 69) and its metabolite R014821 (m/z 257 to m/z 69 and m/z 257 to m/z 125) for quantification and confirmation, respectively. The mobile phase consists of HPLC water (Solvent A) and acetonitrile (Solvent B), each containing 0.01% formic acid. The gradient varies from 90:10 to 5:95, back to 90:10

Results and discussion

Table C.1.3.1-1 Recovery Results in orange

Standards prepared in acetonitrile

Matrix	level (mg/kg)	No samples per level	Range of recoveries (%)	Mean recovery	RSD (%)	Comments
Orange *m/z* 297 → *m/z* 159	0.01	5	74 - 90	81	7	Imazalil (quantification)
Orange *m/z* 297 → *m/z* 69	0.01	5	71 - 88	79	8	Imazalil (confirmation)
Orange *m/z* 257 → *m/z* 69	0.01	5	82 - 89	85	4	R014821 (quantification)
Orange *m/z* 257 → *m/z* 125	0.01	5	78 - 84	81	3	R014821 (confirmation)
Orange *m/z* 297 → *m/z* 159	0.10	5	65 - 87	81	12	Imazalil (quantification)
Orange *m/z* 297 → *m/z* 69	0.10	5	67 - 89	82	11	Imazalil (confirmation)
Orange *m/z* 257 → *m/z* 69	0.10	5	75 - 88	83	6	R014821 (quantification)
Orange *m/z* 257 → *m/z* 125	0.10	5	75 - 86	82	6	R014821 (confirmation)

Table C.1.3.1-2 Characteristics of the analytical method for the quantitation of imazalil and R014821 residues in orange

	Imazalil	R014821
Chromatographic method	LC-MS/MS	LC-MS/MS

	Imazalil	**R014821**
Specificity demonstrated (yes/no, by…)	2 ion transitions in MS	2 ion transitions in MS
Linearity demonstrated (yes/no)	Yes	Yes
Accepted calibration range in concentration units (e.g. in µg/ml or ng/µl)	0.05 – 5.0 ng/mL (corresponding to 0.002 – 0.2 mg/kg)	00.05 – 5.0 ng/mL (corresponding to 0.002 – 0.2 mg/kg)
Calibration consist of at least 3 levels (duplicated points) or 5 levels (single points)? (yes/no)	Yes – 7 levels	Yes – 7 levels
Matrix effects	Yes, matrix matched standards used for calibration	Yes, matrix matched standards used for calibration
Absence of interference >30% of LOQ in blank sample is demonstrated (yes/no)	Yes	Yes
Chromatogram of sample spiked at LOQ demonstrates sufficient S/N ratio? (yes/no)	Yes	Yes
LOQ (mg/kg)	0.01	0.01

Conclusion

The method has been sufficiently validated according to SANCO/3029/99 rev. 4 and SANCO/825/00 rev. 8.1 for the determination of residues of Imazalil and its metabolite R014821 in oranges with an LOQ of 0.01 mg/kg.

Extraction efficiency has not been fully addressed. A monitoring method using acetonitrile as the extraction solvent was fully validated during the MRL Review for imazalil (EFSA 2017, 2018). See Section 1.1 for further information.

C.1.4 Methods for risk assessment for residues in food and feed of animal origin

C.1.5 Methods for risk assessment for toxicological studies

A number of new toxicological studies have been evaluated in the current assessment, for which dose verification methods were required. The dose verification has been performed individually for each analyte and are laid out below. These studies support dose analysis within toxicological studies presented in Section 2.

C.1.5.1 Study 1

Reference:	Development and Validation of an Analytical Method for Analysis of Imazalil in Diet including Trial Diet Analysis. [redacted] 2020a, Study code 20203753
Company reference:	Sponsor no. AGR 5958
Test facility:	[redacted]
Guideline(s):	SANCO Guideline 3029/99 rev. 4 (2000)
Deviations:	No
GLP:	Yes
Study acceptable:	Yes, valid without restrictions

Principles of analytical methods

QC samples are prepared by weighting 5 g blank powder diet in glass vessels and fortified at 500, 1600 and 15000 mg/kg by addition of 1.25 mL of a 2000 mg/L stock solution (for 500 mg/kg samples) or accurate weightings of approx. 8.08 mg test item for 1600 mg/kg samples and approx. 77.2 mg test item for 15000 mg/kg samples. After spiking, the test item is allowed to adsorb to the diet for at least 30 minutes. Samples are extracted by shaking with 50 mL acetonitrile. The solution is filtered through a 0.2 µm Spartan 30/0.2 RC filter. Low fortification samples (500 mg/kg) are analysed directly. Mid and high fortification samples are further diluted into calibration range. Samples were analysed using UPLC-UV. Solvent based calibration solutions were used during the course of this study. Samples were adequately diluted prior to analysis in order to minimize matrix effects. When measuring the matrix extracts using a calibration curve without matrix, acceptable recovery was obtained indicating that significant matrix effect was absent.

Method validation

The analytical method was validated in terms of specificity, linearity of detector's response, LOQ, recovery and repeatability (by means of precision) according to the guidance document SANCO/3029/99 rev. 4. The results are presented in the following table.

Specificity	The chromatogram of the blank QC sample showed no peak at the retention time of the test item. Since no interferences were detected, the specificity requirements were met and the analytical method was found to be specific for the test item.
Linearity	The analytical system was linear within the calibration range of 20 – 120 mg test item/L, which corresponds to concentrations of the test item in diet samples in the range of 200 – 1200 mg/kg. Mid and high fortification samples were diluted approx. 2- and 25-fold into calibration range. y = 0.00346x – 0.00407 (r = 0.9996) Criteria of r ≥ 0.99 as required by the guideline is met.
Accuracy (Recovery) and Precision	The accuracy of the method was assessed by the recovery test by fortifying blank samples with Imazalil at LOQ (500 mg/kg, Low), 1600 mg/kg (Middle) and 15000 mg/kg (High). The criteria of the guideline - accuracy between 70 and 110 %, precision ≤ 20 % - are met. The recovery results are summarized in the following Table C.1.5.1-1.
Limit of Quantification (LOQ)	500 mg/kg

Table C.1.5.1-1 Recovery results from method validation of diet samples using the analytical method. Standards were prepared in acetonitrile

Matrix	Analyte	Fortification level	Recovery			
			Range [%]	Mean [%]	RSD [%]	Sample size (n)
Diet	Imazalil	Blank	Not detected	-	-	2
		500 mg/kg	94.4 – 95.0	94.6	0.23	6
		1600 mg/kg	94.5 – 95.9	95.1	0.57	6
		15000 mg/kg	92.7 – 95.5	93.8	1.10	6
		Overall	92.7 – 95.5	94.5	1.02	18

An analytical method for the determination of Imazalil in diet was successfully validated in terms of specificity, linearity of detector's response, LOQ, recovery and repeatability (by means of precision) according to guidance document(s) SANCO/3029/99 rev. 4 (2000).

Additionally, diets were stable when stored at room temperature under normal laboratory light conditions in open containers for at least 4 days, and in a freezer (≤ -15°C) for at least 25 days (3 weeks).

C.1.5.2 Study 2

Reference: Development and Validation of an Analytical Method for Analysis of R014821 in Diet including Trial Diet Analysis.

[redacted] 2020b, Study code 20192863

Company reference: Sponsor no. AGR 5998

Test facility: [redacted]

Guideline(s): SANCO Guideline 3029/99 rev. 4 (2000)

Deviations: No

GLP: Yes

Study acceptable: Yes, valid without restrictions

Principles of analytical methods

QC samples are prepared by weighting 5 g blank powder diet in glass vessels and fortification at 500, 1600 and 15000 mg/kg by addition of 1.25 mL of a 2000 mg/L or 1.6 mL of a 5000 mg/L stock solution (for 500 and 1600 mg/kg samples respectively) or accurate weighting of approx. 76.1 mg test item for 15000 mg/kg samples. After spiking, the test item is allowed to adsorb to the diet for at least 30 minutes. Samples are extracted by shaking with 50 mL methanol. The solution is filtered through a 0.2 µm Spartan 30/0.2 RC filter and further diluted with methanol into calibration range. Samples were analysed using UPLC-UV. Samples were adequately diluted prior to analysis in order to minimize matrix effects. When measuring the matrix extracts using a calibration curve without matrix, acceptable recovery was obtained indicating that significant matrix effect was absent.

Materials and Methods

Method validation

The analytical method was validated in terms of specificity, linearity of detector's response, LOQ, recovery and repeatability (by means of precision) according to the guidance document SANCO/3029/99 rev. 4. The results are presented in the following table.

Specificity	The chromatogram of the blank QC sample showed no significant interference above 30% of LOQ the retention time of the test item, therefore showing that the method is highly specific.
Linearity	The analytical system was linear within the calibration range of 2.5 – 20 mg test item/L, which corresponds to concentrations of the test item in undiluted diet samples in the range of 25 – 200 mg/kg.

	y = 0.000262x – 0.000251 (r = 0.9997) Criteria of r ≥ 0.99 as required by the guideline is met.
Accuracy (Recovery) and Precision	The accuracy of the method was assessed by the recovery test by fortifying blank samples with R014821 at LOQ (500 mg/kg, Low), 1600 mg/kg (Middle) and 15000 mg/kg (High). The criteria of the guideline - accuracy between 70 and 110 %, precision ≤ 20 % - are met. The recovery results are summarized in the following Table C.1.3.2-1.
Limit of Quantification (LOQ)	500 mg/kg
Limit of Detection (LOD)	n.a.

Table C.1.3.2-1 Recovery results from method validation of diet samples using the analytical method. Standards were prepared in methanol

Matrix	**Analyte**	**Fortification level**	**Recovery**			
			Range [%]	**Mean [%]**	**RSD [%]**	**Sample size (n)**
Diet	R014821	Blank	Not detected	-	-	2
		500 mg/kg	98.8 – 100.1	99.3	0.54	6
		1600 mg/kg	91.1 – 94.0	92.7	1.20	6
		15000 mg/kg	94.9 – 97.5	96.6	0.97	6
		Overall	91.1 – 100.1	96.2	3.02	18

An analytical method for the determination of Imazalil metabolite R014821 in diet was successfully validated in terms of specificity, linearity of detector's response, LOQ, recovery and repeatability (by means of precision) according to guidance document(s) SANCO/3029/99 rev. 4 (2000).

C.1.5.3 Study 3

Reference:	Development and Validation of an Analytical Method for Analysis of FK-284 in Diet including Trial Diet Analysis. [redacted] 2020d, Study code 20203751
Company reference:	Sponsor no. AGR 5996
Test facility:	[redacted]
Guideline(s):	SANCO Guideline 3029/99 rev. 4 (2000)
Deviations:	No
GLP:	Yes
Study acceptable:	Yes, valid without restrictions

Principles of analytical methods

QC samples are prepared by weighting 5 g blank powder diet in glass vessels and fortification at 500, 1600 and 15000 mg/kg by addition of 1.25 mL of a 2000 mg/L stock solution (for 500 mg/kg samples) or accurate weightings of approx. 8.45 mg test item for 1600 mg/kg samples and approx. 76.5 mg test item for 15000 mg/kg samples. After spiking, the test item is allowed to adsorb to the diet for at least 30 minutes. Samples are extracted by shaking with 50 mL dimethyl sulfoxide (DMSO). The solution is filtered through a 0.2 µm Spartan 30/0.2 RC filter and diluted in a 1:4 (v/v) ratio with water to obtain concentrations within the calibration range. High fortification samples (15000 mg/kg) are 10-fold further diluted with 20/80 (v/v) DMSO/water into calibration range. Samples were analysed using UPLC-UV. Samples were adequately diluted prior to analysis in order to minimize matrix effects. When measuring the matrix extracts using a calibration curve without matrix, acceptable recovery was obtained indicating that significant matrix effect was absent.

Materials and Methods

Method validation

The analytical method was validated in terms of specificity, linearity of detector's response, LOQ, recovery and repeatability (by means of precision) according to the guidance document SANCO/3029/99 rev. 4. The results are presented in the following table.

Specificity	The chromatogram of the blank QC sample showed no significant interference above 30% of LOQ the retention time of the test item, therefore showing that the method is highly specific.
Linearity	The analytical system was linear within the calibration range of 1.0 – 40 mg test item/L, which corresponds to concentrations in the range of 50 – 2000 mg/kg of the test item in lowest diluted diet samples. Highest fortified samples were further diluted 10-fold into calibration range. y = 0.00201 – 0.00229 (r = 0.9996) Criteria of r ≥ 0.99 as required by the guideline is met.
Accuracy (Recovery) and Precision	The accuracy of the method was assessed by the recovery test by fortifying blank samples with FK-284 at LOQ (500 mg/kg, Low), 1600 mg/kg (Middle) and 15000 mg/kg (High). The criteria of the guideline - accuracy between 70 and 110 %, precision ≤ 20 % - are met. The recovery results are summarized in the following Table C.1.5.3-1. It is noted that samples were diluted to within the linear range for the higher fortification levels.
Limit of Quantification (LOQ)	500 mg/kg

Table C.1.5.3-1 Recovery results from method validation of diet samples using the analytical method. Standards were prepared in 20/80 (v/v) DMSO/water

Matrix	Analyte	Fortification level	Recovery			
			Range [%]	Mean [%]	RSD [%]	Sample size (n)
Diet	FK-284	Blank	Not detected	-	-	2
		500 mg/kg	95.9 – 99.8	97.6	1.46	6
		1600 mg/kg	97.5 – 100.2	98.7	0.92	6
		15000 mg/kg	98.6 – 100.3	99.5	0.68	6
		Overall	95.9 – 100.3	98.6	1.29	18

An analytical method for the determination of Imazalil metabolite FK-284 in diet was successfully validated in terms of specificity, linearity of detector's response, LOQ, recovery and repeatability (by means of precision) according to guidance document(s) SANCO/3029/99 rev. 4 (2000).

C.1.5.4 Study 4

Reference: Development and Validation of an Analytical Method for Analysis of FK-772 in Diet including Trial Diet Analysis.

[redacted] 2020c, Study code 20203750

Company reference: Sponsor no. AGR 5997

Test facility: [redacted]

Guideline(s): SANCO Guideline 3029/99 rev. 4 (2000)

Deviations: No

GLP: Yes

Principles of analytical methods

QC samples are prepared by weighting 5 g blank powder diet in glass vessels and fortification at 500, 1600 and 15000 mg/kg by addition of 0.5 mL of a 5000 mg/L stock solution (for 500 mg/kg samples) or accurate weightings of approx. 8.05 mg test item for 1600 mg/kg samples and approx. 76.5 mg test item for 15000 mg/kg samples. After spiking, the test item is allowed to adsorb to the diet for at least 30 minutes. Samples are extracted by shaking with 50 mL of a 50/50 (v/v) acetonitrile/water solution. The solution is filtered through a 0.2 µm Spartan 30/0.2 RC filter and further diluted with 50/50 (v/v) acetonitrile water to fit into calibration range. Samples were analysed using UPLC-UV. Samples were adequately diluted prior to analysis in order to minimize matrix effects. When measuring the matrix extracts using a calibration curve without matrix, acceptable recovery was obtained indicating that significant matrix effect was absent.

Method validation

The analytical method was validated in terms of specificity, linearity of detector's response, LOQ, recovery and repeatability (by means of precision) according to the guidance document SANCO/3029/99 rev. 4. The results are presented in the following table.

Specificity	The chromatogram of the blank QC sample showed no peak at the retention time of the test item. Since no interferences were detected, the specificity requirements were met and the analytical method was found to be specific for the test item.
Linearity	The analytical system was linear within the calibration range of 5 – 50 mg test item/L, which corresponds to concentrations of the test item in undiluted diet samples in the range of 50 – 500 mg/kg. Samples were diluted to fit into calibration range. y = 0.00304 – 0.0627 (r = 0.998) Criteria of r ≥ 0.99 as required by the guideline is met.
Accuracy (Recovery) and Precision	The accuracy of the method was assessed by the recovery test by fortifying blank samples with FK-772 at LOQ (500 mg/kg, Low), 1600 mg/kg (Middle) and 15000 mg/kg (High). The recovery results are summarized in the following Table C.1.3.4-1. Samples for the middle fortification level are high, and outside the threshold of 70-110% as stated in the guidance. As this is a

	minor variation at only one fortification level, this is considered acceptable in this case.
Limit of Quantification (LOQ)	500 mg/kg
Limit of Detection (LOD)	n.a.

Table C.1.3.5-1 Recovery results from method validation of diet samples using the analytical method. Standards were prepared in 50/50 (v/v) acetonitrile/water

Matrix	**Analyte**	**Fortification level**	**Recovery**			
			Range [%]	**Mean [%]**	**RSD [%]**	**Sample size (n)**
Diet	FK-772	Blank	Not detected	-	-	2
		500 mg/kg	98.7 – 100.8	99.7	0.72	6
		1600 mg/kg	110.8 – 118.0	116.4	4.52	6
		15000 mg/kg	98.6 – 104.7	101.3	2.15	6
		Overall	98.6 – 118.0	105.8	7.89	18

An analytical method for the determination of Imazalil metabolite FK-772 in diet was successfully validated in terms of specificity, linearity of detector's response, LOQ, and repeatability (by means of precision) according to guidance document(s) SANCO/3029/99 rev. 4 (2000). The method is declared as fit for purpose in terms of recovery.

C.1.5.5 Study 5

An additional method validation is required for the determination of JNJ-123955-AAA (T000824) in propylene glycol to support the toxicological study (Test facility study no. 517992) evaluated within this assessment.

Reference:	Development and Validation of an Analytical Method for Analysis of JNJ-123955-AAA (T000824) in Propylene Glycol including determination of formulation stability. [redacted] 2017, Study code 217993
Test facility:	[redacted]
Guideline(s):	SANCO Guideline 3029/99 rev. 4 (2000)
Deviations:	No
GLP:	Yes
Study acceptable:	Yes, valid without restrictions

Principles of analytical methods

Approximately 500 or 400 mg vehicle was accurately weighed and spiked with the test item at a target concentration of 1 or 200 mg/mL. The QC samples were prepared in volumetric flasks of 10 or 25 mL. The flasks were filled up to mark with acetonitrile. In order to dissolve the test item the solutions were ultrasonicated for 5 minutes. The solutions were diluted to obtain an end solution of 20/80 (v/v) acetonitrile/water and concentrations within the calibration range Samples were analysed using UPLC-UV.

Method validation

The analytical method was validated in terms of specificity, linearity of detector's response, LOQ, recovery and repeatability (by means of precision) according to the guidance document SANCO/3029/99 rev. 4. The results are presented in the following table.

Specificity	The chromatogram of the blank QC sample showed no significant interference above 30% of LOQ the retention time of the test item, therefore showing that the method is highly specific.
Linearity	The analytical system was linear within the calibration range of 0.5 – 20 mg/L. $y = 0.00944x – 0.00480$

	(r = 0.9998) Criteria of r ≥ 0.99 as required by the guideline is met.
Accuracy (Recovery) and Precision	The accuracy of the method was assessed by the recovery test by fortifying blank samples with JNJ-123955-AAA (T000824) at LOQ (1mg/mL, Low), and 200 mg/mL (High). The criteria of the guideline - accuracy between 70 and 110 %, precision ≤ 20 % - are met. The recovery results are summarized in the following Table C.1.5.5-1.
Limit of Quantification (LOQ)	1 mg/mL

Table C.1.5.5-1 Recovery results from method validation of diet samples using the analytical method. Standards were prepared in methanol

Matrix	**Analyte**	**Fortification level**	**Recovery**			
			Range [%]	**Mean [%]**	**RSD [%]**	**Sample size (n)**
Diet	R014821	Blank	Not detected	-	-	2
		1 mg/mL	99.2 – 101.0	99.91	0.79	5
		200 mg/mL	98.6 – 102.5	100.89	1.56	5
		Overall	98.6 – 102.5	100.4	1.28	10

An analytical method for the determination of Imazalil metabolite JNJ-123955-AAA (T000824) in diet was successfully validated in terms of specificity, linearity of detector's response, LOQ, recovery and repeatability (by means of precision) according to guidance document(s) SANCO/3029/99 rev. 4 (2000).

An additional method validation to support study AGR6320 is evaluated below.

C.1.5.6 Study 6

Reference:	Development and Validation of an Analytical Method for Analysis of R014821 in Diet including Trial Diet Analysis. [redacted] 2022, Study code 20321429
Company reference:	Sponsor no. AGR 6296
Test facility:	[redacted]
Guideline(s):	SANCO Guideline 3029/99 rev. 4 (2000)
Deviations:	No
GLP:	Yes
Study acceptable:	Yes, valid without restrictions

Principles of analytical methods

QC samples at a target test material concentration of 200 ppm were prepared by weighing approximately 5 g blank powder into glass vial and adding 500 µL of 2000 mg/L stock solution. QC samples at target concentration 15000 ppm were prepared by accurately weighing approximately 76.1 mg test item and approximately 5 g blank powder diet in a glass vessel. Thereafter the test material was allowed to adsorb to the diet for at least 30 minutes. Then the samples were extracted at 225 rpm with 50 mL of methanol. The shaking time was 30 minutes. Subsequently the extracts were filtered through a 0.2 µm Spartan 30/0.2 RC filter (Whatman, Dassel, Germany) and diluted with methanol to obtain concentrations within the calibration range. Samples were analysed using UPLC-UV. Samples were adequately diluted prior to analysis in order to minimize matrix effects. When measuring the matrix extracts using a calibration curve without matrix, acceptable recovery was obtained indicating that significant matrix effect was absent.

Method validation

The analytical method was validated in terms of specificity, linearity of detector's response, LOQ, recovery and repeatability (by means of precision) according to the guidance document SANCO/3029/99 rev. 4. The results are presented in the following table.

Specificity	The chromatogram of the blank QC sample showed no significant interference above 30% of LOQ the retention time of the test item, therefore showing that the method is highly specific.
Linearity	The analytical system was linear within the calibration range of 1.0 – 20 mg test item/L, which corresponds to concentrations of the test item in undiluted diet samples in the range of 10 – 200 mg/kg. y = 0.000281x – 0.000206 (r = 0.998) Criteria of r ≥ 0.99 as required by the guideline is met.
Accuracy (Recovery) and Precision	The accuracy of the method was assessed by the recovery test by fortifying blank samples with R014821 at LOQ (200 mg/kg, Low), and 15000 mg/kg (High). The criteria of the guideline - accuracy between 70 and 110 %, precision ≤ 20 % - are met. The recovery results are summarized in the following Table C.1.3.2-1.
Limit of Quantification (LOQ)	200 mg/kg

Table C.1.3.7-1 Recovery results from method validation of diet samples using the analytical method. Standards were prepared in methanol

Matrix	Analyte	Fortification level	Recovery			
			Range [%]	Mean [%]	RSD [%]	Sample size (n)
Diet	R014821	Blank	Not detected	-	-	2
		200 mg/kg	98.5 – 101.4	100.24	1.04	6
		15000 mg/kg	90.1 – 93.3	91.23	1.24	6
		Overall	90.1 – 101.4	95.07	5.03	12

An analytical method for the determination of Imazalil metabolite R014821 in diet was successfully validated in terms of specificity, linearity of detector's response, LOQ, recovery and repeatability (by means of precision) according to guidance document(s) SANCO/3029/99 rev. 4 (2000).

C.2 Mammalian toxicology

No new toxicological information on the parent substance imazalil was provided with this application. The conclusions of the studies evaluated in the renewal report by the EU Member State the Netherlands (RAR, 2009) and during the MRL review under Article 12 of Regulation (EC) No 396/2005 (EFSA, 2017) have been re-presented in section 2 of this document for completeness.

A 90-day comparative toxicity study in the rat to compare the toxicity of the three metabolites R014821, FK772 and FK284 with that of the parent imazalil was submitted with this MRL confirmatory data application.

Additional *in vitro* mutagenicity studies were conducted on metabolites R014821, FK284 and FK772 and submitted with this MRL application in order to fully exclude the genotoxic potential of these metabolites.

This new additional information has been fully evaluated by HSE below.

C.2.1 Absorption, distribution, excretion and metabolism

C.2.2 Acute toxicity including irritancy and skin sensitisation

C.2.3 Short-term toxicity

C.2.4 Genotoxicity

C.2.5 Long-term toxicity and carcinogenicity

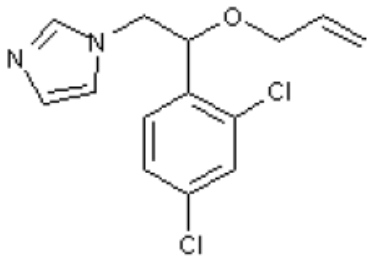
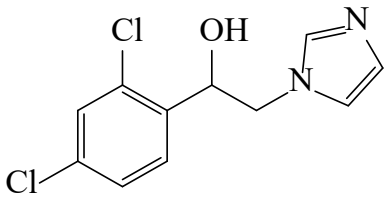
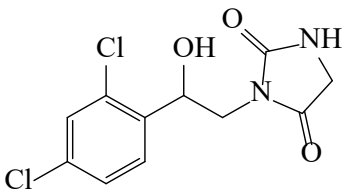
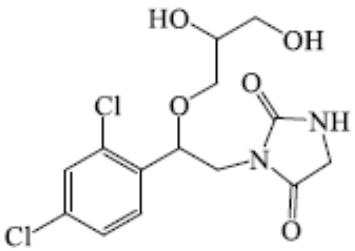
C.2.6 Reproductive toxicity

C.2.7 Neurotoxicity

C.2.8 Further toxicological studies

Comparative toxicity study on imazalil and metabolites R014821, FK772 and FK284

A 90-day comparative toxicity study in the rat has been provided with this MRL application in order to compare the potential toxicity of the three metabolites R014821, FK772 and FK284 with that of the parent compound imazalil when administered via the diet for 90 days to rats. In addition, the toxicokinetic characteristics of imazalil, FK772, FK284 and R014821 were determined. The structure of imazalil and the three metabolites are shown below:

Imazalil (parent compound)	
R014821	
FK284	
FK772	

R014821 is formed by O-dealkylation of imazalil and there is no change in the cyclic groups between the parent and its metabolite. Extensive biotransformation of imazalil occurs in animals, which results in production of FK772 and FK284 (both detected in livestock matrices). The chemical structures of imazalil and the metabolites FK284 and FK772 mostly differ in that the parent contains an aromatic heterocycle (imidazole), while the heterocycles in FK772 and FK284 are saturated (imidazolidine-2,4-dione). FK284 also contains a hydroxy group formed by O-dealkylation and FK772 is a diol formed by reduction of the allyloxy group.

New information submitted with this application

A 90-Day Study of Imazalil, FK772, FK284 and R014821 by Dietary Administration in Wistar Han Rats (2021)

Reference:	(2021)
	Report No. AGR 5959
	Test facility study no. 20192866
Date performed:	Completion date: 19th May 2020
Test facility:	
Guideline(s):	Study design was based on OECD 408
Deviations:	Yes – both major and minor deviations were noted
	Only one dose level of each test substance was administered because the study was designed to compare the toxicity of the three metabolites with the parent compound imazalil. This means that the dose-response cannot be characterised and the study is not suitable to derive a NOAEL.
	Additional minor deviations included a reduced number of results available in males at 1600 ppm imazalil due to clotted blood samples. Results obtained from a previously-conducted 90-day oral gavage rat study on imazalil were compared against those obtained in this study for the three metabolites FK284, FK772 and R014821.
	These deviations do not invalidate the study.
GLP:	Yes
Test item/dose: groups	1600 ppm imazalil (batch no. ZR023979EXA909, purity 99.7%)
	1600 ppm FK772 (batch no. ND19/GF_2357D, purity 96.9%)
	1600 ppm FK284 (batch no. 08-051-000-A-20190506-AWO, purity 94.0%)
	1600 ppm R014821 (batch no. 181101, purity 99.9%)
Validated method of analysis:	Validated analytical methods are available for R014821, FK284, FK772 and imazalil (study reference no. 20192863, 20203751, 20203750 and 20203753 respectively)
Study acceptable:	Yes

Method

In this GLP-compliant study based on the OECD 408 test guideline, the toxicity of the metabolites FK284, FK772 and R014821 were compared to that of the parent compound imazalil by administering each test substance via the diet to groups of Wistar Han rats (10/sex/group) for 90 days. The test groups were as follows: control diet, 1600 ppm imazalil, 1600 ppm FK284, 1600 ppm FK772, and 1600 ppm R014821.

The stability and homogeneity of each test substance in the diet were shown to be acceptable. Fully validated methods of analysis for each test substance in the diet at concentrations covering those tested are available (see Validated methods of analysis above).

An additional investigation into the toxicokinetic characteristics of imazalil, FK772, FK284 and R014821 was conducted as part of this study. Blood samples were taken from the jugular vein from groups of 6-10 animals at timepoints spaced 6 hours apart on two separate days (Day 1 and during the final week of the study), processed and analysed to determine the concentration of the test substance in the plasma. Toxicokinetic parameters (AUC_{last}, AUC_{last}/ dose, $AUC_{(0\text{-}18h)}$, $AUC_{(0\text{-}18h)}$/ dose, $AUC_{(0\text{-}24h)}$, $AUC_{(0\text{-}24h)}$/ dose) were calculated for imazalil, FK772, FK284 and R014821.

Results

Administered doses

Dietary analyses using validated test methods confirmed that formulations of imazalil, FK772, FK284 or R014821 in diets were prepared accurately and homogenously. The table below shows the equivalent doses for the dietary concentration of 1600 ppm.

Table C2.8-1. Test substance intake

Mean intake based on a nominal dietary concentration of 1600 ppm [mg/kg bw/d]				
	Imazalil	**FK772**	**FK284**	**R014821**
Males	100	101	104	101
Females	106	113	109	110

Plasma kinetics

The parent compound and its metabolites were given to the animals and their concentrations were determined in different groups. Toxicokinetic parameters ($AUC_{(last)}$, $AUC_{(last)}$/ Dose) were calculated at Day 1 and Week 13. Time-dependent changes in exposure were evaluated by comparing $AUC_{(0\text{-}18h)}$/dose values at the start of the study and

after repeated administration. Metabolite and parent ratios of $AUC_{(last)}$/Dose were calculated on Day 1 and in Week 13 assuming equal bioavailability between the test items.

Table C2.8-2. Plasma concentrations of test substances on Day 1 and in Week 13

Test substance	Sex	Period		Time (h)			
				0[1]	6	12	18
				Plasma concentration (ng/mL)			
Imazalil	M	Day 1	Mean ± SD	<LLOQ	30.1 ± 39.5	154 ± 71.8	86.9 ± 43.9
Imazalil	M	Week 13	Mean ± SD	5.32 ± 0.953	7.34 ± 6.09	15.5 ± 4.20	11.3 ± 2.10
FK772	M	Day 1	Mean ± SD	<LLOQ	316 ± 279	1530 ± 472	2130 ± 210
FK772	M	Week 13	Mean ± SD	1720 ± 51.3	1180 ± 165	1250 ± 261	1710 ± 537
FK284	M	Day 1	Mean ± SD	<LLOQ	525 ± 382	3510 ± 1440	11100 ± 1940
FK284	M	Week 13	Mean ± SD	8250 ± 1520	5600 ± 1590	5350 ± 2900	8460 ± 1100
R014821	M	Day 1	Mean ± SD	< LLOQ	277 ± 99.6	845 ± 99.6	1470 ± 230
R014821	M	Week 13	Mean ± SD	626 ± 166	402 ± 23.3	446 ± 149	812 ± 58.0
Imazalil	F	Day 1	Mean ± SD	<LLOQ	61.6 ± 58.1	1120 ± 448	67.6 ± 45.5
Imazalil	F	Week 13	Mean ± SD	44.0 ± 41.8	41.3 ± 12.7	22.0 ± 17.4	30.1 ± 10.8
FK772	F	Day 1	Mean ± SD	<LLOQ	528 ± 199	1490 ± 139	2950 ± 737
FK772	F	Week 13	Mean ± SD	3850 ± 789	2570 ± 829	1850 ± 481	2660 ± 474
FK284	F	Day 1	Mean ± SD	<LLOQ	777 ± 179	3910 ± 1100	10600 ± 2870
FK284	F	Week 13	Mean ± SD	11300 ± 2660	8270 ± 2020	6460 ± 653	9750 ± 2120
R014821	F	Day 1	Mean ± SD	< LLOQ	58.3 ± 96.7	1040 ± 251	1130 ± 345
R014821	F	Week 13	Mean ± SD	880 ± 463	648 ± 141	480 ± 395	851 ± 177

[1] t=0 concentrations were also used as t=24h concentrations in Week 13 to enable daily exposure assessment, <LLOQ: below lower limit of quantification of 0.3 ng/mL (value was set as 0 for statistical and kinetic analysis)

Table C2.8-3. Toxicokinetic parameters of imazalil, FK772, FK284 and R014821

	Test substance							
Parameters	**Imazalil**		**FK772**		**FK284**		**R014821**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Day 1	**Mean test item intake (mg/kg bw/d)**							
	127	**126**	**156**	**155**	**144**	**133**	**113**	**188**
$AUC_{(last)}$[1] (h.ng/mL)	1360	7310	17500	21000	57500	59800	11100	9970
$AUC_{(last)}$/ Dose [1] (h. kg.ng/ mL/mg)	10.7	58.0	112	135	399	450	98.6	84.5
Metabolite/Parent ratio of $AUC_{(last)}$/ Dose	n/a	n/a	10	2.3	37	7.8	9.2	1.5
Week 13	**Mean test item intake (mg/kg bw/d)**							
	79	**87**	**79**	**87**	**82**	**88**	**86**	**97**
$AUC_{(0\text{-}18h)}$ (h.ng/mL)	187	602	24800	4600	116000	151000	9410	12000
$AUC_{(0\text{-}18h)}$/ Dose (h.kg.ng/ mL/mg)	2.37	6.92	314	529	1410	1720	109	123
$AUC_{(last)}$[2] (h.ng/mL)	237	825	35100	65600	166000	215000	13700	17200
$AUC_{(last)}$/ Dose [1] (h.kg.ng/ mL/mg)	3.00	9.48	445	754	2020	2440	160	177
Metabolite/Parent ratio of $AUC_{(last)}$/ Dose	n/a	n/a	150	80	670	260	53	19

M = male, F = female, [1] Day 1 AUC_{last} = $AUC_{(0\text{-}18h)}$, [2] Week 13 AUC_{last} = $AUC_{(0\text{-}24h)}$

The toxicokinetic assessment showed that exposure to imazalil (expressed as $AUC_{(0\text{-}18h)}$/dose) was lower after 13 weeks of repeated dosing compared to Day 1. R014821 did not show a significant change in exposure following repeat dosing. FK772 and FK284 showed accumulation in both sexes after repeated dosing.

After dietary administration, higher exposure to imazalil was noted for females compared to males, while for FK772, FK284 and R014821, no significant sex differences were noted during the study.

The metabolites of imazalil showed a higher exposure (AUC_{last}/dose) compared to the parent compound, with the highest exposure noted for FK284, followed by FK772, then R014821.

Mortality
No mortality occurred during the study.

Clinical observations
No clinical signs were noted during the observation period.

Body weight and body weight gain
Females treated with imazalil or FK284 showed statistically significant reductions in body weight and body weight gain throughout the study. Although males in these treatment groups also showed initial weight loss, there was no consistent pattern of body weight reductions in imazalil or FK284-treated males. Animals treated with FK772 did not show any changes in body weight or body weight gain compared to concurrent controls. In contrast, animals of both sexes dosed with R014821 showed statistically significant reductions in body weight and body weight gain throughout the study.

Overall, treatment-related adverse reductions in body weight and body weight gain were observed with imazalil, R014821 and FK284. No body weight effects were seen with FK772.

Table C2.8-4. Body weight and body weight effects

	Test substance									
	Control		**Imazalil**		**FK772**		**FK284**		**R014821**	
	male	female	male	female	male	female	male	female	male	female
Day	**Mean bodyweight ±SD [g] (*Δ% compared to control)***									
1	228 ± 8.1 *(-)*	149 ± 6.8 *(-)*	227 ± 7.0 *(-0.4)*	143 ± 6.9 *(-4)*	225 ± 7.6 *(-1.3)*	154 ± 7.6 *(+3.4)*	223 ± 9.3 *(-2.2)*	142 ± 8.8 *(-4.7)*	225 ± 7.9 *(-1.3)*	146 ± 10.6 *(-2.0)*
8	265 ± 9.8 *(-)*	170 ± 7.5 *(-)*	254 ± 10.7 *(-4.2)*	**158* ± 7.9 *(-7.6)***	263 ± 10.6 *(-0.8)*	173 ± 8.1 *(+1.8)*	**253* ± 8.9 *(-4.5)***	**158* ± 10.4 *(-7.6)***	**244** ± 10.4 *(-7.9)***	**155 ± 10.7** *(-8.8)***

43	381 ± 28.3 *(-)*	224 ± 13.3 *(-)*	367 ± 21.3 *(-3.7)*	**200** ± 12.6** ***(-10.7)***	375 ± 18.4 *(-1.6)*	228 ± 15.7 *(+1.8)*	360 ± 19.7 *(-5.5)*	**189** ± 19.9** ***(-15.6)***	**329** ± 20.2** ***(-13.6)***	**190** ± 14.1** ***(-15.2)***
91	444 ± 39.1 *(-)*	238 ± 14.0 *(-)*	424 ± 30.8 *(-4.5)*	**213* ± 17.4 *(-10.5)***	433 ± 23.0 *(-2.5)*	245 ± 18.4 *(+2.9)*	416 ± 20.1 *(-6.3)*	**199** ± 23.1** ***(-16.4)***	**380** ± 29.7** ***(-14.4)***	**207** ± 18.7** ***(-13.0)***
	Mean body weight gain ±SD [g] (*Δ% compared to control)*									
1-8	16 ± 1.3 *(-)*	14 ± 1.4 *(-)*	**12** ± 1.8** ***(-25.0)***	**11* ± 2.5 *(-21.4)***	17 ± 2.4 *(+6.3)*	13 ± 2.6 *(-7.1)*	**14* ± 2.1 *(-12.5)***	**11* ± 4.2 (-21.4)**	**9** ± 2.1 *(-43.8)***	**6 ± 3.0** *(-57.1)***
1-43	67 ± 8.7 *(-)*	51 ± 4.3 *(-)*	62 ± 6.7 *(-7.5)*	**40** ± 6.1** ***(-21.6)***	67 ± 6.9 *(±0.0)*	48 ± 5.6 *(-5.9)*	61 ± 5.8 *(-9.0)*	**33** ± 10.1** ***(-35.3)***	**47** ± 6.1** ***(-29.9)***	**30** ± 4.2** ***(-41.2)***
1-91	94 ± 11.8 *(-)*	60 ± 5.0 *(-)*	87 ± 9.9 *(-7.4)*	**49** ± 8.9** ***(-18.3)***	93 ± 10.4 *(-1.1)*	60 ± 6.5 *(±0.0)*	87 ± 7.8 *(-7.4)*	**39** ± 11.9** ***(-35.0)***	**69** ± 10.5** ***(-26.6)***	**41** ± 5.0** ***(-31.7)***

* P ≤ 0.05, ** P ≤ 0.01 (Dunnett's test)

Food and water consumption

Slight decreases in food consumption were observed throughout the study in females dosed with imazalil, FK284, or R014821. Males dosed with R014821 also showed a slight decrease in food consumption during the first 8 weeks of the study compared to concurrent controls. For all other groups (males dosed with imazalil or FK284, animals of both sexes dosed with FK772), food consumption was similar to the control level over the entire study period. The observed decreases in food consumption correlated with the effects on body weight and were considered treatment-related and adverse. Overall, treatment-related adverse reductions in food consumption were observed with imazalil, R014821 and FK284. No effects on food consumption were seen with FK772.

Ophthalmology

No treatment-related findings were observed at the end of the administration period.

Haematology

No haematology evaluation could be carried out for males treated with imazalil due to a reduced number of samples, caused by clotting of blood samples. However, results from a previous 90-day oral gavage rat study with imazalil (Gur et al., 1991) showed that only a decrease in monocytes was noted at 1600 ppm imazalil. This finding was not considered treatment-related.

Statistically significant changes were observed in several haematology parameters compared to concurrent controls. Females treated with FK772 showed decreased platelet count and increased red blood cell width distribution, neutrophil, and basophil counts. After administration of R014821, females showed increased red blood cell width distribution, while males had statistically significant decreases in mean cell volume, mean cell haemoglobulin and platelet count. The majority of these findings were not considered biologically relevant due to their small magnitude (< ±10%). In addition, no biologically relevant effects on coagulation parameters were noted during the observation period, suggesting that the decreases in platelet count observed in FK772-treated females (-17%) and in R014821-treated males (-16%) were not adverse.

Overall, no treatment-related and adverse effects on haematological parameters were observed in males or females of any treatment group.

Clinical chemistry
Animals of both sexes dosed with imazalil showed statistically significant changes in serum potassium (-8 / -19 % in males /females respectively), chloride (-3 % in both sexes) and bilirubin (-15 / -31% in males /females). Imazalil-treated female rats also showed statistically significant decreases in AST (-15 %), albumin (-6 %), creatinine (-13 %) and calcium (-3 %). The majority of these changes were not considered biologically relevant due to their small magnitude (< ±10 %) compared to concurrent controls. As a decrease in total bilirubin is generally not considered clinically relevant, decreases in bilirubin were not considered adverse. The decrease in serum potassium in both sexes treated with imazalil was considered treatment-related and potentially adverse. Females treated with FK772 showed a decrease in total bilirubin (-26 %) and increased serum phosphate (+30 %) which were both statistically significant, while females treated with FK284 showed decreases in total protein (-5 %), chloride (-2 %) and an increase in phosphate (+37 %). Males dosed with FK284 and R014821 showed statistically significant increases in urea (+21 % and +27% respectively) and phosphate (+19% and +20 % respectively). Males treated with R014821 also showed a decrease in total bilirubin (-18 %), which is generally not considered clinically relevant. Females dosed with R014821 showed statistically significant changes in several clinical chemistry parameters (decreases in total protein, albumin, bilirubin and chloride; increases in urea and phosphate).

Some of these changes were not considered biologically relevant due to their small magnitude; however, the changes in urea (+18 %) and phosphate (+26 %), indicative of potential impaired kidney function, observed in males treated with FK284 and in animals of both sexes treated with R014821 were considered treatment-related and adverse. In female rats treated with FK772 and FK284, there was only one clinical chemistry parameter (increase in phosphate) which showed a change which was both statistically significant and biologically relevant and considered adverse.

Overall, treatment-related and adverse changes in clinical chemistry parameters indicative of potential impaired kidney function were observed in this study for imazalil and the 3 metabolites. These included increased urea and phosphate with FK284 and R014821, increased phosphate with KF772; and decreased serum potassium and decreased creatinine with imazalil.

Assessment of thyroid hormones
No effects were observed on thyroid hormones in females for any treatment group.

Males treated with FK772 and R014821 showed statistically significantly increased T3 levels (+41 % and +86 % respectively). Although changes in other thyroid parameters, most notably an increase in TSH, were also observed in these animals, (+29 % and +122 % in FK772 and R014821-treated males), they did not reach statistical significance. No statistically significant changes in circulating T4 were observed in either the FK772 or the R014821 treatment groups and the only histological findings observed in the thyroid was a slight increase in the incidence of diffuse follicular cell hypertrophy in males treated with R01482. Overall, treatment-related and adverse increases in T3 and TSH were seen with FK772 and R014821 (mainly in males).

Table C2.8-5. Selected haematology and coagulation parameters after 13-weeks treatment

Test substance	Males (day 92)					Females (day 93)				
	Control	Imazalil	FK772	FK284	R014821	Control	Imazalil	FK772	FK284	R014821
Haematology	parameters									
n	8	2	8	10	10	10	9	10	10	10
RBC [10^{12}/l]	8.893 ± 0.435 *(-)#*	9.210^ ± 0.198 *(+4)* **#**	8.959 ± 0.525 *(+1)#*	8.788 ± 0.352 *(-1)#*	8.495 ± 0.864 *(-4)#*	8.269 ± 0.432 *(-)*	8.581 ± 0.449 *(+4)*	8.142 ± 0.408 *(-2)*	8.165 ± 0.348 *(-1)*	8.260 ± 0.333 *(±0)*
RDWG (%)	12.70 ± 0.43 *(-)*	13.30^ ± 0.85 *(+5)*	12.95 ± 0.78 *(+2)*	12.73 ± 0.49 *(±0)*	13.12 ± 0.56 *(+3)*	11.46 ± 0.54 *(-)*	12.02 ± 0.48 *(+5)*	11.47 ± 0.47 *(±0)*	**12.09* ± 0.42 *(+5)***	**12.31** ± 0.53 *(+7)***
MCV [fl]	50.59 ± 1.26 *(-)*	50.95^ ± 0.21 *(+1)*	49.99 ± 1.48 (-1)	50.55 ± 0.98 *(±0)*	**48.38** ± 1.02 *(-4)***	52.94 ± 1.18 *(-)*	51.88 ± 1.86 *(-2)*	52.45 ± 1.33 *(-1)*	51.58 ± 1.01 *(-3)*	51.83 ± 1.10 *(-2)*
MCH [pg]	17.59 ± 0.53 *(-)*	17.25^ ± 0.21 *(-2)*	17.26 ± 0.40 *(-2)*	17.38 ± 0.38 *(-1)*	**16.89** ± 0.50 *(-4)***	1.14 ± 0.03 *(-)*	1.12 ± 0.04 *(-2)*	1.14 ± 0.03 *(±0)*	1.12 ± 0.03 *(-2)*	1.12 ± 0.03 *(-2)*
PLT [10^9/l]	741.6 ± 87.5 *(-)*	569.0^ ± 36.8 *(-23)*	757.1 ± 99.0 *(+2)*	657.7 ± 90.3 *(-11)*	**620.7* ± 57.7 *(-16)***	836.7 ± 80.2 *(-)*	802.0 ± 64.2 *(-4)*	803.2 ± 91.2 *(-4)*	**697.4** ± 67.9 *(-17)***	777.6 ± 86.4 *(-7)*
NEUT [10^9/l]	0.664 ± 0.222 *(-)*	0.860^ ± 0.184 *(+30)*	0.936 ± 0.189 *(+41)*	0.957 ± 0.487 *(+44)*	0.884 ± 0.243 *(+33)*	0.492 ± 0.172 *(-)*	0.497 ± 0.279 *(+1)*	0.542 ± 0.161 *(+10)*	**0.827* ± 0.415 *(+68)***	0.458 ± 0.152 *(-7)*
MONO [10^9/l]	0.090 ± 0.024 *(-)*	0.070^ ± 0.014 *(-9)*	0.113 ± 0.035 *(+25)*	0.096 ± 0.032 *(+7)*	0.097 ± 0.048 *(+8)*	0.093 ± 0.039 *(-)*	0.077 ± 0.028 *(-18)*	0.066 ± 0.024 *(-29)*	0.115 ± 0.046 *(+24)*	0.074 ± 0.025 *(-20)*
BASO [10^9/l]	0.008 ± 0.005 *(-)*	0.005^ ± 0.007 *(-33)*	0.006 ± 0.005 *(-17)*	0.008 ± 0.006 *(+7)*	0.005 ± 0.007 *(-33)*	0.001 ± 0.003 *(-)*	0.003 ± 0.005 *(+233)*	0.002 ± 0.004 *(+100)*	**0.007* ± 0.005 *(+600)***	0.003 ± 0.005 *(+233)*
Coagulation	parameters									
n	8	9	9	10	10	10	9	10	**10**	7

PT [s]	18.21 ± 0.58 *(-)*	**16.96** ± 0.62 *(-7)*	17.56 ± 0.67 *(-4)*	17.35* ± 0.67 *(-5)*	**17.23** ± 0.58 *(-5)*	16.83 ± 0.58 *(-)*	16.91 ± 0.63 *(±0)*	17.72* ± 0.69 *(+5)*	17.25 ± 0.92 *(+2)*	17.54 ± 0.56 *(+4)*

RBC: red blood cell count, RDWG: red blood cell width distribution, MCV: mean cell volume, MCH: mean cell haemoglobulin, MCHC: mean cell haemoglobulin concentration, PLT: platelet count, PT: prothrombin time, NEUT: neutrophil count, MONO: monocyte count, BASO: basophil count, * p ≤ 0.05, ** p ≤ 0.01 (Anova & Dunnett, # Kruskal-Wallis & Dunn), ^ = inappropriate for statistics

Table C2.8-6. Selected biochemistry parameters

Test substance	Males (day 92)					Females (day 93)				
	Control	Imazalil	FK772	FK284	R014821	Control	Imazalil	FK772	FK284	R014821
n	10	10	9	10	10	10	10	10	10	10
AST [U/l]	84.4 ± 8.0 *(-)*	84.7 ± 33.1 *(+0)*	78.0 ± 11.7 *(-8)*	93.5 ± 13.1 *(+11)*	93.2 ± 30.7 *(+10)*	82.1 ± 6.1 *(-)* #	**69.9* ± 7.8 *(-15)* #**	87.3 ± 19.0 *(+6)* #	119.0 ± 95.6 *(+45)* #	73.5 ± 5.2 *(-10)* #
TPROT [g/l]	63.39 ± 1.40 *(-)* #	62.49 ± 1.39 *(-1)* #	63.90 ± 2.13 *(+1)* #	**60.29* ± 2.50 *(-5)* #**	61.88 ± 3.54 *(-2)* #	68.50 ± 2.40 *(-)*	65.24 ± 2.71 *(-5)*	67.52 ± 3.43 *(-1)*	**64.78* ± 3.85 *(-5)***	**64.08* ± 3.49 *(-6)***
ALB [g/l]	39.96 ± 1.08 *(-)*	39.78 ± 0.96 *(±0)*	40.60 ± 1.44 *(+2)*	38.30 ± 1.59 *(-4)*	39.50 ± 2.22 *(-1)*	44.60 ± 1.81 *(-)*	**41.81* ± 1.66 *(-6)***	43.77 ± 2.38 *(-2)*	42.33 ± 2.45 *(-5)*	**41.99* ± 2.43 *(-6)***
TBIL [µmol/L]	2.07 ± 0.25 *(-)*	**1.76* ± 0.27 *(-15)***	2.07 ± 0.35 *(±0)*	1.88 ± 0.15 *(-9)*	**1.69** ± 0.26 *(-18)*	2.47 ± 0.47 *(-)*	**1.71** ± 0.35 *(-31)***	**1.84** ± 0.27 *(-26)***	2.08 ± 0.36 *(-16)*	**1.99* ± 0.43 *(-19)***
UREA [mmol/L]	5.90 ± 1.01 *(-)* #	6.21 ± 0.61 *(+5)* #	6.47 ± 0.83 *(+10)* #	**7.11* ± 0.69 *(+21)* #**	**7.48* ± 1.47 *(+27)*** #	6.93 ± 1.21 *(-)*	7.47 ± 0.79 *(+8)*	7.14 ± 0.79 *(+3)*	6.79 ± 0.84 *(-2)*	**8.17* ± 0.85 *(+18)***
CREA [µmol/l]	26.9 ± 3.7 *(-)*	26.4 ± 3.2 *(-2)*	27.9 ± 3.1 *(+4)*	29.5 ± 90.3 *(+10)*	28.4 ± 5.3 *(-16)*	33.0 ± 3.3 *(-)*	**28.9* ± 4.0 *(-13)***	35.0 ± 2.8 *(+6)*	31.8 ± 4.0 *(-4)*	29.8 ± 3.1 *(-10)*
K [mmol/l]	3.75 ± 0.26 *(-)*	**3.43* ± 0.35 *(-8)***	3.58 ± 0.19 *(-4)*	3.73 ± 0.29 *(-1)*	3.81 ± 0.16 *(+2)*	3.39 ± 0.18 *(-)*	**2.76** ± 0.31 *(-19)***	3.29 ± 0.26 *(-3)*	3.15 ± 0.19 *(-7)*	3.34 ± 0.29 *(-2)*
Cl [mmol/l]	105.6 ± 1.3 *(-)* #	**102.3** ± 2.0 *(-3)* #**	104.8 ± 1.0 *(-1)* #	104.9 ± 1.0 *(-1)* #	103.9 ± 1.4 *(-2)* #	107.5 ± 1.2 *(-)*	**104.1** ± 1.4 *(-3)***	106.8 ± 1.5 *(-1)*	**105.4** ± 1.6 *(-2)***	**105.5** ± 1.1 *(-2)***
Ca [mmol/l]	2.517 ± 0.052 *(-)*	2.523 ± 0.051 *(±0)*	2.563 ± 0.072 *(-2)*	2.524 ± 0.062 *(±0)*	2.520 ± 0.081 *(±0)*	2.615 ± 0.040 *(-)*	**2.531* ± 0.042 *(-3)***	2.603 ± 0.089 *(±0)*	2.578 ± 0.051 *(-1)*	2.581 ± 0.081 *(-1)*

Test substance	Males (day 92)					Females (day 93)				
	Control	Imazalil	FK772	FK284	R014821	Control	Imazalil	FK772	FK284	R014821
PHOS [mmol/l]	1.512 ± 0.254 *(-)*	1.560 ± 0.147 *(+3)*	1.620 ± 0.197 *(+7)*	**1.797* ± 0.245 *(+19)***	**1.810* ± 0.231 *(+20)***	1.227 ± 0.211 *(-)*	1.329 ± 0.191 *(+8)*	**1.597** ± 0.173 *(+30)***	**1.676** ± 0.254 *(+37)***	**1.551** ± 0.285 *(+26)***
T3 [ng/ml]	0.446 ± 0.114 *(-)*	0.570 ± 0.109 *(+28)*	**0.629** ± 0.160 *(+41)***	0.577 ± 0.121 *(+29)*	**0.742** ± 0.113 *(+66)***	0.488 ± 0.078 *(-)*	0.487 ± 0.097 *(±0)*	0.594 ± 0.151 *(+22)*	0.525 ± 0.123 *(+8)*	0.572 ± 0.116 *(+17)*
T4 [ng/ml]	46.06 ± 10.75 *(-)*	45.61 ± 12.77 *(-1)*	50.49 ± 9.32 *(+10)*	48.48 ± 11.19 *(+5)*	49.78 ± 7.31 *(+8)*	25.25 ± 7.07 *(-)*	32.63 ± 10.62 *(±0)*	26.15 ± 4.32 *(+4)*	23.38 ± *(+4)*	24.48 ± 7.82 *(-3)*
TSH [mU/l]	0.2336 ± 0.1207 *(-)* #	0.2495 ± 0.1207 *(+7)* #	0.3003 ± 0.2033 *(+29)* #	0.2110 ± 0.1330 *(-10)* #	0.5193 ± 0.3332 *(+122)* #	0.1058 ± 0.1170 *(-)*	0.1051 ± 0.0604 *(-1)*	0.1011 ± 0.0837 *(-4)*	0.0664 ± 0.0643 *(-37)*	0.1353 ± 0.1177 *(+28)*

AST: aspartate aminotransferase, TPROT: total protein, ALB: albumin, TBIL: total bilirubin, CREA: creatinine, K: potassium, Cl: chloride, Ca: calcium, PHOS: phosphate, T3: triiodothyronine, T4: thyroxine, TSH: thyroid-stimulating hormone, * $p \leq 0.05$, ** $p \leq 0.01$ (Anova & Dunnett, # Kruskal-Wallis & Dunn)

Organ weights
Statistically significant changes in the weights of several organs were reported.

Increases in relative liver weights were observed in rats of both sexes treated with imazalil (+22 % and +23 % in males and females respectively), male rats treated with R014821 (+23 %) and female rats treated with FK284 (+20 %). These findings were considered treatment-related and adverse. The increases in relative liver weight observed in female rats treated with FK772 (+9%) and R014821 (+13 %) were considered adaptive as they were less than +15 %.

Statistically significant increases in kidney weights were observed in imazalil-treated rats of both sexes (absolute: +12 % in males, relative: +18 % and +14 % in males and females respectively). Rats of both sexes treated with R014821 and males treated with FK284 all showed increases in relative kidney weight (+14 % in FK284-treated males, +18 % and +12% in males and females dosed with R014821 respectively). Although no histopathology was observed, there were corroborating clinical chemistry findings (increase in urea and phosphate) associated with changes in kidney function. Therefore, these findings were considered treatment-related and adverse.

Statistically significant increases in the relative weight of the adrenals were observed in males with FK284 and R014821 (+17 % for both treatment groups) and in females treated with FK284, which were corroborated by histopathological findings (Table C2.8-.8). These findings were considered treatment-related and adverse.

Females showed a statistically significant decrease in pituitary weight with R014821 (absolute: -33 %, relative: -22 %). These findings in females were not corroborated by histopathological findings, changes in secretion of measured pituitary hormones (TSH) or clinical observations indicating deficiencies in pituitary hormones. Therefore, they were not considered adverse.

Males treated with R014821 had a statistically significant reduction in prostate gland weight (absolute: -29%, relative: -17%), which was not accompanied by histopathology. Therefore, the finding was not considered adverse.

All other statistically significant changes in absolute or relative organ weights were secondary to reduced body weight.

Overall, treatment-related and adverse changes in organ weights were observed with imazalil (liver and kidney), R014821 (liver, kidney and adrenals) and FK284 (kidney and adrenals).

Table C2.8-7. Selected organ weights

Test substance		Control		Imazalil		FK772		FK284		R014821	
		mean ±SD	***Δ%***	**mean ±SD**	***Δ%***	**mean ±SD**	***Δ%***	**mean ±SD**	***Δ%***	**mean ±SD**	***Δ%***
Males											
Brain	abs.[g]	2.12 ± 0.08	-	2.12 ± 0.09	±0	2.11 ± 0.06	±0	2.12 ± 0.05	±0	2.10 ± 0.07	-1
	rel.[%]	0.51 ± 0.03	-	0.53 ± 0.03	+4	0.52 ± 0.03	+2	**0.55* ± 0.03**	**+8**	**0.59** ± 0.04**	**+14**
Heart	abs.[g]	1.085 ± 0.082	-	1.095 ± 0.100	+1	1.037 ± 0.065	-4	1.042 ± 0.052	-4	**0.969** ± 0.077**	**-11**
	rel.[%]	0.259 ± 0.02	-	0.275 ± 0.018	+6	0.255 ± 0.012	-2	0.270 ± 0.012	+4	0.270 ± 0.018	+4
Liver	abs.[g]	9.61 ± 1.02	-	**11.07* ± 0.84**	**+15**	9.46 ± 0.86	-2	8.92 ± 0.64	-7	10.15 ± 1.79	+6
	rel.[%]	2.28 ± 0.12	-	**2.78** ± 0.12**	**+22**	2.32 ± 0.19	+2	2.31 ± 0.12	+1	**2.81** ± 0.33**	**+23**
Kidneys	abs.[g]	2.35 ± 0.23	-	**2.64* ± 0.30**	**+12**	2.30 ± 0.16	-2	2.47 ± 0.14	+5	2.37 ± 0.23	+1
	rel.[%]	0.56 ± 0.04	-	**0.66** ± 0.05**	**+18**	0.56 ± 0.04	±0	**0.64** ± 0.06**	**+14**	**0.66** ± 0.04**	**+18**
Adrenals	abs.[g]	0.049 ± 0.006	-	0.051 ± 0.10	+4	0.053 ± 0.011	+6	0.056 ± 0.006	+14	0.051 ± 0.008	+4
	rel.[%]	0.012 ± 0.001	-	0.013 ± 0.002	+8	0.013 ± 0.002	+8	**0.014** ± 0.02**	**+17**	**0.014* ± 0.002**	**+17**
Testes	abs.[g]	3.62 ± 0.31	-	3.73 ± 0.25	+3	3.54 ± 0.21	-2	3.71 ± 0.24	+2	3.61 ± 0.24	+2

Test substance		Control		Imazalil		FK772		FK284		R014821	
		mean ±SD	***Δ%***	**mean ±SD**	***Δ%***	**mean ±SD**	***Δ%***	**mean ±SD**	***Δ%***	**mean ±SD**	***Δ%***
	rel.[%]	0.86 ± 0.07	-	0.94 ± 0.09	+9	0.87 ± 0.06	+1	**0.96* ± 0.07**	**+12**	**1.01** ± 0.06**	**+17**
Prostate	abs.[g]	1.014 ± 0.183	-	0.912 ± 0.141	-10	0.898 ± 0.166	-11	0.927 ± 0.094	-9	**0.721** ± 0.175**	**-29**
	rel.[%]	0.241 ± 0.037	-	0.229 ± 0.032	-5	0.220 ± 0.035	-9	0.240 ± 0.023	±0	**0.200* ± 0.045**	**-17**
Epididymides	abs.[g]	1.179 ± 0.098	-	1.210 ± 0.070	+3	1.181 ± 0.088	+2	1.205 ± 0.085	+2	1.154 ± 0.103	-2
	rel.[%]	0.281 ± 0.027	-	0.305 ± 0.027	+9	0.290 ± 0.018	+3	**0.313* ± 0.027**	**+11**	**0.322** ± 0.020**	**+15**

Test substance		Control		Imazalil		FK772		FK284		R014821	
		mean ±SD	Δ%	mean ±SD	Δ%	mean ±SD	Δ%	mean ±SD	Δ%	mean ±SD	Δ%
Females											
Brain	abs.[g]	1.91 ± 0.04	-	1.92 ± 0.09	+1	1.92 ± 0.05	+1	1.90 ± 0.08	-1	1.89 ± 0.07	-1
	rel.[%]	0.86 ± 0.06	-	**0.97** ± 0.08**	**+15**	0.84 ± 0.06	-2	**1.00** ± 0.08**	**+16**	**0.98** ± 0.07**	**+14**
Pituitary	abs.[g]	0.0133 ± 0.016	-	0.0118 ± 0.0023	-11	0.0140 ± 0.0024	+5	**0.0104** ± 0.0019**	**-22**	**0.0089** ±0.0017**	**-33**
	rel.[%]	0.0059 ± 0.0007	-	0.0060 ± 0.014	+2	0.0061 ± 0.012	+3	0.0054 ± 0.0008	-8	**0.0046* ± 0.0006**	**-22**
Heart	abs.[g]	0.683 ± 0.048	-	0.642 ± 0.077	-6	0.708 ± 0.064	+4	**0.595** ± 0.056**	**-13**	0.613 ± 0.061	-10
	rel.[%]	0.306 ± 0.019	-	0.323 ± 0.023	+6	0.310 ± 0.018	+1	0.312 ± 0.017	+2	0.317 ± 0.025	+4
Liver	abs.[g]	5.15 ± 0.55	-	5.62 ± 0.65	+9	5.73 ± 0.35	+11	5.29 ± 0.66	+3	5.06 ± 0.73	-2
	rel.[%]	2.30 ± 0.14	-	**2.82** ± 0.20**	**+23**	**2.51* ± 0.10**	**+9**	**2.77** ± 0.20**	**+20**	**2.61** ± 0.23**	**+13**
Thymus	abs.[g]	0.291 ± 0.050	-	0.271 ± 0.054	-7	0.292 ± 0.059	±0	**0.225* ± 0.049**	**-23**	0.260 ± 0.034	-10
	rel.[%]	0.130 ± 0.019	-	0.136 ± 0.026	+5	0.127 ± 0.20	-2	0.117 ± 0.021	-10	0.135 ± 0.019	+4
Kidneys	abs.[g]	1.46 ± 0.08	-	1.49 ± 0.14	+2	1.55 ± 0.17	+6	1.33 ± 0.10	-9	1.43 ± 0.14	-2
	rel.[%]	0.66 ± 0.04	-	**0.75** ± 0.07**	**+14**	0.68 ± 0.03	+3	0.70 ± 0.04	+6	**0.74** ± 0.06**	**+12**

Test substance		Control		Imazalil		FK772		FK284		R014821	
		mean ±SD	***Δ%***	**mean ±SD**	***Δ%***	**mean ±SD**	***Δ%***	**mean ±SD**	***Δ%***	**mean ±SD**	***Δ%***
Adrenals	abs.[g]	0.061 ± 0.01	-	0.054 ± 0.009	-11	0.066 ± 0.012	+10	0.064 ± 0.012	+8	0.053 ± 0.08	-13
	rel.[%]	0.028 ± 0.004	-	0.027 ± 0.003	-4	0.029 ± 0.04	+4	**0.034** ± 0.006**	**+21**	0.027 ± 0.003	-4
Spleen	abs.[g]	0.397 ± 0.088	-	0.382 ± 0.060	-4	0.431 ± 0.041	+9	0.383 ± 0.052	-4	0.383 ± 0.052	-4
	rel.[%]	0.177 ± 0.025	-	0.193 ± 0.029	+11	0.189 ± 0.016	+7	**0.213** ± 0.012**	**+20**	0.197 ± 0.014	+11
Ovaries	abs.[g]	0.122 ± 0.019	-	0.139 ± 0.033	+14	0.139 ± 0.021	+14	0.131 ± 0.022	+7	0.131 ± 0.022	+7
	rel.[%]	0.055 ± 0.008	-	**0.070* ± 0.014**	**+27**	0.061 ± 0.010		**0.072** ± 0.012**	**+31**	**0.068* ± 0.012**	**+24**

*p < 0.05, ** p < 0.01 (Dunnett's test, based on pooled variance)

Gross pathology
Animals of both sexes treated with FK284 showed discolouration of the adrenal gland, which was considered treatment-related and adverse. No macroscopic findings were observed in any of the other treatment groups.

Histopathology
The following treatment-related histopathological findings were observed.

Hepatocellular vacuolation (minimal to moderate in males, minimal in females) was observed in the liver of animals of both sexes treated with imazalil. Males dosed with R014821 also showed minimal to moderate hepatocellular vacuolation, which was accompanied by minimal to slight centrilobular hepatocellular hypertrophy in most instances. All of the histopathological findings observed in the liver were considered treatment-related and adverse.

Micro- and macrovesicular vacuolation was observed in the inner zone of the zona fasciculata of the adrenals in the majority of females treated with FK284 and in some animals treated with imazalil or R014821 (2/10 and 4/10 males treated with imazalil and R014821 respectively, 4/10 females treated with imazalil). Multifocal vacuolation was also observed in animals of both sexes treated with imazalil and in males treated with FK284. All of these findings were considered treatment-related and adverse.

Increased apoptosis of lymphocytes (minimal to slight) was observed in the thymus of 7/10 and 5/10 females treated with FK772 or FK284. As no statistically or biologically significant reductions in white blood cell counts or clinical effects were observed, these findings were not considered adverse.

Hypertrophy/vacuolation of interstitial cells (minimal to moderate) was observed in the ovary of all female rats treated with FK284. These findings were considered treatment-related and adverse.

Males treated with R014821 showed a slight increase in the incidence and severity of diffuse follicular cell hypertrophy in the thyroid (5/10 animals, minimal to slight). No other animals were affected. Thyroid hormone measurements showed that circulating T4 was unaffected; however, levels of T3 and TSH were elevated (+66 % and +122 %) compared to controls. Overall, adverse histopathological findings were seen in the thyroid of males treated with R014821.

All other histopathological findings were either observed in single animals, or were within the expected incidence range for animals of this age and strain.

Table C2.8-8. Selected histopathological findings

Sex		Males					Females				
Test substance		CTL	IZA	FK 772	FK 284	R014 821	CTL	IZA	FK 772	FK 284	R014 821
Dose (ppm)				1600					1600		
Dose (mg/kg bw/day)		-	100	101	104	101	-	106	113	109	110
		Histopathology grading§									
Liver	[N]	10	10	10	10	10	10	10	10	10	10
- Multifocal/ midzonal hepatocellular vacuolation diffuse	[n]	0	**8**	0	1	**9**	0	**4**	0	0	1
	Minimal	-	**5**	-	1	**3**	-	**4**	-	-	1
	Slight	-	**3**	-	-	**2**	-	-	-	-	-
	Moderate	-	**1**	-	-	**4**	-	-	-	-	-
- Centrilobular, hepatocellular hypertrophy	[n]	0	0	0	0	**8**	0	0	0	0	0
	Minimal	-	-	-	-	**7**	-	-	-	-	-
	Slight	-	-	-	-	**1**	-	-	-	-	-
Adrenals	[N]	10	10	10	10	10	10	10	10	10	10
- Multifocal vacuolation zona fasciculata	[n]	3	**4**	3	**5**	**3**	0	**4**	0	0	1
	Minimal	3	**2**	3	**4**	3	-	**4**	-	-	1
	Slight	-	**2**	-	**1**	**-**	-	-	-	-	-
- Micro/ macrovesicular vacuolation of inner zone, zona fasciculata	[n]	0	**2**	0	0	**4**	0	0	0	**9**	0
	Minimal	-	**1**	-	-	**2**	-	-	-	**3**	-
	Slight	-	**1**	-	-	**2**	-	-	-	**4**	-
	Moderate	-	-	-	-	-	-	-	-	**2**	-
Ovaries	[N]						10	10	10	10	10
- Hypertrophy/ vacuolation, interstitial cells	[n]						0	0	0	**10**	0
	Minimal						-	-	-	**4**	-
	Slight						-	-	-	**4**	-
	Moderate						-	-	-	**2**	**-**
Thymus	[N]	10	10	10	10	10	10	10	10	10	10
- Increased apoptosis of lymphocytes	[n]	-	-	-	-	1	0	1	**7**	**5**	1
	Minimal	-	-	-	-	1	-	1	**6**	**3**	1
	Slight	-	-	-	-	-	-	-	**-**	**2**	-
Thyroid	[N]	10	10	10	10	10	10	10	10	10	10
- Diffuse follicular cell hypertrophy	[n]	2	3	2	2	**5**	0	0	1	0	1
	Minimal	2	2	2	2	**3**	-	-	1	-	1
	Slight	0	1	-	-	**2**	-	-	-	-	-

CTL = control, IZA = imazalil, N = number of animals examined, n = number of animals affected, § = Severity grading; histopathological findings were graded minimal, slight, moderate, marked, and severe, values in **bold text** were considered treatment-related

Overall, treatment-related and adverse histopathological findings were observed with imazalil (liver and adrenals), R014821 (liver, adrenals and thyroid) and FK284 (adrenals and ovaries).

Summary

Under the conditions of this OECD and GLP-compliant 90-day comparative study in rats, dietary administration of imazalil or metabolites FK772, FK284, and R014821 at the single dose of 1600 ppm induced no mortalities and no treatment-related clinical signs of toxicity.

Treatment-related adverse reductions in body weight, body weight gain and food consumption were observed with imazalil, R014821 and FK284. No body weight and food consumption effects were seen with FK772. Adverse changes in clinical chemistry parameters indicative of potential impaired kidney function were observed with imazalil and the 3 metabolites. Treatment-related and adverse increases in T3 and TSH were seen with FK772 and R014821 (mainly in males). Organ weights were adversely affected with imazalil (liver and kidney), R014821 (liver, kidney and adrenals) and FK284 (kidney and adrenals). Histopathological findings were observed with imazalil (liver and adrenals), R014821 (liver, adrenals and thyroid) and FK284 (adrenals and ovaries).

As only one dose level was investigated in this comparative toxicity study, a NOAEL cannot be set for imazalil or metabolites FK772, FK284 and R014821 based on the data from this study. Previous repeat dose studies conducted with imazalil in the rat resulted in a NOAEL of 4 and 5 mg/kg bw/d after 90-days and 6-months of treatment respectively.

Comparison of the observed treatment-related findings in the different treatment groups showed that rats which were treated with imazalil or its metabolites FK772, FK284, and R014821 showed similar effects on similar target organs. However, some treatment-related and adverse effects on organ weight and/or histopathology were observed in one or both sexes in selected metabolite treatment groups that did not occur in imazalil-treated rats. These were: thyroid histopathology (with associated increases in T3 and TSH) in R014821 males and ovarian histopathology in FK284 females. In addition, liver histopathological findings in males occurred at a higher incidence and severity in R014821-treated rats than in imazalil treated rats.

In addition, more pronounced effects on body weight and body weight gain were observed in males and females treated with R014821 and in females dosed with FK284 than in rats treated with imazalil.

Conclusion

Overall, taking all the findings together, metabolite FK772 showed a slightly lower toxicity compared to the parent imazalil, while FK284 showed comparable toxicity to the parent. Metabolite R014821 showed a slightly higher toxicity compared to the parent imazalil.

As the comparative toxicity study did not allow the derivation of NOAELs, an additional 90-day dietary study in the rat was conducted with R014821 in order to derive reference values for this relatively more toxic metabolite.

Metabolite R014821

It should be noted that an Ames test, a chromosome aberration study, a mammalian cell gene mutation assay and an acute oral toxicity test on the metabolite were already considered previously (see Table below). The oral LD50 of R014821 in rats was determined to exceed 2000 mg/kg bw. The Ames and the chromosome aberration tests were negative, but the mammalian cell gene mutation assay produced an equivocal result without S9. To address the equivocal result, an in vivo Comet assay was provided. In addition, an in vitro micronucleus study was submitted to investigate aneugenicity.

Table C2.8-9. Previous studies on R014821

Study Type	Species / test system	Result	Reference
Acute oral LD_{50}	Rat	> 2000 mg/kg bw	report no. 25876/1 (2011)
In vitro Ames	*S. typhimurium* 3.16 to 1000 µg/plate	± S9: negative	[redacted] (2010)
In vitro chromosome aberration	Chinese hamster V79 lung cells	± S9: negative	[redacted] (2017a)
Mammalian cell gene mutation	CHO K1 Chinese hamster ovary cells, *hprt* locus	-S9: equivocal +S9: negative	[redacted] (2017b)

In addition to the 90-day comparative toxicity study, four new studies on metabolite R014821 were provided with this MRL confirmatory data application. These included two additional genotoxicity studies (in vitro micronucleus study and in vivo Comet assay) that were submitted in order to fully exclude the genotoxic potential of the metabolite, a 90-day dietary study in the rat, and a combined 28-day repeated dose toxicity study with reproduction/ developmental toxicity screening (OECD 422) in the rat. The repeated dose studies were conducted in order to derive reference values for this metabolite, as the comparative toxicity study was not suitable for this purpose. This additional information has been fully evaluated by HSE below.

New information submitted with this application

An in vitro Micronucleus Assay with R014821 in Cultured Peripheral Human Lymphocytes ([redacted] 2019)

Reference: An in vitro Micronucleus Assay with R014821 in Cultured Peripheral Human Lymphocytes, [redacted] (2019)
Report No. AGR 5957
study no. 20192868

Date performed:	Completion date: 9th Sep 2019
Test facility:	[redacted]
Guideline(s):	Yes, OECD 487 (2016)
Deviations:	No deviations that affected the reliability of the study
GLP:	Yes
Test material:	R014821
	Batch: 181101
	Purity: 99.9%
	Vehicle: DMSO
Study acceptable:	Yes

Method

The potential for R014821 to induce micronuclei in human lymphocytes was investigated *in vitro* in a GLP and OECD TG compliant study. A preliminary assay ('pre-test') was performed to determine the test substance concentrations for the main study.

In the main study, cells were incubated with R014821 for 3 hours in the presence and absence of metabolic activation (S9 mix) at concentrations of 50, 100, 200, 300, 400, 500, 600 and 700 µg/ml. No appropriate concentration levels could be selected for scoring of micronuclei in this experiment because not enough cytotoxicity was observed at a concentration of 500 µg/ml (37% and 47% in the absence and presence of S9-mix respectively), whereas the next higher concentration of 600 µg/ml was too toxic for scoring (70% and 63% in the absence and presence of S9 mix respectively). The experiment was repeated in Experiment IA at the following concentrations: 100, 400, 450, 500, 525, 550, 575, 600 and 650 µg/ml. In Experiment II, cells were incubated continuously for 24 hours in the absence of metabolic activation at concentrations of 10, 20, 30, 40, 50, 60 and 70 µg/ml. In each experiment, concurrent solvent (DMSO) and positive controls (mitomycin C (MMC) and colchicine (Colch) without metabolic activation, cyclophosphamide (CP) with metabolic activation) were included. In each experimental group, two parallel cultures were analysed. At least 1000 binucleate cells per culture (2000 cells per test group) were scored and recorded as percent micronucleated cells. To investigate cytotoxicity, the cytokinesis-block proliferation index (CBPI) was determined in at least 500 cells per culture. CBPI was then used to determine percent cytostasis and assess cytotoxicity, with a reduction in CBPI to 45 ± 5 % of the concurrent negative control considered an indication of cytotoxicity.

Results
All relevant experimental conditions were tested and an adequate number of cells and concentrations were analysed in accordance with the acceptability criteria of the OECD guideline.

Cytotoxicity and solubility
Cytotoxicity was observed in the pre-test without S9 mix from a concentration of 500 µg/ml and 63 µg/ml following an exposure period of 3 and 24 hours respectively. In Experiment IA, cytotoxicity was observed from a concentration of 525 µg/ml and 550 µg/ml with and without S9 mix respectively. In Experiment II, cytotoxicity was observed from a concentration of 50 µg/ml.

Precipitation was observed in the pre-test following 3 hours exposure in the presence and absence of S9 mix at 1000 µg/ml and following 24 hours exposure in the absence of S9 mix at 2000 µg/ml. However, no precipitation was observed in the main study at up to the highest tested concentrations (650 and 70 µg/ml in Experiments I and II respectively).

Micronucleus assay
No statistically significant or biologically relevant increases in micronucleated cells were observed in Experiments IA and II when compared to the solvent control at any concentration level, with or without metabolic activation.

The concurrent negative controls produced no increase in the number of micronucleated cells, with results within the 95% control limits of the negative HCD. The concurrent positive controls (mitomycin C, CP and colchicine) induced clear increases in the number of micronucleated cells, which were also within the 95% control limits of the positive HCD. The criteria in the TG for cell proliferation were fulfilled and all relevant experimental conditions were tested. An adequate number of cells and concentrations were analysed and testing was performed up to the cytotoxicity limit (CBPI of 45± 5% of the concurrent negative control). Therefore, as all the acceptability criteria have been met, the test is considered to be acceptable.

Table C2.8-10. Summary of results of the micronucleus assay with R014821
Exposure period 3 hrs without S9 mix

Exp.	Test item concentration (µg/ml)	Proliferation index (CBPI)	Cytostasis in % [a]	Micronucleated cells [b]	HCD (%) (mean ± SD) [95% control limit]
IA	Solvent control[1]	1.60	0	5	3.46 ± 2.31 [-1.57 – 8.50]
	0.25 MMC	1.38	36	**18****	24.93 ± 9.85 [4.58 – 45.27]
	0.38 MMC	1.26	56	**30*****	
	0.1 Colchicine	1.09	85	**43*****	
	100	1.59	1	5	
	400	1.46	24	n.s..	
	450	1.42	30	4	
	500	1.38	37	n.s.	
	525	1.34	43	n.s.	
	550	1.28	54	5	
	575	1.24	59	n.s.	
	600	1.23	62	n.s.	
	650	1.09	86	n.s.	

Exposure period 3hrs with S9 mix

Exp.	Test item concentration (µg/ml)	Proliferation index (CBPI)	Cytostasis in % [a]	Micronucleated cells [b]	HCD (%) (mean ± SD) [95% control limit]
IA	Solvent control[1]	1.65	0	5	3.46 ± 2.30 [-1.69 – 8.62]
	15 CP	1.21	68	**24*****	18.48 ± 8.68 [2.07 – 34.88]
	100	1.56	14	4	
	400	1.50	23	n.s..	
	450	1.42	36	5	
	500	1.36	44	n.s.	
	525	1.34	47	n.s.	
	550	1.30	54	7	
	575	1.28	57	n.s.	
	600	1.22	66	n.s.	

Exp.	Test item concentration (µg/ml)	Proliferation index (CBPI)	Cytostasis in % [a]	Micronucleated cells [b]	HCD (%) (mean ± SD) [95% control limit]
	650	1.14	79	n.s.	

Exposure period 24 hrs without S9 mix

Exp.	Test item concentration (µg/ml)	Proliferation index (CBPI)	Cytostasis in % [a]	Micronucleated cells [b]	HCD (%) (mean ± SD) [95% control limit]
II	Solvent control[1]	1.66	0	6	3.35 ± 2.48 [-2.53 – 9.23]
	0.15 MMC	1.38	43	55***	22.77 ± 11.94 [-2.06 – 47.60]
	0.23 MMC	1.26	61	n.s.	
	0.05 Colchicine	1.02	16.7	4	
	10	1.55	17	6	
	20	1.51	22	n.s.	
	30	1.44	33	n.s.	
	40	1.40	40	n.s.	
	50	1.36	46	8	
	60	1.27	60	6	
	70	1.20	69	n.s.	

CBPI = Cytokinesis-block proliferation index; HCD = Historical control data based on experiments performed between 2015 – 2018; [a] Values are relative to the solvent controls for the positive control groups and test item treatment groups, [b] Number of micronucleated cells was determined in a sample of 2000 binucleated cells; [c] Number of cells with micronuclei per 1000 cells; * Significantly different from the corresponding solvent control (*P <0.05, **P <0.01, ***P <0.001); n.s. not scored; [1] DMSO 1.0% (v/v); MMC = Mitomycin C; CP = Cyclophosphamide

Conclusion

In conclusion, in this GLP and OECD test guideline compliant study, the test item did not induce micronuclei in human lymphocytes under any experimental conditions. Overall, R014821 showed no clastogenic or aneugenic potential under the conditions of this study either in the presence or absence of metabolic activation, up to the limit of cytotoxicity.

The Alkaline in vivo Comet Assay with R014821 in Cultured Liver, Duodenum and Stomach of Wistar Han rats (2020a)

Reference:	The Alkaline in vivo Comet Assay with R014821 in Liver, Duodenum and Stomach of Wistar Han Rats; (2020a) Report No. AGR 5964 study no. 20202368
Date performed:	Completion date: 19th Nov 2019
Test facility:	
Guideline(s):	Yes; OECD 489 (2016)
Deviations:	No deviations that affected the reliability of the study
GLP:	Yes
Test material:	R014821 Batch: 181101 Purity: 99.9% Vehicle: Corn oil
Study acceptable:	Yes

Method

The potential for R014821 to cause DNA damage (strand breaks) in the liver, stomach and duodenum of Wistar Han rats was investigated in a GLP and OECD TG compliant Comet assay. The homogeneity/ stability of the test materials and dose accuracy was confirmed using a validated analytical method.

A dose range finding assay was conducted prior to the main study. Two groups comprising of 1 male and 1 female were dosed by oral gavage with 1000 mg/kg bw and 2000 mg/kg bw R014821. One additional dose group, comprising of 3 males and females, was dosed for two consecutive days (once daily) with 1500 mg/kg bw. The observation period after dosing was one to three days, during which mortality and physical condition were recorded at least once a day. Since there were no substantial differences in toxicity between sexes based on the results of the dose-range finding test, the main study was conducted in one sex. Bioanalysis was conducted in animals dosed with the vehicle and at 1500 mg/kg bw at 1, 2, 4 and 6 h after administration of the first dose in order to confirm exposure to R014821.

In the main study, male Wistar Han rats (5 animals/group, with an additional 3 animals in the top dose group) were treated with metabolite R014821 orally by gavage at dose levels

of 375, 750 and 1500 mg/kg bw on two subsequent days. The dose range selected was based on the preliminary test, where the maximum tolerated dose (MTD) was considered to be 1500 mg/kg bw in males and females (see Results). The second dose was administered 21 hours after the first dose and sampling occurred 3 hours after the second dose. Bioanalysis was conducted in animals dosed with the vehicle and at the MTD. As the initial assay did not fulfil the vehicle criteria for stomach and duodenum, the experiment was repeated for these tissue types.

The negative control group received the vehicle (corn oil) and the positive control group received 200 mg/kg bw ethyl methanesulphonate (EMS) dissolved in physiological saline. The liver was selected as a target organ because it is the primary site of metabolism and is often highly exposed to the parent material and metabolite(s). As the test item was administered orally (by gavage) the stomach and duodenum are a site of direct contact; therefore they were also selected for sampling.

Following electrophoresis three slides per tissue per animal were analysed for comets. The slides were visualised by staining with SYBR GOLD® and examined by fluorescence microscopy. One hundred and fifty cells were analysed for the presence of comets per animal per tissue. DNA strand breaks were assessed by comparing the mean and median % tail intensity (% TI) values from the treated animals with the vehicle control values. The frequency of excessively damaged (hedgehog) cells was determined and documented based on the visual scoring of at least 150 cells per tissue per animal in the repeat experiment. No hedgehog cells were detected during scoring in the first or repeated assay.

Results

Preliminary toxicity test

Both animals dosed at 2000 mg/kg bw R014821 died or were euthanised for humane reasons during the post-dosing observation period following administration of the first dose. At a dose of 1000 mg/kg bw, mild clinical signs of toxicity (ataxia, hunched posture, rough coat) were observed in both animals. Male and female rats dosed with 1500 mg/kg bw R014821 showed several clinical signs of toxicity, which included ataxia, lethargy, hunched posture, blood crust around nose and/or eye, rough coat, pale skin, slow breathing, and diarrhoea. Based on the clinical observations, 1500 mg/kg bw was identified as the maximum tolerated dose (MTD) in both sexes and this dose was selected as the top dose for the main test.

Bioanalysis and Toxicokinetic Evaluation

R014821 was detected in the plasma of all animals dosed with the test substance, while all animals that were dosed with the vehicle (except one) showed a plasma level of R014821 that was below the lower limit of quantification (LLOQ). As only one animal in the vehicle control group showed R014821 in the plasma at a single timepoint, this finding was not considered relevant. Therefore, it can be demonstrated that R014821 was systemically bioavailable following oral administration by gavage.

Main assay

No mortality was observed in any treatment groups. Clinical signs of toxicity (ataxia, lethargy) were reported at the top dose of 1500 mg/kg bw R014821, but not in the low or medium dose groups or control animals.

No statistically significant increases in the mean Tail Intensity (% TI) were observed in liver, duodenum and stomach cells of male rats treated with R014821 compared to concurrent vehicle control values. In the liver, the mean % TI values of liver cells from all treated groups were also within the 95% CL of the negative HCD. In the stomach and the duodenum, the mean % TI values were slightly above the 95% CL of the negative HCD; however, it was noted that the concurrent vehicle control groups also had %TI values that were above the 95% CL of the negative HCD.

The positive control produced a statistically significant ($p<0.001$) increase in the mean % TI when compared to vehicle control values in all of the analysed tissue types and all % TI values were comparable to the positive HCD range.

As not all of the acceptability criteria was met for all tissue types, the assay was repeated in stomach and duodenum tissues.

Table C2.8-11. Summary of results from the Comet assay with R014821

Test item dose (mg/kg bw)	Liver	Glandular Stomach	Duodenum
Negative control [1]	3.09 ± 0.51	6.12 ± 0.87	8.18 ± 1.36
HCD negative control [95% confidence limits]	1.96 ± 0.92 [0.27 – 3.65]	2.45 ± 1.39 [-1.07 – 5.96]	3.06 ± 1.52 [-0.86 – 6.97]
375	3.49 ± 0.86	6.36 ± 1.47	14.91 ± 6.62
750	2.93 ± 0.83	5.28 ± 1.01	11.86 ± 8.70
1500	2.81 ± 1.08	5.80 ± 2.47	11.34 ± 6.60
Positive control [2]	94.07*** ± 1.37	71.75*** ± 2.48	68.79*** ± 5.96
HCD positive control [95% confidence limits]	89.53 ± 6.89 [79.70 – 99.36]	55.16 ± 14.23 [34.74 – 78.58]	41.17 ± 14.03 [20.78 – 61.56]

[1] Corn oil; [2]200 mg/kg EMS; HCD on experiments performed from Jan 2018 – July 2019; *** P<0.001 compared to vehicle control (Student t-test)

Repeat assay

No mortality was observed in any treatment groups and no clinical signs were seen at the low dose of 375 mg/kg bw/d or in the control groups. Mild clinical signs were reported in the mid dose group following the second dose (ataxia, lethargy) and more significant clinical indications of toxicity (ataxia, lethargy, hunched posture, rough coat, ventral

recumbency) were observed at the top dose of 1500 mg/kg bw/d R014821 on the second day of the experiment.

No statistically significant increases in the mean Tail Intensity (% TI) were observed in duodenum cells of male rats treated with R014821 compared to concurrent vehicle control values and the mean %TI values were also within the 95% CL of the negative HCD for all treatment groups. In the stomach, the mean % TI of the low and high dose group were comparable to control animals, but the mid dose group showed a statistically significant increase in % TI compared to concurrent controls, which was also above the 95% CL of the HCD. However, no dose-response relationship was observed and the % TI value in the mid dose group was far lower than both the concurrent positive control and the lower 95% CL of the positive HCD. Therefore, this finding was not considered treatment-related. In addition, the % TI value in the stomach cells of the mid dose group was far lower than that obtained for the concurrent positive control group and below the 95% CL of the positive HCD.

The positive control produced a statistically significant ($p<0.001$) increase in the mean % TI when compared to vehicle control values in all of the analysed tissue types and all % TI values were comparable to the positive HCD range.

Although not all of the acceptability criteria was met for a clearly negative result in the stomach tissues following the repeat assay, the mean % TI value of the mid dose group was far lower than the concurrent positive control animals and was also below the lower 95% CL of the positive HCD. When this is taken into consideration, along with the absence of a dose-response relationship in both the main and the repeat assay, it can be concluded that based on the weight of evidence, metabolite R014821 did not induce DNA strand breakage in any of the tissues studied in this test system.

Table C2.8-12. Summary of results from the repeated Comet assay with R014821

Test item dose (mg/kg bw)	Glandular Stomach	Duodenum
Negative control [1]	8.05 ± 2.36	5.36 ± 1.69
HCD negative control [95% control limits]	2.45 ± 1.39 [-1.07 – 5.96]	3.06 ± 1.52 [-0.86 – 6.97]
375	9.17 ± 4.14	4.71 ± 1.20
750	13.58* ± 5.34	4.53 ± 1.00
1500	6.69 ± 1.29	4.87 ± 0.45
Positive control [2]	58.93*** ± 3.64	52.37*** ± 9.17
HCD positive control [95% control limits]	55.16 ± 14.23 [34.74 – 78.58]	41.17 ± 14.03 [20.78 – 61.56]

Corn oil; [2]200 mg/kg EMS; HCD on experiments performed from Jan 2018 – July 2019, * P< 0.05 compared to vehicle control and test item groups (one-sided Dunnett's test); *** P<0.001 compared to vehicle control (one-sided Student's t-test)

Conclusion

Under the experimental conditions reported in this GLP and OECD test guideline compliant study, metabolite R014821 did not induce DNA strand breaks in the liver, glandular stomach and duodenum of the rat following administration by oral gavage up to the maximum tolerated dose, at which exposure was demonstrated. Therefore, it is concluded that R014821 does not show genotoxic potential in rat in the Comet assay.

Combined 28-Day Repeated Dose Toxicity Study with the Reproduction/ Developmental Toxicity Screening Test of JNJ-123955-AAA (T000824) in Rats by Oral Gavage (2018)

Reference:	Combined 28-Day Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test of JNJ-123955-AAA (T000824) in Rats by Oral Gavage, 2018 study no. 517992
Date performed:	Completion date: 7th Nov 2017
Test facility:	
Guideline(s):	OECD 422 (2016)
Deviations:	Blood sampling was conducted at different timepoints for top dose animals (immediately after the end of pregnancy, after dosing for 41-47 days) and control animals (after at least 2 weeks of lactation, after dosing for 50-62 days). This deviation invalidated part of the study as haematology and clinical chemistry results could not be compared between top dose animals and controls.
GLP:	Yes
Test item/dose: groups	0, 30, 100 and 330 mg/kg bw/d R014821 in propylene glycol vehicle Batch: M15KB5305 Purity: 97.5%

Validated method of analysis:	Validated method (Test Facility Study No. 517993).
Study acceptable:	Yes

Method

In this GLP and OECD TG-compliant study, metabolite R014821 was administered via oral gavage to groups of male and female Wistar Han rats (10/sex/dose) at dose levels of 0, 30, 100 and 330 mg/kg bw/d. The dose levels in this study were selected based on results from a dose-range finding study conducted in the same test facility (Test facility study no. 517995). R014821 was dissolved in a propylene glycol vehicle. The stability of the test substance in the vehicle had been demonstrated (Test Facility Study No. 517993).

Males were treated for 29 days (2 weeks prior to mating, during mating and up to termination). Dosing of females was carried out for 41-47 days, 2 weeks prior to mating, during mating and pregnancy (if no healthy offspring delivered) or for 50-62 days, 2 weeks prior to mating, during mating, during pregnancy and at least 2 weeks during lactation (if offspring delivered). Male animals were sacrificed after the end of the mating period. Females were sacrificed either soon after the end of failed pregnancy (if no offspring delivered) or after the lactation period (if offspring delivered). Blood samples were collected on the day of termination from 5 animals/sex/group. Blood samples were taken from 2 pups/litter (one male and one female, if possible) during planned necropsy on PND 14-16 and analysed to measure serum thyroxine (T4).

Results

General toxicity

Mortality

At a dose of 330 mg/kg bw/d, there were three premature decedents that were euthanised in a moribund condition. One male and one female showed significant clinical signs of toxicity in the early stages of the study (hunched posture, piloerection of the fur, rales, ptosis, and rapid body weight loss of 10-12% between days 1-8). Another top dose female was euthanized at study day 38 (day 19 post-coitum) after showing red discoloration in the inguinal region, increased respiratory rate, pale appearance, hunched posture, lethargy, piloerection and body weight loss (nearly 10% between days 17-19 post-coitum). Post-mortem examination showed two early resorptions and one dead foetus in the left uterus horn and slight inflammation of the endometrium of the uterus, which were considered related to the moribundity. These deaths were considered treatment-related.

At a dose of 100 mg/kg bw/d, one female showed laboured respiration, rales, ptosis, excessive salivation and felt cold to the touch after dosing, therefore was sacrificed for humane reasons. Microscopic examination revealed lesions indicative of a dosing error in the form of moderate to marked necrosis of the bronchus and tracheal epithelium, therefore the moribundity of this animal was not considered test-item related.

Overall, treatment-related mortality occurred at the top dose.

Clinical observations

In addition to the clinical observations in premature decedents described in the above section, clinical signs (piloerection, hunched posture, and rales) were observed in all treatment groups during the study. Piloerection showed a dose-response relationship, with the highest incidence and duration observed in top dose females. Several mid dose females had piloerection for 1-2 weeks, while it was present in a few low dose females only at specific timepoints. Therefore, piloerection was not considered adverse at the low dose. In contrast, there was no dose-response observed for rales and it was suggested that it was associated with the dosing method. Yellow discoloration of the faeces was also observed in all top dose animals during the second and third weeks of the study. As this finding was transitory and did not coincide with other clinical indications of malabsorption (e.g. weight loss), it was considered treatment-related, but not adverse.

Overall, treatment-related clinical signs of toxicity (piloerection) were observed in animals from the mid dose of 100 mg/kg bw/d.

Table C2.8-13. Selected clinical observations with R014821 treatment

Sex	Males				Females			
Dose (mg/kg bw/d)	0	30	100	330	0	30	100	330
Premating period								
Week 1								
Animals examined (N)	10	10	10	10	10	10	10	10
Piloerection	0	0	0	2	0	0	0	6
Hunched posture	0	0	0	0	0	0	0	7
Rales	0	0	0	2	0	0	0	4
Faeces discoloration	0	0	0	**0**	0	0	0	0
Week 2								
Animals examined (N)	10	10	10	10	10	10	10	10
Piloerection	0	0	1	7	0	0	1	10
Hunched posture	0	0	0	1	0	0	0	6
Rales	0	0	0	3	0	1	1	3
Faeces discoloration	0	0	0	10	0	0	0	10
Reproduction period								
Week 1								
Animals examined (N)	10	10	10	9	10	10	10	9
Piloerection	0	0	1	2	0	0	1	9
Hunched posture	0	0	0	0	0	0	0	6

Sex	Males				Females			
Rales	0	0	1	1	0	3	3	3
Faeces discoloration	0	0	0	9	0	0	0	9
Week 2								
Animals examined (N)	10	10	10	9	10	10	9	9
Piloerection	0	1	4	4	0	0	6	9
Hunched posture	0	0	0	0	0	0	0	0
Rales	0	1	0	0	0	0	0	2
Faeces discoloration	0	0	0	0	0	0	0	0
Week 3								
Animals examined (N)	0	0	0	0	10	10	9	9
Piloerection					0	4	8	7
Hunched posture					0	0	0	0
Rales					0	0	0	0
Week 4								
Animals examined (N)					10	10	9	9
Piloerection					0	1	2	7
Hunched posture					0	0	0	4
Rales					0	1	0	0
Week 5								
Animals examined (N)					10	10	7	2
Piloerection					0	1	1	2
Hunched posture					0	0	0	0
Rales					0	0	0	0
Week 6								
Animals examined (N)					10	10	6	0
Piloerection					0	1	1	
Hunched posture					0	0	0	
Rales					0	2	0	

Functional observations

The mean hind limb grip strength values of treated males were statistically significantly lower compared to concurrent controls. However, as no dose-response relationship was observed, this finding was not considered treatment-related. Females in the mid dose group showed higher mean values for total movement compared to concurrent controls, but due to an absence of a dose-response relationship, this finding was not regarded as treatment-related. All other parameters did not show any differences between treated animals and controls.

Overall, no treatment-related effects on functional observations were observed up to the top dose of 330 mg/kg bw/d.

Body weight and body weight gain

Males and females at the top dose showed statistically significantly reduced body weight gain during the first week of treatment, which was reversed until the end of treatment in males and until day 7 post-coitum in females. Thereafter, top dose females showed reduced body weight gain compared to control animals, reaching a maximum of -22% at day 20 post-coitum. The reduction in body weight gain during gestation in top dose females was due to abnormal pregnancies (3 non-pregnant females and 6 with implantations only) and was therefore considered not a direct effect of treatment.

Overall, adverse effects on body weight and body weight gain were observed in top dose males and females during the pre-mating period.

Table C2.8-14. Body weight with R014821 treatment

Sex		**Males**				**Females**			
Dose (mg/kg bw/d)		0	30	100	330	0	30	100	330
		Body weight (g)							
Pre-mating									
Day 1	Mean	291	288	286	287	221	226	226	218
	SD	10.7	8.4	10.6	10.7	7.5	6.0	10.3	11.7
	Δ%	-	-1.0	-1.7	-1.3	-	+2.3	+2.3	+1.4
	N	10	10	10	10	10	10	10	10
Day 8	Mean	319	312 ±	306 ±	**291****	223	226	225	212
	SD	12.1	12.1	10.6	19.7	12.9	5.7	10.8	15.1
	Δ%	-	-2.2	-4.1	-8.8	-	+1.3	+0.9	-4.2
	N	10	10	10	10	10	10	10	10
Mating period									
Day 1	Mean	342	338	334	327	227	229	230	222
	SD	15.4	15.1	13.5	19.8	13.4	8.3	13.1	13.4
	Δ%	-	-0.1	-2.3	-4.4	-	+0.9	+1.3	-2.2
	N	10	10	10	9	10	10	10	9

Sex		Males				Females			
Day 8	Mean	354	351	349	343	252	259		236
	SD	16.3	20.5	16.4	20.8	16.2	n/a		n/a
	Δ%	-	-0.8	-1.4	-3.1	-	+2.8		-6.4
	N	10	10	10	9	2	1		1
Post-coitum									
Day 0	Mean	37	369	366	360	235	241	237	223
	SD	18.3	26.0	19.3	21.1	16.4	8.4	14.4	11.3
	Δ%	-	-0.3	-1.1	-2.4	-	+2.6	+0.9	-5.1
	N	10	10	10	9	9	9	10	6
Day 7	Mean					250	258	253	240
	SD					12.9	10.6	17.8	13.3
	Δ%					-	+3.2	+1.2	-4.0
	N					9	9	9	6
Day 11	Mean					267	274	265	**247***
	SD					13.8	12.6	19.3	11.3
	Δ%					-	+2.6	+0.7	-7.5
	N					9	9	9	6
Day 14	Mean					278	283	276	**255***
	SD					14.3	10.6	22.3	12.4
	Δ%					-	+1.8	-0.8	-8.3
	N					9	9	9	6
Day 17	Mean					300	306	297	**258****
	SD					18.5	13.7	27.3	11.4
	Δ%					-	+2.0	-1.0	-14.0
	N					9	9	9	6
Day 20	Mean					338	345	331	**264****
	SD					23.0	15.8	35.6	17.3
	Δ%					-	+2.1	-2.1	-21.9
	N					9	9	9	6
Lactation									
Day 1	Mean					262	261	258	

Sex		Males				Females			
	SD					11.1	15.0	19.5	
	Δ%					-	-0.4	+1.5	
	N					9	10	8	
Day 7	Mean					282	294	285	
	SD					12.4	17.2	16.0	
	Δ%					-	+4.3	+1.1	
	N					9	10	5	
Day 13	Mean					301	305	300	
	SD					14.5	22.2	19.0	
	Δ%					-	+1.3	-0.3	
	N					9	10	5	

Dunnett-test: * $p \leq 0.05$; ** $p \leq 0.01$

Food consumption

A sizable (-26/-27%) reduction in food consumption was observed in male and female animals at the top dose during the first week of treatment. In females, food consumption was also reduced at the top dose during the post-coitum period compared to controls. This latter finding was related to their abnormal pregnancies (i.e. 3 non-pregnant females and 6 females with implantations only) and was not a direct effect of treatment.

Food consumption was also statistically significantly reduced during the lactation period for mid dose females. Within this treatment group, two animals had very small litters and consequently lower food consumption, while the other three animals with normal litter sizes showed normal food consumption. Therefore, the reduced food consumption in mid dose females during the lactation period was not regarded as directly treatment-related, but a secondary effect due to the small litter sizes.

The higher food consumption observed in low dose females at the beginning of lactation period was considered unrelated to treatment as food consumption of these animals was similar to controls at all other time points.

Overall, R014821 treatment resulted in reduced food consumption in top dose males and females during the pre-mating period.

Table C2.8-15. Food consumption with R014821 treatment

Sex		Males				Females			
Dose (mg/ kg bw/d)		0	30	100	330	0	30	100	330
		Food consumption (g/animal/day)							
Pre-mating period									
Day 1-8	Mean	23	22	21	17	15	14	14	11
	SD	0.7	0.0	0.4	0.1	0.2	0.1	0.1	0.8
	Δ%	-	-4.3	-8.7	-26.1	-	-6.7	-6.7	-26.7
	N (cage)	2	2	2	2	2	2	2	2
Day 8-15	Mean	23	22	23	22	15	15	15	13
	SD	1.5	0.7	0.5	0.4	0.2	0.2	0.3	0.1
	Δ%	-	-4.3	0	-4.3	-	0	0	-13.3
	N (cage)	2	2	2	2	2	2	2	2
Mating period									
Day 1-8	Mean	24	24	25	21				
	SD	1.2	1.2	2.1	2.9				
	Δ%	-	0	+4.2	-12.5				
	N (cage)	2	2	2	2				
Day 8-15	Mean	23	23	24	22				
	SD	0.5	0.5	1.0	1.3				
	Δ%	-	0	+4.3	-4.3				
	N (cage)	2	2	2	2				
Post coitum									
Day 0-4	Mean					17	17	16	15
	SD					2.8	2.1	2.4	1.9

Sex		Males				Females			
	Δ%					-	0	-5.8	-11.8
	N					9	9	10	6
Day 4-7	Mean					17	16	16	14
	SD					1.5	3.2	3.5	3.4
	Δ%					-	-5.9	-5.9	-17.6
	N					9	9	9	6
Day 7-11	Mean					20	20	20	**16****
	SD					2.0	1.7	2.7	2.1
	Δ%					-	0	0	-20.0
	N					9	9	9	6
Day 11-14	Mean					21	20	20	**17****
	SD					1.3	2.4	3.3	2.2
	Δ%					-	-4.8	-4.8	-19.0
	N					9	9	9	6
Day 14-17	Mean					22	21	20	**17****
	SD					2.3	2.1	2.5	2.2
	Δ%					-	-4.5	-9.1	-22.7
	N					9	9	9	6
Day 17-20	Mean					23	23	22	**16****
	SD					2.7	2.9	3.3	3.9
	Δ%					-	0	-4.3	-30.4
	N					9	9	9	5
Lactation									
Day 1-4	Mean					25	**31***	23	
	SD					3.9	5.2	9.5	
	Δ%					-			
	N					9	10	6	
Day 4-7	Mean					38	41	35	
	SD					4.4	5.3	8.9	
	Δ%					-	+7.9	-7.9	
	N					9	10	5	

Sex		Males				Females			
Day 7-13	Mean					51	49	**40***	
	SD					3.7	8.0	11.0	
	Δ%					-	-3.9	-21.6	
	N					9	10	5	

Dunnett test * p ≤ 0.05; ** p ≤ 0.01

Haematology

Male rats showed statistically significant decreases in mean corpuscular haemoglobin (MCH) at the top dose and in platelet count (PLT) at the mid dose. As these changes were either of a small magnitude (MCH) or did not show a dose-response relationship (PLT), they were not considered treatment-related.

Top dose female rats showed statistically significant changes in several haematological changes compared to control animals. These included increases in total white blood cell (WBC) and reticulocyte (RET) counts, and decreases in haemoglobulin concentration (HGB), mean cell volume of erythrocytes (MCV), MCH, and mean corpuscular haemoglobin concentration (MCHC). This was accompanied by a non-statistically significant decrease (-12%) in haematocrit (HCT).

Due to the difference in physiological status between controls and top dose females (lactating controls versus failed pregnancies at the top dose – see reproductive section below), no percentage differences from control values were calculated. Since pregnancy and lactation (partially occurring or not occurring at all respectively in the top dose females) are known to cause changes in many haematological parameters, it cannot be determined whether these findings were directly caused by R014821 treatment rather than by differences in physiological status at sacrifice.

Mid dose females also showed a statistically significant decrease in MCH (-8%) compared to controls; however, due to the small magnitude of this change, it was not regarded as treatment-related.

Overall, the haematological findings in the top dose females are uninterpretable and no conclusions can be drawn.

Table C2.8-16. Selected haematological parameters

Sex		**Males**				**Females**			
Dose (mg/kg bw/d)		0	30	100	330	0	30	100	330
RBC [10^{12}/L]	Mean ± SD Δ% N	8.75 0.43 - 4	8.89 0.15 +2 5	8.86 0.33 +1 5	9.10 0.24 +4 5	7.08 0.38 - 5	7.29 0.35 +3 5	7.61 0.41 +7 5	7.37 1.38 N/C 4
RET [10^9//L]	Mean ± SD Δ% N	267.3 35.2 - 4	238.2 44.4 -11 5	235.2 37.7 -12 5	262.8 81.2 -2 5	235.9 32.6 - 5	219.5 31.8 -7 5	263.2 47.3 +12 5	**512.7+ 277.0 NC 5**
HGB (g/L)	Mean ± SD Δ% N	10.1 0.6 - 4	9.9 0.2 -2 5	9.8 0.2 -3 5	9.8 0.3 -3 5	8.9 0.2 - 5	9.0 0.4 +1 5	8.8 0.4 -1 5	**7.4* 1.3 NC 4**
HCT (L/L)	Mean ± SD Δ% N	0.463 0.021 - 4	0.468 0.013 +1 5	0.450 0.010 -3 5	0.459 0.015 -1 5	0.409 0.014 - 5	0.427 0.020 +4 5	0.416 0.020 +2 5	0.359 0.063 NC 4
MCV (fL)	Mean ± SD Δ% N	52.9 0.6 - 4	52.6 2.1 -1 5	50.9 1.3 -4 5	50.5 1.7 -5 5	57.8 1.5 - 5	58.7 2.7 +2 5	54.8 2.3 -5 5	**48.9** 0.9 NC 4**
MCH (fmol)	Mean ± SD Δ% N	1.15 0.04 - 4	1.12 0.04 -3 5	1.10 0.03 -4 5	**1.07* 0.04 -7 5**	1.26 0.08 - 5	1.23 0.05 +2 5	**1.16* 0.04 -8 5**	**1.00** 0.03 NC 4**
MCHC (mmol/L)	Mean ± SD Δ% N	21.71 0.56 - 4	21.24 0.26 -2 5	21.59 0.34 -1 5	21.24 0.25 -2 5	21.78 0.81 - 5	20.97 0.44 -4 5	21.23 0.52 -3 5	**20.51* 0.35 NC 4**
WBC [10^9//L]	Mean ± SD Δ% N	8.9 0.7 - 4	7.9 0.5 +11 5	7.7 1.6 -13 5	9.1 1.6 +2 5	4.4 1.1 - 5	4.6 0.7 +5 5	5.2 0.9 +18 5	**6.6* 1.2 NC 5**
PLT [10^9//L]	Mean ± SD Δ% N	831 133 - 4	748 58 -10 5	**676* 71 -19 5**	717 73 -14 5	740 27 - 5	683 77 -8 5	762 63 +3 5	949 324 NC 5

SD = standard deviation, Δ% = percentage change compared to control, ; Dunnett test * p ≤ 0.05; ** p ≤ 0.01, Steel-test + p ≤ 0.05; ++ p ≤ 0.01, RBC = red blood cell count, RET = reticulocyte count, HGB = haemoglobulin, HCT = haematocrit, MCV = mean cell volume,

MCH = mean cell haemoglobulin, MCHC = mean cell haemoglobulin concentration WBC = white blood cell count, PLT = platelet count, NC = not calculated

Clinical chemistry

Males showed statistically significant reductions in total thyroxine (T4) in all treatment groups, which was dose-dependent. The value for top dose males was below the range of the laboratory historical control data (HCD), therefore this finding was considered treatment-related and adverse. Males also showed an increase in GGT at all doses; however, the statistical significance and magnitude of difference from control could not be determined as all control values were below the limit of detection (0.5 U/L). The values for the low and mid dose males were within the range of the laboratory HCD; however; the values for top dose males were outside the HCD range. Consequently, the increased GGT observed in top dose males was considered treatment-related and adverse. At the top dose, males also showed statistically significant reductions in AST and TBIL. However, these findings were not considered adverse as decreases are not considered clinically relevant.

In top dose females, several clinical chemistry parameters were statistically significantly different compared to concurrent controls. These included increases in total protein (TPROT), albumin (ALB), creatinine (CREA), glucose, sodium, and calcium, and decreases in alanine aminotransferase (ALT), urea, potassium, and chloride. Due to the difference in physiological status between controls and top dose females (lactating controls versus failed pregnancies at the top dose), no percentage differences from control values were calculated. Since pregnancy and lactation (partially occurring or not occurring at all respectively in the top dose females) are known to cause changes in many clinical chemistry parameters, it cannot be determined whether these findings were directly caused by R014821 treatment rather than by differences in physiological status at sacrifice.

Overall, treatment-related and adverse changes in clinical chemistry parameters (decrease in T4 and increase in GGT) were observed at the top dose in males. The findings in top dose females are uninterpretable and no conclusions can be drawn.

Table C2.8-17. Selected clinical chemistry parameters

Sex		**Males**				**Females**			
Dose (mg/kg bw/d)		0	30	100	330	0	30	100	330
ALT [U/L]	Mean ± SD Δ% N	50.9 13.5 - 5	54.5 6.3 +7 5	58.1 11.6 +14 5	50.2 11.2 -1 5	97.3 14.5 - 5	104.4 28.5 +7 5	91.1 28.1 -6 5	**52.3*** **6.6** **NC** **5**

Sex		Males				Females			
AST [U/L]	Mean ± SD Δ% N	85.8 11.7 - 5	87.1 14.1 +2 5	85.4 9.0 0 5	**59.8*** **17.4** **-30** **5**	101.8 9.6 - 5	98.9 11.4 -3 5	85.0 16.4 -17 5	107.5 20.3 NC 5
GGT [U/L]	Mean ± SD Δ% N	<LOD -- -- 0	0.6 0.1 -- 5	0.8 0.1 -- 5	1.2 0.3 -- 5	0.7 0.1 - 2	1.0 0.4 +43 3	0.8 0.2 +14 5	3.3 2.4 NC 5
	HCD£	Mean = 0.7 U/L	*[95% C.I.= 0.5 – 0.9 U/L]*						
TPROT [g/L]	Mean ± SD Δ% N	63.9 2.3 - 5	62.5 2.2 -2 5	63.0 0.7 -1 5	65.2 1.3 +2 5	58.1 1.9 - 5	58.3 5.7 0 5	60.1 5.5 +3 5	**75.3**** **5.1** **NC** **5**
ALB [g/L]	Mean ± SD Δ% N	32.1 0.8 - 5	31.7 0.8 -1 5	31.8 0.6 -1 5	31.2 1.0 -3 5	30.3 0.8 - 5	30.1 2.4 -1 5	30.9 2.7 +2 5	**39.2**** **2.3** **NC** **5**
TBIL [µmol/L]	Mean ± SD Δ% N	2.5 0.2 - 5	2.6 0.3 +4 5	2.4 0.3 -4 5	**2.0**** **0.2** **-20** **5**	2.1 0.2 - 5	1.9 0.3 -10 5	2.0 0.3 -5 5	1.8 0.4 NC 5
Urea [mmol/L]	Mean ± SD Δ% N	7.7 1.1 - 5	9.5 1.2 +23 5	9.2 0.9 +19 5	8.0 1.4 +4 5	9.9 0.9 - 5	10.4 2.3 +5 5	10.8 1.1 +9 5	**7.1*** **0.8** **NC** **5**
CREA [mmol/L]	Mean ± SD Δ% N	37.3 3.6 - 5	39.0 2.7 +5 5	40.0 1.6 +7 5	40.3 2.3 +8 5	36.3 2.4 - 5	37.1 1.7 +2 5	38.6 2.4 +6 5	**41.0*** **2.4** **NC** **5**
GLUC [mmol/L]	Mean ± SD Δ% N	8.65 2.46 - 5	7.72 1.05 -11 5	8.27 1.00 -4 5	7.46 0.89 -14 5	5.90 0.64 - 5	6.86 1.17 +16 5	7.30 1.05 +24 5	**7.52*** **0.63** **NC** **5**
Na [mmol/L]	Mean ± SD Δ% N	141.0 1.5 - 5	141.6 0.6 0 5	141.6 1.4 0 5	141.6 1.4 0 5	139.3 2.1 - 5	139.1 0.9 0 5	137.6 1.2 -1 5	**141.8*** **1.1** **NC** **5**
K	Mean	3.91	3.82	4.07	4.09	4.82	4.60	4.91	**3.43****

Sex		Males				Females			
[mmol/L]	± SD Δ% N	0.21 - 5	0.15 -2 5	0.33 +4 5	0.38 +5 5	0.29 - 5	0.63 -5 5	0.39 +2 5	**0.47** **NC** **5**
Cl [mmol/L]	Mean ± SD Δ% N	100 2 - 5	101 2 +1 5	101 1 +1 5	99 2 -1 5	102 1 - 5	102 3 0 5	101 2 -1 5	**96**** **3** **NC** **5**
Ca [mmol/L]	Mean ± SD Δ% N	2.56 0.08 - 5	2.55 0.05 0 5	2.14 0.26 -16 5	2.58 0.05 +1 5	2.42 0.09 - 5	2.36 0.14 -2 5	2.42 0.10 0 5	**2.79**** **0.09** **NC** **5**
Total T4 [µg/dL]	Mean ± SD Δ% N	5.25 0.89 - 10	**3.99**** **0.96** **-24** **10**	**3.16**** **0.55** **-40** **10**	**2.75**** **0.46** **-48** **9**	-- -- -- 0	-- -- -- 0	-- -- -- 0	-- -- -- 0
HCD$		**Mean = 4.65 µg/dL**		***[95% C.I 3.01 – 6.52 µg/dL]***					

SD = standard deviation, Δ% = percentage change compared to control, Dunnett test * p ≤ 0.05; ** p ≤ 0.01, ALT = alanine aminotransferase, AST = aspartate aminotransferase, GGT = gamma-glutaryltransferase, TPROT = total protein, ALB = albumin, TBIL = total bilirubin, CREA = creatinine, GLUC = glucose, Na = sodium, K = potassium, Cl = chloride, Ca = calcium, T4 = thyroxine, NC = not calculated, LOD = Limit of Detection, HCD£: Historical control data for male Wistar Han rats 2015-Dec 2017 (n=14), C.I. = confidence interval, HCD$: Historical control data for adult male Wistar Han rats 2015-Dec 2017 (n=724).

Gross pathology

At the top dose, treatment-related and adverse macroscopic findings were observed during necropsy in the liver (males) and uterus (females). All other observed macroscopic findings occurred at a comparable rate to that expected for rats of this age and strain, therefore they were not considered treatment-related. Overall, treatment-related macroscopic pathology was observed in the liver (males) and the uterus (females) at the top dose.

Table C2.8-18. Selected gross pathology findings following R014821 treatment

Sex	Males				Females			
Dose (mg/kg bw/d)	0	30	100	330	0	30	100	330
Liver [N]	10	10	10	10	10	10	10	10
- Enlargement				**7**				

- Accentuated lobular patterns				**1**				
Uterus [N]					10	10	10	10
- Thickened horn and/or contents								**4**

Findings in bold text were considered treatment-related

Organ weight

Top dose males showed a statistically significant increase in liver weight (absolute: +39%, relative: +44%), which was corroborated by gross pathology and histopathology findings. This finding was considered treatment-related and adverse.

Absolute ovary weight was statistically significantly increased from the mid dose but was still within the range of the laboratory HCD, therefore this finding was not considered treatment-related.

Two top dose animals had very high uterus weights due to the presence of a dead foetus in their uterus, leading to a statistically significant increase in uterus weight in top dose females compared to concurrent controls. Terminal body weight was statistically significantly reduced in females at the top dose due to their abnormal pregnancies. Therefore these effects on the uterus were not genuine adverse increases.

Changes in the absolute or relative organ weights of adrenals, brain, heart, kidney, liver and thymus were also observed in top dose females. As the terminal body weight of the top dose females was also lower than controls due to the differences in their physiological status (failed pregnancy in top dose females compared to lactation in controls), all other changes in organ weight were considered secondary to reduced body weight, rather than treatment-related.

Overall, treatment-related and adverse effects on liver weights were observed in males at the top dose.

Table C2.8-19. Selected organ weights following R014821 treatment

Sex	**Males**					**Females**			
Dose (mg/kg bw/d)		0	30	100	330	0	30	100	330
Body	Mean [g] ± SD Δ%	352 18 -	343 22 -3	343 17 -3	336 19 -5	288 20 -	294 21 -2	269 30 -7	**238**** **16** **-17**
Adrenals (abs.)	Mean [g] ± SD Δ%	0.066 0.007 -	0.061 0.004 -8	0.059 0.005 -11	0.069 0.004 +4	0.078 0.004 -	0.081 0.008 -4	0.083 0.008 +1	0.081 0.011 -4

Sex	Males					Females			
Adrenals (rel.)	Mean [g] ± SD Δ%	0.019 0.002 -	0.018 0.002 -5	0.018 0.002 -5	0.021 0.002 +11	0.027 0.001 -	0.028 0.003 +4	0.029 0.002 +7	**0.034**** **0.005** **+26**
Brain (abs.)	Mean [g] ± SD Δ%	2.08 0.06 -	2.03 0.09 -2	2.03 0.05 -2	2.02 0.08 -3	1.85 0.03 -	1.95 0.15 +5	1.89 0.05 +2	1.90 0.05 +3
Brain (rel.)	Mean [%] ± SD Δ%	0.60 0.04 -	0.60 0.05 ±0	0.61 0.04 +2	0.61 0.06 +2	0.64 0.03 -	0.66 0.03 +8	0.65 0.04 +6	**0.80**** **0.06** **+25**
Heart (abs.)	Mean [g] ± SD Δ%	0.907 0.038 -	0.950 0.090 +5	0.898 0.099 -10	0.918 0.071 +1	0.866 0.033 -	0.846 0.099 -2	0.806 0.058 -7	**0.743*** **0.031** **-14**
Heart (rel.)	Mean [%] ± SD Δ%	0.262 0.011 -	0.281 0.011 +7	0.267 0.017 +2	0.273 0.016 +4	0.300 0.015 -	0.287 0.018 -4	0.278 0.024 -7	0.313 0.021 +4
Kidney (abs.)	Mean [g] ± SD Δ%	2.46 0.29 -	2.53 0.27 -3	2.49 0.19 +1	2.59 0.29 +5	2.02 0.13 -	2.01 0.22 ±0	2.05 0.20 +1	1.94 0.09 -4
Kidney (rel.)	Mean [%] ± SD Δ%	0.71 0.08 -	0.75 0.07 +6	0.75 0.06 +6	0.77 0.08 +8	0.70 0.04 -	0.68 0.04 -3	0.70 0.05 ±0	**0.82**** **0.05** **+17**
Liver (abs.)	Mean [g] ± SD Δ%	9.20 1.10 -	8.83 1.35 -4	9.76 0.86 +6	**12.79**** **1.10** **+39**	12.61 0.62 -	13.27 1.24 +5	13.41 0.94 +6	**9.94**** **0.57** **-22**
Liver (rel.)	Mean [%] ± SD Δ%	2.65 0.011 -	2.60 0.20 -2	2.91 0.11 +10	**3.81**** **0.16** **+44**	4.36 0.07 -	4.52 0.36 +4	4.62 0.36 +6	4.19 0.22 -4
Ovary (abs.)	Mean [g] ± SD Δ%					0.114 0.011 -	0.106 0.014 -7	**0.140*** **0.040** **+23**	**0.159**** **0.020** **+39**
	HCD£					*Mean: 0.114g*	*[95% C.I. = 0.095 – 0.590]*		
Ovary (rel.)	Mean [%] ± SD Δ%					0.039 0.002 -	0.036 0.004 -8	0.048 0.005 +23	**0.067**** **0.011** **+72**
Spleen (abs.)	Mean [g] ± SD Δ%	0.630 0.035 -	0.547 0.078 -13	0.578 0.088 -8	0.662 0.074 +5	0.507 0.067 -	0.461 0.040 -9	**0.414*** **0.040** **-18**	0.450 0.055 -11
	HCD£					*Mean: 0.479g*	*[95% C.I. = 0.387*		

Sex	Males					Females			
							– *0.590]*		
Spleen (rel.)	Mean [%] ± SD Δ%	0.182 0.010 -	0.162 0.002 -11	0.172 0.021 -5	0.197 0.010 +8	0.175 0.019 -	0.158 0.016 -10	0.143 0.014 -18	0.191 0.034 +9
Thymus (abs.)	Mean [%] ± SD Δ%	0.415 0.046 -	0.357 0.103	0.352 0.103	0.376 0.091	0.207 0.039 -	0.185 0.003 -11	0.237 0.075 +14	0.314 0.143 +52
Thymus (rel.)	Mean [%] ± SD Δ%	0.120 0.013 -	0.104 0.023	0.104 0.017	0.111 0.024	0.072 0.014 -	0.062 0.016 -14	0.081 0.024 +13	**0.130* 0.053 +81**
Uterus (abs.)	Mean [g] ± SD Δ%					0.361 0.055 -	0.334 0.073 -7	0.503 0.136 +39	**1.981* 1.624 +449**
Uterus (rel.)	Mean [%] ± SD Δ%					0.125 0.016 -	0.113 0.021 -10	0.174 0.052 +39	**0.628** 0.641 +402**

SD = standard deviation, Δ% = percentage change compared to control, abs. = absolute, rel. = relative, Dunnett test * p ≤ 0.05; ** p ≤ 0.01, HCD£ Historical control data for female Wistar Han rats (2015- Jan 2018)

Histopathology

In the liver, hepatocellular vacuolation and hypertrophy were observed in mid dose males and top dose animals of both sexes. Although these findings were not indicative of degenerative or inflammatory hepatic changes, they were accompanied by sizable increases in organ weight in top dose males (absolute: +39%, relative: +44%). Therefore, the findings in males were considered treatment-related and adverse.

Top dose males showed an increase in hyaline droplet accumulation in the kidney, which was considered likely to be caused by α2μ-globulin, a protein specific to male rats. However, as no confirmation was provided, this finding cannot be dismissed as not relevant to humans. Intravascular macrophage-like cellular emboli (characterised by oval/ polygonal nuclei, vacuolated cytoplasm, and frequent pigment granules) were observed in the kidney of top dose females. Overall, there were adverse findings in the kidney in top dose males and females.

In the lung, intravascular macrophage-like cellular emboli (minimal to moderate) were observed in top dose female animals, all of which had lost embryos. This finding at the top dose was considered treatment-related and adverse.

In the spleen, mild-to-moderate yellow-brown pigmentation (identified as haemosiderin, a degradation product of haemoglobulin) was observed in females at all doses. A similar

effect was seen in the subsequent 90-day study. Therefore, these histopathological changes in the spleen in females are considered treatment-related and adverse from the lowest dose.

In the adrenal gland, focal (single-cell) necrosis was observed in 4/5 females at the top dose. This finding may potentially be secondary to other pathological processes rather than a direct effect of R014821 administration, as it was observed in only the top dose group, where all animals have undergone pregnancy loss. In addition, this finding was not observed in females treated with R014821 for 90 days at a dose of 133 mg/kg bw/d. Nevertheless, as this finding was observed solely in top dose females, it was considered treatment-related and adverse.

In the ovary, cysts were observed in one mid dose female (1/9) and in 6/8 top dose females (vs 0/6 in controls), suggesting a dose-response relationship. As multiple animals were affected at the top dose, the findings at the top dose were considered treatment-related and adverse. The significance of one single female affected at the mid dose out of 9 animals is difficult to interpret as in controls only 6 females were examined; it is most likely the ovary cyst at the mid dose was part of normal variation.

Overall, treatment-related and adverse histopathological findings were observed in the liver from the mid dose in males and at the top dose in females. Adverse findings were also seen in the kidneys at the top dose (males and females), in the lung, adrenal and ovary in top dose females and in the spleen in females from the lowest dose.

Table C2.8-20. Selected histopathology findings following R014821 treatment

Sex	Males					Females			
Dose (mg/kg bw/d)		0	30	100	330	0	30	100	330
Liver [N]		5	5	5	7	5	5	5	5
- Hypertrophy, hepatocellular		0	0	**4**	**7**	0	0	1	**5**
	Minimal	-	-	**4**	**1**	-	-	1	**-**
	Slight	-	-	-	**6**	-	-	**-**	**5**
- Vacuolation, hepatocellular		0	0	**2**	**7**	0	0	0	**5**
	Minimal	-	-	**2**	**-**	-	-	-	**4**
	Slight	-	-	**-**	**3**	-	-	-	**1**
	Moderate	-	-	**-**	**4**	-	-	-	**-**
Kidney [N]		5	5	5	5	5	5	5	**5**
- Hyaline droplets accumulation		3	5	5	**5**	0	0	0	0
	Minimal	2	3	3	**-**	-	-	-	-
	Slight	1	2	2	**5**	-	-	-	-
- Intravascular macrophage-like cellular emboli									**4**
	Minimal								**4**
Lung [N]						5	5	**5**	**5**
- Intravascular macrophage-like cellular emboli						0	0	**0**	**5**
	Minimal								**1**
	Slight								**2**
	Moderate								**2**

Sex	Males					Females			
Spleen [N]						6	**5**	**5**	**5**
- Yellow-brown pigmentation						3	**5**	**5**	**5**
	Minimal					3	**-**	**-**	**4**
	Mild					-	**3**	**2**	**1**
	Moderate					-	**2**	**3**	**-**
Adrenal gland [N]						5	5	5	**5**
- Single cell necrosis						0	0	0	**4**
Minimal						-	-	-	**4**
Ovaries [N]						6	5	9	**8**
- Cyst						0	0	1	**6**
	Minimal					-	-	1	**2**
	Slight					-	-	-	**4**

[N] = number of tissues examined per group, findings in bold text were considered treatment-related

Reproductive parameters

All females in the control, low dose and mid dose groups had regular oestrus cycles of 4 or 5 days. Only 3/10 top dose females had regular 5-day oestrous cycles during the pre-mating period, whilst 4/10 animals had an irregular cycle and 2/10 were in persistent dioestrus. The oestrous stage/length of the animal euthanized *in extremis* on day 8 could not be determined. Overall, treatment-related and adverse effects on the oestrus cycle were observed at the top dose.

Mating index and pre-coital time were not affected by treatment. The fertility index was 90% for the control group, 100% for the low and mid dose groups and 67% for the top dose group. One of the control animals was not pregnant after mating and three of the nine mated females in the top dose group were not pregnant. No abnormalities were observed in the reproductive organs of either sex to explain the cases of non-pregnancy, therefore the reduction in fertility index at the top dose was considered treatment-related and adverse.

Nine out of ten control animals and all females in the low dose group delivered healthy pups. In the mid dose group, one female did not deliver offspring, but had implantation sites in the uterus and similar microscopic findings in the uterus and vagina as those with total litter loss, suggesting former pregnancy. All other mid dose females delivered

offspring, but 3/9 females had total litter loss. These animals showed findings in their reproductive organs at necropsy, which included inflammation in the uterus (endometrium), luminal cellular debris and/or haemorrhage, and increased mucification of vaginal epithelium. Of the females in the mid dose group with live offspring, one lost one of her three pups at PND 2, while all offspring of the other dams survived to their scheduled sacrifice. None of the 6/9 pregnant top dose females had live offspring at the first litter check. Therefore reduced litter size was observed from the mid-dose, with no live litter delivered at the top dose.

The mean number of implantation sites was statistically significantly decreased at the top dose and also slightly (but not statistically significantly) decreased at the mid dose compared to concurrent controls. These findings correlated with the reduction in litter size, therefore the reduction in implantation sites was considered treatment-related and adverse from the mid dose.

Post-implantation survival index was significantly reduced to 52% and 0% at the mid and top dose respectively compared to 90% in control animals. At the top dose, there were no live offspring. Litter size (4.9 pups), live birth index (76%) and viability index (90%) were reduced at the mid dose compared to controls (11.7 pups, 98% and 97% respectively), with post-natal loss increased at the mid dose, whereas no effects on litter size and survival of the offspring were observed at the low dose. The lactation index was 100% in all treatment groups with litters.

Gestation index was reduced to 78% and 0% at the mid and top dose respectively compared to 100% in the low dose and control groups, with affected females showing histopathological changes in the uterus (mostly inflammation of the endometrium) which was related to their reproductive failure. These findings noted from the mid dose were considered to be treatment-related and adverse.

Gestation length was statistically significantly higher in the low and mid dose groups compared to concurrent controls; however, as there was no dose-related trend and the mean duration of gestation in these groups was within the range of historical control data, these findings were not considered treatment-related.

Overall, treatment-related adverse effects on the length and regularity of the oestrus cycle and on the fertility index were observed in female rats at the top dose and on the implantation index, gestation index, litter size, post-implantation survival index and survival of offspring (live birth index, viability index, post-implantation loss) from the mid dose. No live litter were delivered at the top dose.

Table C2.8-21. Reproduction parameters of female rats following R014821 treatment

	Dose (mg/kg bw/d)			
	0	30	100	330
# females placed with males	10	10	10	9
# females pregnant	9	10	10	6
Female fertility index [%]	90	100	100	67
Pre-coital interval [mean days]	4.2	4.1	**3.3**	3.2
Duration of gestation [mean days]	21.0	21.9	22.0	-
HCD	*21.4 [95% C.I. = 21.0 – 22.0]*			
Females with liveborn pups [N]	9	10	7	0
Females with all stillborn [N]	0	0	1	0
Gestation index [%]	100	100	78	0
Implantation sites, total	119	126	99	51
- mean (± SD) per dam	13.2 ± 2.2	12.6 ± 1.3	11.0 ± 2.7	8.5## ± 4.0
# of offspring born	107	104	51	0
Post-implantation survival index	90	83	52	0
# living pups delivered	105	103	39	0
- mean (± SD) per dam	11.7 ± 1.8	10.3 ± 1.3	4.9## ± 4.6	
Live birth index [%]	98	99	76	0
Postnatal loss, total [%]	2.9	0.0	10.3	0
# litters affected	1	0	3	0
Viability index [%]	97	100	90	0
Lactation index [%]	100	100	100	0

#/## Steel-test significant at 5% (+) or 1% (++) level, HCD = Historical control data for duration of gestation in Wistar Han rats (period 2015-June 2017): mean = 21.4, P5 - P95 = 21.0 – 22.0 (n=484).

Development of pups

There were no live pups at the top dose to follow up. Clinical signs of dehydration were observed in three pups from the mid dose treatment group. As two of these three pups were sacrificed *in extremis* on PND 3-4, these clinical signs contributed to the reduced viability index and were therefore considered adverse. No treatment-related clinical signs in pups from the low dose treatment group were noted.

All developmental signs examined in pups (body weights, anogenital distance, areola/ nipple retention, serum T4) were unaffected by R014821 treatment. The statistically significant decrease in mean bodyweight in female pups of the mid dose at PND 1 was not considered treatment-related as there was no dose-response relationship. Although top

dose female pups showed a statistically significantly higher serum T4 level (+36 %) compared to concurrent controls, this finding was not considered treatment-related as it was within the range of the laboratory HCD. Overall, no treatment-related adverse effects were observed in the development of pups, but clinical signs of general toxicity were observed at the mid dose.

Table C2.8-22. Selected development parameters of pups

	Males			Females			Combined		
Dose (mg/kg bw/d)	0	30	100	0	30	100	0	30	100
	Mean body weight ± SD [g]								
PND 1	6.1 ± 0.5	6.6 ± 0.6	5.7 ± 0.7	5.8 ± 0.4	**6.5* ± 0.5**	5.9 ± 0.5	6.0 ± 0.5	6.5 ± 0.6	5.7 ± 0.5
PND 4	8.8 ± 1.0	9.6 ± 0.8	8.8 ± 1.4	8.5 ± 0.6	9.4 ± 0.8	8.5 ± 1.6	8.7 ± 0.7	9.5 ± 0.8	8.4 ± 1.6
PND 7	14.3 ± 1.7	15.0 ± 1.6	14.0 ± 2.4	14.1 ± 0.8	14.9 ± 1.4	14.6 ± 1.3	14.3 ± 1.0	15.0 ± 1.4	14.3 ± 1.7
PND 13	28.4 ± 2.7	27.9 ± 1.9	27.2 ± 1.9	28.2 ± 1.4	27.5 ± 2.9	27.9 ± 2.1	28.4 ± 2.7	27.8 ± 3.1	27.5 ± 1.5
	Mean total serum T4 ± SD [µg/dL]								
PND 14-16	6.33 ± 1.35	7.31 ± 1.81	7.93 ± 1.37	5.84 ± 0.99	6.76 ± 0.99	7.95* ± 2.07			

Dunnett test: * $p \leq 0.05$; ** $p \leq 0.01$

Conclusion

Under the conditions of this GLP-compliant and OECD 422 study in rats, administration of R014821 by oral gavage resulted in treatment-related mortality at the top dose of 330 mg/kg bw/d. Treatment-related clinical signs of toxicity (piloerection) were observed in animals from the mid dose of 100 mg/kg bw/d.

Adverse effects on body weight, body weight gain and food consumption were observed in top dose males and females during the pre-mating period.

A decrease in T4 and an increase in GGT were seen in males at the top dose. In top-dose females, the clinical-chemistry and haematological findings were uninterpretable and no conclusions can be drawn.

Treatment-related and adverse histopathological findings were observed in the liver from the mid dose in males and at the top dose in females. Adverse findings were also seen in the kidneys at the top dose (males and females), in the lung, adrenal and ovary in top dose females and in the spleen in females from the lowest dose.

With regard to reproductive toxicity, treatment-related adverse effects on the length and regularity of the oestrus cycle and on the fertility index were observed in female rats at the top dose and on the implantation index, gestation index, litter size, post-implantation survival index and survival of offspring (live birth index, viability index, post-implantation loss) from the mid dose. No live litters were delivered at the top dose.

Treatment-related clinical signs of toxicity were observed in pups at the mid dose. Body weights and developmental signs (anogenital distance, areola/ nipple retention, serum T4) examined in pups were unaffected by R014821 treatment up to the mid dose.

Based on these findings, a **LOAEL of 30 mg/kg bw/d** was established by HSE for **general parental toxicity** based on histopathological findings in the spleen in females.

Treatment with R014821 impacted reproductive performance, with decreases in the fertility index, implantation index, litter size, gestation index and survival of offspring (live birth index, viability index) observed from the mid dose. At the top dose, treatment-related adverse effects on the length and regularity of the oestrus cycle were also observed in female rats and there were no successful pregnancies. No treatment-related effects on reproductive parameters were observed at the low dose. Therefore, a **NOAEL of 30 mg/kg bw/day** is identified for effects on **reproduction**.

With regard to offspring toxicity, treatment-related and adverse clinical signs were observed in pups at the mid dose. No live pups were available for follow up at the top dose. No treatment-related effects on body weight, or on developmental landmarks in either males or females were observed in pups at up to the mid dose. A **NOAEL of 30 mg/kg bw/d** for **offspring toxicity** is therefore identified by HSE.

A 90-Day Study of R014821 by Dietary Administration in Wistar Han Rats (2022)

Reference: A 90-Day Study of R014821 by Dietary Administration in Wistar Han Rats; (2022)

Report No. AGR 6320

	Test facility study no. 20361822
Date performed:	Completion date: 15th Aug 2022
Test facility:	[redacted]
Guideline(s):	Yes; OECD 408
Deviations:	No deviations that affected the reliability of the study.
GLP:	Yes
Test item/dose: groups	0, 200, 600 and 2000 ppm R014821 Batch: 2102010-714 Purity: 99.9%
Validated method of analysis:	Validated analytical methods are available for R014821 (study reference no. 20321429)
Study acceptable:	Yes

Method

In this GLP and OECD TG-compliant study, metabolite R014821 was administered via the diet to groups of Wistar Han rats (10/sex/dose) for 90 days at dose levels of 0, 200, 600 and 2000 ppm (equivalent to 0, 14/15, 42/45 and 137/144 mg/kg bw/d in males/females respectively). The dose levels in this study were selected based on information provided by the sponsor and results from a 14-day repeated dose toxicity study with oral exposure of R014821 via diet in rats (Test facility study no. 20321431), a limited 90-day repeated dose toxicity study with oral exposure of R014821 via diet in rats at 1600 ppm (i.e. the comparative toxicity study presented above), and the OECD 422 study in rats by gavage (also described above). R014821 was mixed in a standard powder rodent diet using propylene glycol as a vehicle. The stability and homogeneity of the test substance in the diet was shown to be acceptable. Fully validated methods of analysis for R014821 in the diet at concentrations covering those tested are available (see Validated methods of analysis above).

Results

Mortality

No mortality occurred during the study.

Clinical observations

No treatment-related clinical signs were noted during the observation period.

Body weight and body weight gain

Body weight and body weight gain was consistently and statistically significantly reduced in females from the mid dose of 600 ppm. Although effects on body weight and body weight effects were not statistically significant in males, due to their magnitude, the reductions in body weight in males from the mid dose were considered biologically significant, treatment-related, and adverse.

Overall, there were treatment-related and adverse reductions in body weight and body weight gain in males and females from the mid dose of 600 ppm.

Table C2.8-23. Body weight and body weight gain following R014821 treatment

Sex		**Males**			**Females**			
Dose (ppm)	**0**	**200**	**600**	**2000**	**0**	**200**	**600**	**2000**
Day	**Mean bodyweight ± SD [g] (*Δ% compared to control*)**							
5	174.8 ± 11.9 *(-)*	191.8 ± 22.0 *(+9.7)*	170.8 ± 13.0 *(-2.3)*	177.7 ± 17.6 *(+1.7)*	151.6 ± 11.5 *(-)*	147.9 ± 8.6 *(-2.4)*	146.0 ± 7.0 *(-3.7)*	**138.7** ± 8.7 *(-8.5)*
8	199.2 ± 13.7*(-)*	214.5 ± 23.2 *(+7.7)*	186.2 ± 14.8 *(-6.5)*	194.7 ± 18.9 *(-2.3)*	163.0 ± 12.5 *(-)*	156.2 ± 9.0 *(-4.2)*	**151.5* ± 6.3 (-7.1)**	**144.8** ± 12.1 (-11.2)**
15	238.0 ± 18.0 *(-)*	250.9 ± 27.6 *(+5.4)*	218.9 ± 17.6 *(-8.0)*	219.8 ± 20.3 *(-7.6)*	177.4 ± 13.6 *(-)*	170.2 ± 10.2 *(-4.1)*	**162.2* ± 8.7 *(-8.6)***	**157.1** ± 11.1 *(-11.4)***
29	286.3 ± 24.2 *(-)*	304.3 ± 34.6 *(+6.3)*	263.6 ± 27.8 *(-7.9)*	262.3 ± 24.5 *(-8.4)*	202.2 ± 17.1 *(-)*	194.5 ± 11.0 *(-3.8)*	**186.5* ± 8.7 *(-7.8)***	**171.4** ± 13.2 *(-15.2)***
43	322.7 ± 29.8 *(-)*	336.1 ± 42.3 *(+4.2)*	296.0 ± 37.7 *(-8.3)*	**279.6* ± 28.3 *(-13.4)***	217.7 ± 17.9 *(-)*	208.1 ± 13.6 *(-4.4)*	**195.6** ± 12.6 *(-10.2)***	**181.5** ± 15.6 *(-16.6)***
61	353.8 ± 34.2 *(-)*	362.7 ± 48.0 *(+2.5)*	324.9 ± 43.7 *(-8.2)*	**305.7* ± 35.5 *(-13.6)***	230.3 ± 20.3 *(-)*	222.2 ± 12.4 *(-3.5)*	**210.6** ± 10.7 *(-9.9)***	**195.4** ± 16.5 *(-15.2)***
91	398.6 ± 41.2 *(-)*	399.4 ± 53.6 *(+0.2)*	360.5 ± 47.0 *(-9.6)*	333.1 ± 39.7 *(-16.4)*	243.7 ± 23.0 *(-)*	233.4 ± 17.2 *(-4.2)*	**218.7* ± 16.7 *(-10.3)***	**201.5** ± 19.2 *(-17.3)***
	Mean body weight gain ±SD [g]							

Sex	Males				Females			
Dose (ppm)	0	200	600	2000	0	200	600	2000
	(Δ% compared to control)							
1-5	18.4 ± 4.2 *(-)*	17.5 ± 4.6 *(-4.9)*	15.4 ± 4.0 *(-16.3)*	**8.5** ± 5.7 *(-53.8)***	10.9 ± 1.7 *(-)*	8.8 ± 1.8 *(-19.3)*	**8.2* ± 2.8 *(-24.8)***	**4.2** ± 2.7 *(-61.5)***
5-8	24.4 ± 3.5 *(-)*	22.7 ± 6.4 *(-7.0)*	**15.4## ± 2.5 *(-36.9)***	**17.0## ± 3.6 *(-30.3)***	11.4 ± 5.4 *(-)*	8.3 ± 2.7 *(-23.9)*	**5.5* ± 4.0 *(-51.8)***	**6.1* ± 4.6 *(-46.5)***
1-91	242.2 ± 33.5 *(-)*	225.1 ± 44.3 *(-7.1)*	205.1 ± 42.9 *(-15.3)*	**163.9** ± 33.6 *(-32.3)***	103.0 ± 15.7 *(-)*	94.3 ± 11.8 *(-8.4)*	**80.9** ± 13.4 *(-21.5)***	**67.0** ± 14.2 *(-35.0)***

Anova & Dunnett: * p ≤ 0.05; ** p ≤ 0.01, Kruskal-Wallis & Dunn: ## p ≤ 0.01

Food consumption

Reductions in food consumption were observed in male and female rats from the mid dose (at 600 ppm: from day 5 of the study onwards; at 2000 ppm: throughout the study). These effects were considered treatment-related and adverse. In addition, a slight reduction in food consumption (-7%) was observed in females at 200 ppm; however, this finding was not considered adverse as it was not accompanied by biologically or statistically significant changes in body weight in this treatment group. Low dose males showed comparable food consumption to the concurrent controls.

Overall, treatment-related and adverse reductions in food consumption was observed in males and females from the mid dose of 600 ppm.

Ophthalmic examinations

No treatment-related effects were observed following administration of R014821.

Functional observational battery (FOB) and measurement of motor activity

No statistically or biologically significant changes in functional observations were reported in the FOB in male or female rats.

Overall, there were no treatment-related effects on functional observations or motor activity in male or female rats.

Haematology

Several slight (<10%) but statistically significant changes were also observed in red blood cell parameters. These included increased red blood cell distribution width (RDWG) in top dose males and females (+7 % and +6 % respectively), lower haemoglobulin (HGB) at the

top dose in males (-5 %) and at the mid dose in females (-4 %), reduced haematocrit (HCT) in top dose males (-6 %) and lowered reticulocyte count (RET), mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) in top dose females (-22 %, -5 % and -6 % respectively). RET were also lower (-25%) in the mid-dose females. With the exception of the decrease in RET in females from the mid dose (which was corroborated by histopathological findings in the spleen), the majority of these changes were of small magnitude (<10 %) and were not associated with clinical pathology, therefore they were not considered adverse.

In top dose males, there was a reduction in platelet count (-12 %) and in absolute monocyte count (-35 %). As the reduction in monocytes was not corroborated by histopathology findings or clinical observations, they were not regarded as adverse. Similarly, the decrease in platelet count in top dose males was not accompanied by changes in other coagulation parameters.

Overall, no consistent pattern of treatment-related adverse effects on haematological and coagulation parameters was observed in male rats. In females, a treatment-related decrease in reticulocyte count, corroborated by histopathological findings in the spleen, was observed from the mid dose.

Table C2.8-24. Selected haematology and coagulation parameters

Sex		**Males**				**Females**			
Dose (ppm)		0	200	600	2000	0	200	600	2000
Haematology parameters									
RBC [10^{12}/L]	Mean ± SD Δ%	9.107 0.339 -	8.925 0.254 -2	8.945 0.538 -2	8.845 0.658 -3	7.831 0.372 -	7.849 0.458 ±0	7.635 0.626 -3	7.806 0.899 ±0
RET [10^{9}//L]	Mean ± SD Δ%	176.88 25.81 -	172.85 12.54 -2	183.79 26.20 +4	199.05 27.16 +13	197.87 40.25 -	170.64 35.61 -14	**148.59*** **42.56** **-25**	**153.51*** **34.57** **-22**
RDWG (%)	Mean ± SD Δ%	11.91 0.35 -	12.09 0.45 +2	12.29 0.49 +3	**12.80**** **0.80** **+7**	10.94 0.57 -	10.88 0.68 -1	11.11 0.44 +2	**11.61*** **0.59** **+6**
HGB (g/L)	Mean ± SD Δ%	159.7 3.8 -	156.0 4.1 -2	154.5 6.3 -3	**151.8*** **8.4** **-5**	145.3 4.2 -	146.2 3.6 +1	**140.2#** **3.4** **-4**	135.6 12.4 -7
HCT (L/L)	Mean ± SD Δ%	0.4651 0.0119 -	0.4510 0.0117 -3	0.4484 0.0232 -4	**0.4386*** **0.0280** **-6**	0.4137 0.0192 -	0.4161 0.0164 +1	0.3980 0.0167 -4	0.3898 0.0408 -6

MCV (fL)	Mean ± SD Δ%	51.12 2.24 -	50.54 1.15 -1	50.15 1.00 -2	49.65 1.68 -3	52.83 1.18 -	53.08 1.48 ±0	52.30 2.60 -1	**50.01** **1.42** **-5**
MCH (pg)	Mean ± SD Δ%	17.54 0.64 -	17.48 0.51 ±0	17.28 0.47 -1	17.20 0.64 -2	18.57 0.56 -	18.69 0.94 +1	18.44 1.23 -1	**17.44*** **0.69** **-6**
WBC [10^9//L]	Mean ± SD Δ%	6.019 1.127 -	5.378 0.870 -11	5.727 1.014 -5	5.852 1.467 -3	2.463 0.770 -	2.258 0.368 -8	2.304 0.554 -6	2.751 1.223 +12
MONO [10^9//L]	Mean ± SD Δ%	0.130 0.038 -	0.108 0.016 -17	0.132 0.029 +2	**0.085**** **0.020** **-35**	0.035 0.015 -	0.041 0.014 +30	0.039 0.014 +11	0.053 0.036 +51
PLT [10^9//L]	Mean ± SD Δ%	719.5 71.0 -	696.5 55.0 -3	679.7 53.4 -6	**636.6*** **72.6** **-12**	728.8 60.0 -	707.4 81.0 -3	680.6 80.9 -7	703.2 60.8 -4
Coagulation parameters									
PT [s]	Mean ± SD Δ%	17.83 0.51 -	17.58 0.84 -1	17.25 0.72 -3	17.35 0.88 -3	17.42 0.63 -	16.85 0.97 -3	17.63 0.71 +1	17.50 0.68 ±0
APTT [s]	Mean ± SD Δ%	17.20 1.38 -	**19.76*** **2.24** **+15**	16.04 2.33 -7	15.66 2.04 -9	18.25 2.07 -	17.47 1.79 -4	15.83 1.89 -13	15.55 3.67 -15

SD = standard deviation, Δ% = percentage change compared to control, * $p \leq 0.05$; ** $p \leq 0.01$; Anova and Dunnett, # $p \leq 0.05$; Kruskal-Wallis & Dunn, RBC = red blood cell count, RET = reticulocyte count, RDWG = red blood cell distribution width, HGB = haemoglobulin, HCT = haematocrit, MCV = mean cell volume, MCH = mean cell haemoglobulin, WBC = white blood cell count, MONO = monocyte count, PLT = platelet count, PT = prothrombin time, APPT = activated partial prothrombin time

Clinical chemistry

Several clinical chemistry parameters showed statistically or biologically significant changes.

Males showed reduced triglycerides at the top dose and lowered total bilirubin from the mid dose. Although the reductions in total bilirubin and triglycerides are indicative of possible changes in lipid metabolism, neither hypobilirubinaemia nor low triglycerides are generally regarded as clinically significant, therefore these findings were not considered as adverse. Calcium was increased from the mid dose in males (+3 to +5 %), but this increase is not considered adverse due to its small magnitude.

In females, total protein (-6 to -9 %) and glucose (-18 to -20%) concentrations were lowered from the mid dose, albumin was reduced at the top dose (-6 %), and all R014821

treatment groups showed a reduction in inorganic phosphate concentration (ranging from -20 to -24%). Since serum glucose did not show a clear dose-response, these findings were not considered treatment-related. In addition, the reductions in total protein and albumin were slight (<10 %) and were therefore not regarded as adverse. As only one clinical chemistry parameter (inorganic phosphate) showed a biologically and statistically significantly effect in females (although with no clear dose-response) and there was no organ weight or histopathology findings in the relevant organ (kidney) in these animals, this finding was not regarded as adverse.

No statistically significant changes were observed in T3 or TSH in male or female rats, and T4 was unaffected in animals of both sexes at the low and mid dose. At the top dose, male rats showed a statistically significant decrease (-23%) in serum T4 while females showed a sizable, albeit not statistically significant, increase (+25%). However, these findings were not considered treatment-related as they were within the range of the laboratory HCD.

All other clinical chemistry findings were not considered treatment-related due to an absence of a dose-response relationship or were within the expected range of control values.

Overall, no treatment-related and adverse clinical chemistry findings were observed in male or female rats.

Table C2.8-25. Selected clinical chemistry parameters

Sex		**Males**				**Females**			
Dose (ppm)		0	200	600	2000	0	200	600	2000
Clinical chemistry parameters									
ALT [U/L]	Mean ± SD Δ%	56.9 12.2 -	73.6 62.9 +29	**46.2#** **4.8** **-19**	56.3 10.3 -1	45.9 18.3 -	43.1 8.2 -6	40.4 10.0 -12	49.3 18.1 +8
ALP [U/L]	Mean ± SD Δ%	92.7 13.5 -	82.0 16.3 -12	86.5 23.4 -7	94.1 13.4 +1	32.7 8.4 -	38.6 9.2 +18	42.1 13.7 +29	**53.6**** **10.3** **+64**
TPROT [g/L]	Mean ± SD Δ%	62.53 1.91 -	61.61 1.47 -1	**59.08**** **2.20** **-6**	60.81 1.64 -3	64.15 2.07 -	62.76 2.31 -2	**60.41**** **3.23** **-6**	**59.48**** **2.36** **-9**
ALB [g/L]	Mean ± SD Δ%	37.75 1.01 -	37.90 1.11 ±0	**36.30*** **1.83** **-4**	36.90 0.90 -2	39.72 1.47 -	39.36 1.21 -1	37.71 2.27 -5	**37.42#** **1.76** **-6**

Sex		Males				Females			
TBIL [µmol/L]	Mean ± SD Δ%	2.07 0.25 -	1.88 0.35 -9	**1.77*** **0.24** **-14**	**1.68**** **0.15** **-19**	2.36 0.43 -	2.17 0.38 -8	2.14 0.29 -9	2.10 0.35 -11
GLUC [mmol/L]	Mean ± SD Δ%	10.166 2.191 -	8.952 1.192 -12	8.552 1.215 -16	9.355 2.249 -8	8.378 1.185 -	6.963 0.455 -17	**6.714#** **0.926** **-20**	**6.853#** **1.347** **-18**
TRIG [mmol/L]	Mean ± SD Δ%	1.317 0.561 -	1.155 0.304 -12	0.914 0.254 -31	**0.508##** **0.180** **-61**	0.538 0.309 -	0.611 0.188 +14	0.449 0.152 -17	0.528 0.198 -2
Ca [mmol/L]	Mean ± SD Δ%	2.562 0.042 -	2.592 0.088 +1	**2.649#** **0.188** **+3**	**2.680#** **0.094** **+5**	2.617 0.072 -	2.592 0.064 -1	2.661 0.142 +2	2.701 0.119 +3
PHOS [mmol/L]	Mean ± SD Δ%	1.674 0.137 -	**1.475*** **0.144** **-12**	1.651 0.174 -1	1.701 0.227 +2	1.481 0.166 -	**1.189**** **0.162** **-20**	**1.177**** **0.155** **-21**	**1.121**** **0.117** **-24**
Hormone measurements									
T3 [ng/mL]	Mean ± SD Δ%	0.340 0.137 -	0.332 0.032 -2	0.298 0.028 -12	0.337 0.059 -1	0.383 0.073 -	0.407 0.067 +6	0.433 0.075 +13	0.387 0.046 +1
T4 [ng/mL]	Mean ± SD Δ%	44.43 7.94 -	45.28 5.84 +2	37.74 5.99 -15	**34.68**** **6.32** **-23**	22.07 4.01 -	24.30 6.57 +10	26.20 7.86 +19	27.67 8.78 +25
HCD [a]		mean = 50.07	*95% CI [32.5 – 66.8]*			mean = 29.87	*95% CI [16.2 – 51.4]*		
TSH [mU/L]	Mean ± SD Δ%	0.1509 0.1348 -	0.0836 0.0647 -45	0.1150 0.1065 -24	0.1630 0.1633 +8	0.0516 0.0278 -	0.0789 0.1206 +53	0.0768 0.0698 +49	0.0581 0.0856 +13

SD = standard deviation, Δ% = percentage change compared to control, * p ≤ 0.05; ** p ≤ 0.01; Anova & Dunnett, # p ≤ 0.05; Kruskal-Wallis & Dunn, HCD a historical control data from 2019-2022, ALT = alanine transferase, ALP = alkaline phosphatase, TPROT = total protein, ALB = albumin, TBIL = total bilirubin, GLUC = glucose, TRIG = triglyceride, Ca = calcium, PHOS = phosphate, T3 = triiodothyronine, T4 = thyroxine, TSH = thyroid-stimulating hormone

Organ weight

At the top dose, body weight was statistically significantly reduced in males and females. Relative liver weight was also statistically significantly increased (+23%) in top dose males,

which was corroborated by histopathological findings. These effects were considered treatment-related and adverse.

Several organs (brain, kidney, testes and epididymis in males; brain, pituitary gland, heart, liver, spleen, and uterus/cervix in females) also showed reductions in either absolute or relative weights. However, as these findings were secondary to the low terminal body weight, they were not regarded as treatment-related.

Overall, male rats showed a treatment-related increase in relative liver weight at the top dose, which was corroborated by histopathological findings

Table C2.8-26. Selected organ weights

Sex		Males				Females			
Dose (ppm)		0	200	600	2000	0	200	600	2000
Body	Mean [g] ± SD Δ%	377.0 38.8 -	374.9 50.2 -0.6	336.8 42.3 -10.7	**313.2**** **36.0** **-16.9**	222.9 22.5 -	215.1 14.4 -3.5	204.1 13.1 -8.4	**189.1**** **17.3** **-15.2**
Brain (abs.)	Mean [g] ± SD Δ%	1.9692 0.1265 -	2.0075 0.1006 +1.9	1.9748 0.1678 +0.3	1.9655 0.1350 -0.2	1.9002 0.0766 -	1.9045 0.1021 +0.2	1.9216 0.0756 +1.1	1.8428 0.0542 -3.0
Brain (rel.)	Mean [%] ± SD Δ%	0.5260 0.0510 -	0.5428 0.0644 +3.2	**0.5909*** **0.0585** **+12.3**	**0.6319**** **0.0529** **+20.1**	0.8578 0.0640 -	0.8879 0.0600 +3.5	**0.9432*** **0.0366** **+10.0**	**0.9812**** **0.0868** **+14.4**
Epididymis (abs.)	Mean [g] ± SD Δ%	1.1210 0.0833 -	1.1190 0.0938 -0.2	1.1216 0.0986 +0.1	1.0524 0.0869 -6.1				
Epididymis (rel.)	Mean [%] ± SD Δ%	0.2990 0.0248 -	0.3015 0.0304 +0.8	**0.3350*** **0.0260** **+12.0**	**0.3379**** **0.0271** **+13.0**				
Heart (abs.)	Mean [g] ± SD Δ%	0.9973 0.1158 -	0.9744 0.1140 -2.3	0.9266 0.1428 -7.1	0.8528 0.1233 -14.5	0.7006 0.0827 -	0.6614 0.0830 -5.6	0.6292 0.0603 -10.2	**0.6031*** **0.0627** **-13.9**
Heart (rel.)	Mean [%] ± SD Δ%	0.2651 0.0232 -	0.2607 0.0092 -1.7	0.2752 0.0283 +3.8	0.2723 0.0220 +2.7	1.5159 0.1704 -	1.5440 0.2097 -2.5	1.4904 0.1601 -2.0	1.3836 0.1002 +1.5.
Kidney (abs.)	Mean [g] ± SD	2.3674 0.1312 -	2.3949 0.4001 +1.2	2.2941 0.2692 -3.1	2.2057 0.2882 -6.8	1.5159 0.1704 -	1.5440 0.2097 +1.9	1.4904 0.1601 -1.7	1.3836 0.1002 -8.7

Sex		Males			Females				
	Δ%								
Kidney (rel.)	Mean [%] ± SD Δ%	0.6319 0.0507 -	0.6373 0.0429 +0.9	0.6834 0.0583 +8.2	**0.7039**** **0.0362** **+11.4**	0.68060 .0480 -	0.7195 0.1068 +5.7	0.7303 0.0618 +7.3	0.7346 0.0577 +7.9
Liver (abs.)	Mean [g] ± SD Δ%	8.6832 1.3090 -	8.8417 1.5753 +1	7.8623 1.0340 -9	8.8927 1.5528 +2	5.4934 0.5344 -	5.2081 0.4086 -5.2	**4.9429*** **0.3820** **-10.0**	**4.9300*** **0.5704** **-10.3**
Liver (rel.)	Mean [%] ± SD Δ%	2.2979 0.1888 -	2.3517 0.1954 +2	2.3346 0.0639 +2	**2.8274##** **0.2287** **+23**	2.4681 0.1443 -	2.4230 0.1445 -1.8	2.4219 0.1068 -1.9	2.6102 0.2244 +5.8
Pituitary (abs.)	Mean [g] ± SD Δ%	0.0085 0.0013 -	0.0077 0.0010 -9.0	0.0076 0.0011 -9.9	0.0074 0.0014 -13.1	0.6245 0.1062 -	**0.5323*** **0.0553** **-14.8**	**0.5228*** **0.0704** **-16.3**	**0.4737**** **0.0729** **-24.1**
Pituitary (rel.)	Mean [%] ± SD Δ%	0.0023 0.0003 -	0.0021 0.0002 -8.0	0.0023 0.0004 +1.5	0.0023 0.0003 +4.1	0.0053 0.0009 -	0.0047 0.0006 -11.6	0.0049 0.0006 -7.9	0.0046 0.0005 -13.7
Spleen (abs.)	Mean [g] ± SD Δ%	0.5732 0.0826 -	0.5509 0.0683 -3.9	0.5191 0.0984 -9.4	0.5095 0.0881 -11.1	0.4128 0.0673 -	0.3854 0.0494 -6.6	**0.3559*** **0.0292** **-13.8**	**0.3323**** **0.0497** **-19.5**
Spleen (rel.)	Mean [%] ± SD Δ%	0.1518 0.0118 -	0.1480 0.0159 -2.5	0.1536 0.0153 +1.2	0.1624 0.0165 +7.0	0.1847 0.0203 -	0.1799 0.0261 -2.6	0.1746 0.0120 -5.5	0.1753 0.0163 -5.1
Testis (abs.)	Mean [g] ± SD Δ%	3.6715 0.3026 -	3.5301 0.2900 -3.9	3.6867 0.2647 +0.4	3.5600 0.2189 -3.0				
Testis (rel.)	Mean [%] ± SD Δ%	0.9769 0.0546 -	0.9516 0.0998 -2.6	1.1027** 0.0898 +12.9	1.1452** 0.1002 +17				
Uterus/ cervix (abs.)	Mean [g] ± SD Δ%					0.9188 0.4085 -	0.7744 0.3406 -15.7	0.5881 0.2827 -36.0	**0.4581#** **0.1366** **-50.1**

Sex		Males				Females			
Uterus/ cervix (rel.)	Mean [%] ± SD Δ%					0.4066 0.1687 -	0.3569 0.1492 -12.2	0.2829 0.1235 -30.4	0.2413 0.0655 -40.6

SD = standard deviation, Δ% = percentage change compared to control, abs. = absolute, rel. = relative, * p ≤ 0.05; ** p ≤ 0.01; Anova & Dunnett, # p ≤ 0.05; ## p ≤ 0.01 Kruskal-Wallis & Dunn

Gross pathology and histopathology

No treatment-related macroscopic pathology findings were observed in male or female rats in any treatment groups.

Treatment-related histopathological findings were observed in the liver and spleen of both sexes, adrenal glands of males from the mid dose (vacuolation of zona fasciculata), and the bone marrow of top dose females (adipocyte accumulation).

In the liver, an increased incidence of centrilobular hypertrophy was observed in top dose females and in males from the mid dose. Midzonal macrovesicular vacuolation (minimal to moderate grade) was also observed in the majority (8/10) of top dose males. These findings were considered treatment-related and adverse.

In the spleen, there was an increase in the incidence and/or severity of fine granular, golden-brown pigmentation in the cytoplasm of red pulp macrophages in females from the mid dose and in top dose males. It was postulated that this pigment was haemosiderin, which typically occurs following breakdown of red blood cells. This was accompanied by a decrease in extramedullary haematopoiesis and a statistically significant decrease in reticulocytes in females (but not males) from the mid dose (see haematology section above), suggesting that these animals are unable to apply this compensatory response following increased erythrocyte breakdown. Therefore, the histopathological findings observed in the spleen in females from the mid dose are considered treatment-related and adverse.

In the adrenals, males showed increased incidence of zona fasciculata vacuolation (minimal to mild from the mid-dose). These consisted of micro- and macrovesicular vacuoles (with a similar appearance to vacuolation observed in the liver of males) and were considered treatment-related and adverse.

In the bone marrow, increased accumulation of adipocytes was present in 5/10 female rats at the top dose. This finding was also observed in 3/10 and 1/10 females at 200 ppm and 600 ppm respectively. Since there was no clear dose-response and the findings were of minimal severity, they were not regarded as treatment-related and adverse.

Overall, treatment-related and adverse histopathological findings were observed from the mid dose in males (adrenal glands, liver) and females (spleen). Histopathological findings were also observed in the liver of top dose females.

Table C2.8-27. Selected histopathological findings

Sex	Males				Females			
Dose (ppm)	0	200	600	2000	0	200	600	2000
Liver [N]	10	10	10	10	10	10	10	10
- Hypertrophy, centrilobular	0	0	**3**	**9**	0	0	0	**4**
Minimal	-	-	**3**	**4**	-	-	-	**4**
Mild	-	-	-	**4**	-	-	-	-
Moderate	-	-	-	**1**	-	-	-	-
Vacuolation, hepatocellular	0	0	0	**8**	0	0	0	1
Minimal	-	-	-	**1**	-	-	-	1
Mild	-	-	-	**2**	-	-	-	-
Moderate	-	-	-	**5**	-	-	-	-
Spleen [N]	10	10	10	10	10	10	10	10
Pigment	8	10	8	9	7	6	**10**	**10**
Minimal	7	8	6	2	4	6	**7**	**0**
Mild	1	2	2	7	3	-	**1**	**4**
Moderate	-	-	-	-	-	-	**2**	**6**
Extramedullary haematopoiesis	4	4	1	3	6	5	**1**	**0**
Minimal	4	4	1	2	4	4	**1**	**-**
Mild	-	-	-	1	2	1	**-**	**-**
Adrenal gland [a]	10	10	10	10	10	-	-	10
Vacuolation, zona fasciculata	0	1	**5**	**6**	0			0
Minimal	-	1	**5**	**5**	-			-
Mild	-	-	**-**	**1**	-			-
Bone marrow, sternum [a]	10	-	-	10	10	10	10	10
Adipocyte accumulation					0	3	1	**5**
Minimal					-	3	1	**5**

a = number of tissues examined per group, findings in bold text were considered treatment-related

Conclusion
Under the conditions of this GLP- and OECD-compliant 90-day study in rats, dietary administration of metabolite R014821 resulted in no observed mortality or treatment-related clinical signs of toxicity.

Treatment-related and adverse reductions in body weight and body weight gain, accompanied by reduced food consumption, were observed in males and females from the mid dose of 600 ppm (equivalent to 42 and 45 mg/kg bw/d in males and females respectively). A treatment-related and potentially adverse decrease in reticulocyte count, corroborated by histopathological findings in the spleen, was observed in females from the mid dose.

The target organs identified in this study were the liver, spleen and adrenal glands. Treatment-related and adverse effects on histopathology were observed in animals of both sexes in the liver (males: from the mid dose, females: top dose only) and the spleen (females: from the mid dose), with additional liver relative organ weight changes reported in top dose males (+23 %). Males also showed treatment-related and adverse histopathological findings in the adrenal gland (vacuolation of zona fasciculata) from the mid dose. These findings were supported by similar histopathological findings which had previously been reported in the animals treated with 1600 ppm R014821 in the 90-day comparative toxicity study on imazalil and its metabolites.

Based on these findings, a NOAEL of 200 ppm (equivalent to 14 and 15 mg/kg bw/d in males and females) was established by HSE as no adverse effects were seen at this dose. A LOAEL of 600 ppm in males and females (equivalent to 42 and 45 mg/kg bw/d respectively) was established based on reductions in body weight and body weight gain in both sexes. At this dose, females also showed haematological changes and histopathological findings in the spleen, while males showed histopathological findings in the liver and adrenal glands.

R014821- Summary of toxicological data and derivation of dietary reference values

The table below summarises all the data available on metabolite R014821.

Table C2.8-28. All available data on R014821

Study Type	Species / test system	Result	Reference
Acute oral LD_{50}	Rat	> 2000 mg/kg bw	report no. 25876/1 (2011)
90-day comparative study	Rat (Wistar Han)	May have slightly higher toxicity than imazalil	[redacted] 2021 (20192866; AGR 5959)

Study Type	Species / test system	Result	Reference
In vitro Ames	*S. typhimurium* 3.16 to 1000 µg/plate	± S9: negative	[redacted] (2010)
In vitro chromosome aberration	Chinese hamster V79 lung cells	± S9: negative	[redacted] (2017)
Mammalian cell gene mutation	CHO K1 Chinese hamster ovary cells, *hprt* locus	-S9: equivocal +S9: negative	[redacted] (2017)
In vitro micronucleus	Human lymphocytes	± S9: negative	[redacted] 2019 (20192868; AGR 5957)
In vivo Comet	Rat (Wistar Han)	Negative	[redacted] 2020a (20202368; AGR 5964)
Combined 28-day repeated dose toxicity study with reproduction/ developmental toxicity screening, oral gavage	Rat (Wistar Han)	Parental LOAEL: 30 mg/kg bw/d (spleen histopathology) Reproductive and offspring NOAEL: 30 mg/kg bw/d (reduction in implantation sites, fertility index, gestation index and post-implantation survival at 100 mg/kg bw/d)	[redacted] 2018 (517992)
90-day dietary study	Rat (Wistar Han)	NOAEL: 200ppm, (equivalent to 14/15 mg/kg bw/d in M/F respectively), based on reductions in body weight and body weight gain in both sexes, haematological changes and histopathological findings in the spleen in females, and histopathological findings in the liver and adrenal glands in males at the LOAEL of 42 mg/kg bw/d	[redacted] 2022 (20361822; AGR6321)

Based on a comprehensive genotoxicity package, it can be concluded that R014821 is not genotoxic. With regard to general toxicity, the acute oral LD50 was > 2000 mg/kg bw. Compared to the acute oral LD50 for imazalil of 227 mg/kg bw (EFSA, 2010), R014821 is less acutely toxic than imazalil. In a OECD 422 gavage study in rats, the target organs were the liver, kidney, adrenals and spleen. Effects on the oestrus cycle, the fertility index, implantation index, gestation index, litter size, post-implantation survival index and survival of offspring were observed from a dose of 100 mg/kg bw/d. A LOAEL of 30 mg/kg bw/d was identified for parental toxicity and a NOAEL of 30 mg/kg bw/d was identified for reproductive toxicity and offspring toxicity. In a subsequent 90-day dietary study in the rat, the target organs were the liver, the adrenals and the spleen. A NOAEL of 14 mg/kg bw/d was identified based on reductions in body weight and body weight gain in both sexes, haematological changes and histopathological findings in the spleen in females, and histopathological findings in the liver and adrenal glands in males at the LOAEL of 42 mg/kg bw/d. These data indicate that R014821 may be (quantitatively) slightly more toxic than the parent imazalil. However, more importantly, the data show that qualitatively R014821 has a different toxicological profile compared to imazalil, with effects on the haematological system and the spleen, effects on the adrenals and effects on reproduction not seen with the parent substance. On this basis, metabolite-specific dietary reference values should be derived.

The most appropriate NOAEL for the derivation of the ADI for R014821 is the NOAEL of 14 mg/kg bw/d from the 90-day study. By applying the standard safety factor of 100 for inter- and intra-species variability and an additional factor of 10 for the lack of dog data, cancer studies, multi-generation and developmental studies, an ADI of 0.014 mg/kg bw/d is established.

ADI = 0.014 mg/kg bw/d

For the derivation of the ARfD, the most relevant NOAEL is the reproductive and offspring toxicity NOAEL of 30 mg/kg bw/d from the OECD 422 study. By applying the standard safety factor of 100 for inter- and intra-species variability and an additional factor of 10 for lack of acute neurotoxicity and developmental toxicity studies, an ARfD of 0.03 mg/kg bw is established.

ARfD = 0.03 mg/kg bw

It should be noted that in line with the conclusion that R014821 may be slightly more toxic than the parent compound imazalil, both these reference values are lower than those established for imazalil (ADI = 0.025 mg/kg bw/d; ARfD = 0.05 mg/kg bw).

Metabolite FK284

Metabolite FK284 is not a major rat metabolite. Four studies have been previously evaluated and found acceptable for FK284; an acute oral toxicity study and a standard package of 3 in vitro mutagenicity studies (Ames, chromosome aberration and mammalian cell gene mutation). Based on the outcome of these studies the acute oral LD50 of FK284 was greater than 2000 mg/kg bw and there was no evidence of genotoxicity in the 3 in vitro tests. These studies are summarised below:

Table C2.8-29. Previous studies on FK284

Study Type	Species / test system	Result	Reference
Acute oral LD_{50}	Rat	> 2000 mg/kg bw	report no. 25985 (2010)
Ames	*S. typhimurium* 31.6 to 5000 µg/plate	± S9: negative	[redacted] (2010)
In vitro chromosome aberration	Chinese hamster V79 lung cells	± S9: negative	[redacted] (2017)
Mammalian cell gene mutation	CHO K1 Chinese hamster ovary cells *hprt* locus	± S9: negative	[redacted] (2017)

As part of this MRL confirmatory data application, in addition to the 90-day comparative toxicity study, the applicant submitted two micronucleus studies (one *in vitro* and one *in vivo*) in order to fully exclude the genotoxic potential of FK284 by addressing the endpoint of aneugenicity. This additional information has been fully evaluated by HSE below.

New information submitted with this application

An in vitro Micronucleus Assay with FK284 in Cultured Peripheral Human Lymphocytes ([redacted] 2020b)

Reference: An in vitro Micronucleus Assay with FK284 in Cultured Peripheral Human Lymphocytes, [redacted] (2020b)
Report No. AGR 5955
Test facility study no. 20202367

Date performed: Completion date: 26th Jan 2020

Test facility: [redacted]

Guideline(s):	Yes; OECD 487 (2016)
Deviations:	A reduced number of binucleate cells were sampled for the cultures incubated with colchicine. This deviation was not considered to affect the reliability of the study.
GLP:	Yes
Test material:	FK284 Batch: 308-051-000-A-20190506-AWO Purity: 94% Vehicle: DMSO
Study acceptable:	Yes

Method

The potential of FK284 to induce micronuclei in human lymphocytes in vitro was investigated in a GLP and OECD TG compliant study. A preliminary assay ('pre-test') was performed to assess the cytotoxicity of FK284 in the absence and presence of S9 mix and to determine the test substance concentration ranges for the main study.

In the main study, cells were incubated with FK284 for 3 hours in the presence and absence of metabolic activation (S9 mix) at concentrations of 500, 1000 and 2000 µg/ml. No appropriate concentration levels could be selected for scoring of micronuclei because the top concentration of 2000 µg/ml was too toxic (cytokinesis-block proliferation index (CBPI) of 98 % and 95 % in the absence and presence of S9 mix respectively). The experiment was repeated (with and without S9 mix) in Experiment IA at the following concentrations: 500, 1000, 1200, 1400, 1600, 1800 and 2000 µg/ml. In Experiment II, cells were incubated continuously with FK284 for 24 hours in the absence of metabolic activation at concentrations of 50, 100, 125, 150, 175, 200, 225, 250 and 300 µg/ml. In each experiment, concurrent solvent (DMSO) and positive controls (mitomycin C (MMC) and colchicine (Colch) without metabolic activation, cyclophosphamide (CP) with metabolic activation) were included. In each experimental group, two parallel cultures were analysed. At least 1000 binucleate cells per culture (2000 cells per test group) were scored and recorded as percent micronucleated cells. To investigate cytotoxicity, the CBPI was determined in at least 500 cells per culture. CBPI was then used to determine percent cytostasis and assess cytotoxicity, with a reduction in CBPI to 45 ± 5 % of the concurrent negative control considered an indication of cytotoxicity.

Results

All relevant experimental conditions were tested, the criteria in the TG for cell proliferation were fulfilled, an adequate number of cells and concentrations were analysed and testing

was performed up to the cytotoxicity limit (CBPI of 45 ± 5% of the concurrent negative control).

Cytotoxicity and solubility
Precipitation was observed in the pre-test and in Experiments I and IA following 3 hours exposure in the presence and absence of S9 mix at the highest tested concentration of 2000 µg/l. No precipitation was observed at any other point in the experiment.

No cytotoxicity was observed in the pre-test at up to the limit concentration of 2000 µg/ml. However, cytotoxicity was observed from a concentration of 1400 µg/ml in Experiment IA (with and without S9 mix), and from 250 µg/ml FK284 following 24 hours exposure without S9 mix in Experiment II.

Micronucleus assay
In experiment IA (short treatment), statistically significant increases in the number of binucleated cells with micronuclei were observed in the presence and absence of S9 mix compared to concurrent controls. These increases were concentration-related and the reported values were comparable to those induced by the positive control substances. In experiment II (long treatment), no statistically significant or biologically relevant increases in micronucleated cells were observed compared to solvent control at any concentration level without metabolic activation.

The concurrent negative controls produced no increase in the number of micronucleated cells, with results within the 95% control limits of the laboratory negative HCD. The concurrent positive controls (mitomycin C and CP) induced clear increases in the number of micronucleated binucleate cells, which were also within the 95 % control limits of the laboratory positive HCD. For the positive control substance colchicine, the number of binucleated cells with micronuclei was statistically significantly increased after both 3h and 24h exposure. This was within 95 % control limits of the laboratory positive HCD after 3h exposure but after 24h, the values obtained were below the lower 95% control limit of the laboratory positive HCD. Due to the high degree of cytostasis in the samples exposed to colchicine, smaller samples of binucleate cells were scored (1811 after 3h exposure, 400 after 24h exposure) than that prescribed in the OECD guideline. The smaller sample size may account for the low number of binucleated cells with micronuclei in the colchicine 24h treatment group. Therefore, although the acceptability criteria were not met for colchicine after 24 h exposure, the study was already positive with and without S9 following the 3h treatment and it is hence valid.

Table C2.8-30. Summary of results of the in vitro micronucleus assay with FK284

Exp.	Test item concentration in µg/ml	Proliferation index (CBPI)	Cytostasis in % [a]	Micronucleated cells [b]	HCD [c] (mean ± SD) [95% control limit]
IA	Solvent control[1]	1.58	0	5	6.5 ± 4.3 [-2 – 15]
	0.25 MMC	1.33	44	**69*** **	47.0 ± 19.6 [9 – 85]
	0.38 MMC	1.28	52	n.s.	
	0.1 Colchicine	1.10	83	**39*** ** [2]	
	500	1.55	6	5	
	1000	1.47	20	n.s.	
	1200	1.41	29	**22*** **	
	1400	1.30	49	**30*** **	
	1600	1.10	82	n.s.	
	1800	1.10	83	n.s.	
	2000[P]	1.04	93	n.s.	

Exposure period 3 hrs without S9 mix

Exp.	Test item concentration in µg/ml	Proliferation index (CBPI)	Cytostasis in % [a]	Micronucleated cells [b]	HCD [c] (mean ± SD) [95% control limit]
IA	Solvent control[1]	1.63	0	7	6.6 ± 4.3 [-2 – 15]
	15 CP	1.20	69	**37*** **	35.3 ± 14.4 [7 – 64]
	17.5 CP	1.17	74	n.s.	
	500	1.59	7	10	
	1000	1.46	27	**15***	
	1200	1.44	30	n.s.	
	1400	1.29	53	**33*** **	
	1600	1.20	68	n.s.	
	1800	1.12	82	n.s.	
	2000 [P]	1.08	87	n.s.	

Exposure period 3hrs with S9 mix

Exp.	Test item concentration in µg/ml	Proliferation index (CBPI)	Cytostasis in % [a]	Micronucleated cells [b]	HCD [c] (mean ± SD) [95% control limit]
II	Solvent control[1]	1.66	0	3	5.9 ± 4.1 [-2 – 14]
	0.15 MMC	1.38	43	**39*** **	41.3 ± 17.4 [7 – 75]
	0.05 Colchicine	1.02	16.7	**3*** [3]	
	50	1.36	46	5	
	175	1.27	60	3	
	225	1.20	69	8	

Exposure period 24 hrs without S9 mix

CBPI = Cytokinesis-block proliferation index; HCD = Historical control data based on experiments performed between 2016 – 2019, [a] Values are relative to the solvent controls for the positive control groups and test item treatment groups, [b] Number of micronucleated cells was determined in a sample of 2000 binucleated cells, [c] Number of cells with micronuclei per 1000 cells, *P* Test item precipitation in the culture medium, * Significantly different from the corresponding solvent control (*P <0.05, **P <0.01, ***P <0.001), n.s. not scored, [1] DMSO 1.0% (v/v), [2] 964 and 847 binucleated cells were scored for the presence of micronuclei in each replicate respectively, [3] 219 and 181 binucleated cells were scored for the presence of micronuclei in each replicate respectively, MMC = Mitomycin C,CP = Cyclophosphamide

Conclusion

In conclusion, under the conditions of this GLP and OECD test guideline compliant in vitro study, FK284 induced micronuclei in human lymphocytes in the absence and presence of metabolic activation in the short-treatment experiment. As the study produced positive results, an in vivo follow-up micronucleus study was performed.

Micronucleus test in Bone Marrow Cells of the mouse with FK284 ([redacted] 2020d)

Reference:	An in vivo Micronucleus Assay with FK284, [redacted] (2020d) Report No. AGR 6184 Test facility study no. 20247349
Date performed:	Completion date: 26th Jul 2020
Test facility:	[redacted]
Guideline(s):	Yes; OECD 474 (2016)
Deviations:	No deviations that affected the reliability of the study (dose volume was 10 ml/100g body weight; recommended maximum in OECD 474 is 2 ml/100 g)
GLP:	Yes
Test material:	FK284 Batch: 308-051-000-A-20190506-AWO Purity: 94 % Vehicle: 1 % hydroxypropyl methylcellulose (HPMC) in water
Study acceptable:	Yes

Method

The potential of FK284 to induce micronuclei in immature erythrocytes (PCE) in the bone marrow of NMRI mice was investigated in vivo in a GLP and OECD TG compliant study. A pre-test was performed in mice (3/sex/dose) to determine the doses for the main study. In the pre-test, animals were dosed with 2000 mg/kg bw FK284 by oral gavage on 2 consecutive days and examined for clinical signs of toxicity for 3 days.

In the main study, male mice (5/dose) were administered two doses spaced 24 hours apart of FK284 at 500, 1000, and 2000 mg/kg bw by oral gavage in a vehicle of 1% HPMC in water. The total dose volume was 10ml/100g body weight, which is higher than the maximum of 2ml/100g body weight for aqueous solutions recommended in the OECD test guideline. However, this deviation was not expected to have an impact on the results or the reliability of the study. Additional groups of 10 animals were dosed with the vehicle or 2000 mg/kg bw FK284 for bioanalysis/ toxicokinetic (TK) studies. Animals were examined for clinical signs of toxicity at regular intervals after administration up to sacrifice at 24 hours after the final dose, after which bone marrow cells were collected from both femora for micronuclei analysis. Blood was sampled at necropsy for the vehicle and top dose animals in the main study. To determine whether FK284 was systemically available, and the target tissue was exposed, blood samples were taken 1, 2, 4, and 6 hours after the second dose in TK animals and the plasma was analysed to determine the concentration of FK284. Concurrent vehicle (1 % HPMC in water) and positive (40 mg/kg bw cyclophosphamide (CP), 24 h sacrifice only) controls were included. At least 4000 PCE cells per animal were examined for micronucleus formation. The ratio of polychromatic to normochromatic erythrocytes was also determined for each animal.

Fully validated methods of analysis for FK284 in the vehicle (1% HPMC in water) at concentrations covering those tested and in the plasma are available (see validated method of analysis above).

Results

Clinical observations

No mortality was observed in the pre-test. Mild clinical signs of toxicity (ataxia, lethargy) were observed in one male and one female mouse within 2.5h of dosing, which persisted to day 3 of the study. Similar clinical observations were recorded for animals dosed with 2000 mg/kg bw FK284 in the main study and the bioanalysis group. No clinical observations were noted in the low and mid dose groups in the main study and no effects on body weight were recorded in any of the test groups.

Cytotoxicity

The ratio of polychromatic to normochromatic erythrocytes was not reduced following treatment with FK284 compared to the vehicle control, indicating that FK284 did not have any cytotoxic properties in the bone marrow. Animals treated with the positive control

cyclophosphamide showed an expected decrease in the ratio of polychromatic to normochromatic erythrocytes, demonstrating toxic effects on erythropoiesis.

Bioavailability

FK284 was detected at high levels (in most instances above the upper limit of quantification (ULOQ) of 20,000 ng/ml) in plasma samples taken from test item-treated animals at 1, 2, 4, and 6h after dosing. Plasma samples taken at necropsy (24h after the last dose) showed plasma concentrations of FK284 ranging from 70.5 – 2180 ng/ml in the top dose group. In contrast, most samples taken from vehicle-dosed animals were under the lower limit of quantification (LLOQ), ranging from 0.433 – 2.43 ng/ml. Samples from vehicle-dosed animals that were above the LLOQ were re-analysed to confirm the result. Therefore, the bioavailability of FK284 was quantitatively confirmed and it can be concluded that bone marrow exposure was achieved.

Table C2.8-31. Bioavailability of FK284 in mouse

	Subject #	Sampling time (hours after 2nd dose)	Plasma concentration [ng/ml]
Vehicle control	01	24h / at necropsy	*2.43*
	02		*<LLOQ*
	03		*<LLOQ*
	04		*<LLOQ*
	05		*<LLOQ*
	26	1h	*0.433*
	27		*<LLOQ*
	28		0.581
	29	2h	*<LLOQ*
	30		*<LLOQ*
	31		0.635
	32	4h	*<LLOQ*
	33		*<LLOQ*
	34		*<LLOQ*
	35	6h	*<LLOQ*
	36		*<LLOQ*
	37		*<LLOQ*
FK284 2000 mg/kg bw	16	24h / at necropsy	2190
	17		57.9
	18		331
	19		224
	20		70.5
	39	1h	>ULOQ
	40		>ULOQ
	41		>ULOQ
	42	2h	19,800
	43		>ULOQ
	43		>ULOQ
	44	4h	>ULOQ
	45		>ULOQ

	Subject #	Sampling time (hours after 2nd dose)	Plasma concentration [ng/ml]
	46		>ULOQ
	47	6h	18,800
	48		>ULOQ
	49		19,200

LLOQ = lower limit of quantification = 0.3 ng/ml; ULOQ = upper limit of quantification = 20,000 ng/ml

Micronucleus assay

No biological or statistically significant increases in the number of micronucleated cells were observed in mice following treatment with FK284 up to the limit dose of 2000 mg/kg bw. The concurrent negative control also produced no increase in the number of micronucleated cells, while the concurrent positive control (CP) induced a clear increase in the number of micronucleated PCE. The results of both the negative and positive controls were within the 95 % control limits of the laboratory HCD. An appropriate number of doses and cells were analysed, and testing was performed up to the limit dose of 2000 mg/kg bw. Therefore, all the acceptability criteria have been met and the test is considered acceptable.

Table C2.8-32. Summary of results from the micronucleus test in mouse bone marrow cells with FK284

Substance	Dose (mg/kg bw)	PCE with MN	Ratio PCE/NCE	HCD MN in PCE (%)
		Mean ± SD (%)	Mean ± SD	Mean ± SD [95% CL][a]
Vehicle control	-	1.4 ± 1.1	1.02± 0.07	*3.0 ± 1.0 [1 - 5]*
FK284	500	2.4 ± 1.9	0.99 ± 0.09	
	1000	2.0 ± 1.9	0.96 ± 0.11	
	2000	3.6 ± 2.8	0.95 ± 0.13	
CP	40	22.8** ± 6.4	0.63 ± 0.14	*37.6 ± 9.6 [19 - 256]*

MN = Micronucleated cells; PCE = Polychromatic (immature) erythrocytes; NCE = Normochromatic (mature) erythrocytes; SD = Standard deviation; CP = Cyclophosphamide; [a] HCD (Historical control data) obtained in the performing laboratory between Nov 2016 – Jun 2020; statistically significant at * $p \leq 0.05$, ** $p \leq 0.01$

Conclusion

In conclusion, under the conditions of this GLP and OECD test guideline compliant study, FK284 did not induce micronuclei in the bone marrow of NMRI mice up to the limit dose of 2000 mg/kg bw. Evidence of bone marrow exposure to FK284 (measurement of high plasma blood levels of FK284 demonstrating systemic bioavailability) was provided. Therefore, it is concluded that FK284 is not clastogenic or aneugenic in mice in the micronucleus assay up to the limit dose of 2000 mg/kg bw, at which exposure of the bone marrow was demonstrated. Hence, the positive result seen in vitro was not expressed in vivo and it can be concluded that FK284 is not genotoxic.

FK284 – Summary of toxicological data and derivation of dietary reference values

The table below summarises all the data available on metabolite FK284.

Table C2.8-33. All available data on FK284

Study Type	Species / test system	Result	Reference
Acute oral LD_{50}	Rat	> 2000 mg/kg bw	report no. 25985 (2010)
Comparative 90-day study	Rat (Wistar Han)	Similar toxicity to imazalil	[redacted] 2021 (20192866; AGR 5959)
Ames	*S. typhimurium* 31.6 to 5000 µg/plate	± S9: negative	[redacted] (2010)
In vitro chromosome aberration	Chinese hamster V79 lung cells	± S9: negative	[redacted] (2017)
Mammalian cell gene mutation	CHO K1 Chinese hamster ovary cells *hprt* locus	± S9: negative	[redacted] (2017)
In vitro micronucleus study	Human lymphocytes	± S9: positive	[redacted] 2020b (20202367; AGR 5955)
In vivo micronucleus study	Mouse (NMRI)	negative	[redacted] 2020d (20247349; AGR 6184)

Based on a comprehensive genotoxicity package, it can be concluded that FK284 is not genotoxic. With regard to general toxicity, the acute oral LD50 in the rat was > 2000 mg/kg bw. Compared to the acute oral LD50 for imazalil of 227 mg/kg bw (EFSA, 2010), FK284 is less acutely toxic than imazalil. In a comparative 90-day study in the rat, FK284 showed similar toxicity to imazalil. On this basis, the dietary reference values of imazalil (ADI = 0.025 mg/kg bw/d; ARfD = 0.05 mg/kg bw) can be used in the risk assessment of FK284 if required.

Metabolite FK772

Metabolite FK772 is not a major rat metabolite. Four studies have been previously evaluated and found acceptable for FK772; an acute oral toxicity study and a standard battery of 3 in vitro mutagenicity tests (Ames, chromosome aberration and mammalian cell gene mutation). Based on the outcome of these studies, the acute oral LD_{50} of FK772 was greater than 2000 mg/kg bw. FK772 was negative in the Ames test and in the mammalian cell gene mutation assay; however, it gave an equivocal response in the absence of S9 in the chromosome aberration test. These studies are summarised below:

Table C2.8-34. Previous studies on FK772

Study Type	Species / test system	Result	Reference
Acute oral LD_{50}	Rat	> 2000 mg/kg bw	report no. 25983 (2010)
Ames	*S. typhimurium* 31.6 to 5000 µg/plate	± S9: negative	[redacted] (2012)
In vitro chromosome aberration	Chinese hamster V79 lung cells	+S9: negative -S9: equivocal	[redacted] (2017)
Mammalian cell gene mutation	CHO K1 Chinese hamster ovary cells *hprt* locus	± S9: negative	[redacted] (2017)

As part of this MRL confirmatory data application, in addition to the 90-day comparative study, the applicant submitted two in vitro studies (micronucleus study and mammalian gene cell mutation study) in order to fully exclude the genotoxic potential of FK277. The in vitro micronucleus test was performed to clarify the equivocal response seen in the chromosome aberration test and to address the endpoint of aneugenicity. It is unclear why a second mammalian cell gene mutation assay was performed as the first one gave a negative result. This additional information has been fully evaluated by HSE below.

New information submitted with this application

An in vitro Micronucleus Assay with FK772 in Cultured Peripheral Human Lymphocytes ([redacted] 2020c)

Reference:	An in vitro Micronucleus Assay with FK772 in Cultured Peripheral Human Lymphocytes, [redacted] (2020c) Report No. AGR 5956 Test facility study no. 20202366
Date performed:	Completion date: 29th Oct 2019
Test facility:	[redacted]
Guideline(s):	Yes; OECD 487 (2016)
Deviations:	Minor deviations (e.g. reduced number of binucleated cells were sampled for the positive control colchicine), which did not impact the reliability of the study.
GLP:	Yes

Test material:	FK772 Batch: ND19/ALOPES_0150E Purity: 96.9% Vehicle: DMSO
Study acceptable:	Yes

Method

The potential of FK772 to induce micronuclei in human lymphocytes in vitro was investigated in a GLP and OECD TG compliant study. A preliminary assay ('pre-test') was performed to assess the cytotoxicity of FK772 in the absence and presence of S9 mix and to determine the test substance concentration ranges for the main study.

In Experiment I of the main study, cells were incubated with FK772 for 3 hours in the absence and presence of metabolic activation at concentrations of 500, 1000 and 2000 µg/ml and 500, 1000, 1250, 1500, 1750 and 2000 µg/ml respectively. In Experiment II of the main study, cells were incubated with FK772 continuously for 24 hours in the absence of metabolic activation at concentrations of 50, 250, 300, 350, 400, 450, 500 and 550 µg/ml. Concurrent solvent (DMSO) and positive controls (mitomycin C (MMC) and colchicine (Colch) without metabolic activation, cyclophosphamide (CP) with metabolic activation) were included in each experiment and two parallel cultures were analysed in each experimental group. At least 1000 binucleate cells per culture (2000 cells per test group) were scored and recorded as percent micronucleated cells. To investigate cytotoxicity, the cytokinesis-block proliferation index (CBPI) was determined in at least 500 cells per culture. CBPI was then used to determine percent cytostasis and assess cytotoxicity, with a reduction in CBPI to 45 ± 5 % of the concurrent negative control considered an indication of cytotoxicity.

Results

All relevant experimental conditions were tested and an adequate number of cells and concentrations were analysed in accordance with the acceptability criteria of the OECD 487 test guideline.

Cytotoxicity and solubility

In the pre-test, an acceptable cytotoxicity was observed at the highest tested concentration of 2000 µg/ml following an exposure period of 3 hours with metabolic activation. No cytotoxicity was observed up to the limit concentration of 2000 µg/ml after 3 hours without metabolic activation; however, after 24 hours exposure without S9 mix, cytotoxicity was observed from 500 µg/ml.

No precipitation of the test substance was observed in the pre-test or in the main study up to the highest tested concentrations (2000 µg/ml and 550 µg/ml in Experiments I and II respectively).

Micronucleus assay
In the absence of S9 mix, exposure to FK772 for 3h or 24h did not induce a statistically significant or biologically relevant increase in the number of cells with micronuclei compared to concurrent controls. In the presence of S9 mix, FK772 induced a statistically significant and concentration-related increase in the number of binucleated cells with micronuclei at the highest tested concentration of 2000 µg/ml. However, the number of cells with micronuclei in each duplicate (7/1000 and 6/1000) was within the laboratory HCD range of solvent controls. Therefore, the increase in the number of micronucleated cells in the presence of S9 mix was not considered biologically relevant.

The concurrent negative controls produced no increase in the number of micronucleated cells, with results within the 95% control limits of the laboratory negative HCD. The concurrent positive controls (mitomycin C, cyclophosphamide and colchicine) induced clear increases in the number of micronucleated cells, which were also within the 95% control limits of the positive HCD. The criteria in the TG for cell proliferation were fulfilled and all relevant experimental conditions were tested. An adequate number of cells and concentrations were analysed and testing was performed up to the cytotoxicity limit (CBPI of 45 ± 5% of the concurrent negative control). Therefore, as all the acceptability criteria have been met, the test is considered to be acceptable.

Table C2.8-35. Summary of results from in vitro micronucleus assay with FK772

Exp.	Test item concentration in µg/ml	Proliferation index (CBPI)	Cytostasis in % [a]	Micronucleated cells [b]	HCD [c] (mean ± SD) [95% control limit]
I	Solvent control[1]	1.60	0	2	3.46 ± 2.31 [-1.57 – 8.50]
	0.25 MMC	1.35	41	**27*** **	24.93 ± 9.85 [4.58 – 45.27]
	0.38 MMC	1.28	54	n.s.	
	0.1 Colchicine	1.22	63	**23*** **	
	500	1.58	3	6	
	1000	1.51	15	4	
	2000	1.31	49	4	

Exposure period 3 hrs without S9 mix

Exp.	Test item concentration in µg/ml	Proliferation index (CBPI)	Cytostasis in % [a]	Micronucleated cells [b]	HCD [c] (mean ± SD) [95% control limit]
I	Solvent control[1]	1.65	0	4	3.46 ± 2.30 [-1.69 – 8.62]
	15 CP	1.21	68	**22*** **	18.48 ± 8.68 [2.07 – 34.88]
	17.5 CP	1.18	72	n.s.	
	500	1.63	3	2	
	1000	1.55	16	n.s.	
	1250	1.52	20	n.s.	
	1500	1.51	21	n.s.	
	1750	1.43	34	3	
	2000	1.27	59	**13***	

Exposure period 3hrs with S9 mix

Exp.	Test item concentration in µg/ml	Proliferation index (CBPI)	Cytostasis in % [a]	Micronucleated cells [b]	HCD [c] (mean ± SD) [95% control limit]
II	Solvent control[1]	1.80	0	15	3.35 ± 2.48 [-2.53 – 9.23]
	0.15 MMC	1.41	49	**51*** **	22.77 ± 11.94 [-2.06 – 47.60]
	0.23 MMC	1.35	57	n.s.	
	0.05 Colchicine	1.04	95	**11** ** [d]	
	50	1.72	10	14	
	250	1.60	25	n.s.	
	300	1.51	37	12	
	350	1.46	43	n.s.	
	400	1.39	52	14	
	450	1.30	62	n.s.	
	500	1.27	66	n.s.	
	550	1.24	70	n.s.	

Exposure period 24 hrs without S9 mix

CBPI = Cytokinesis-block proliferation index; HCD = Historical control data based on experiments performed between 2015 – 2018; [a] Values are relative to the solvent controls for the positive control groups and test item treatment groups, [b] Number of micronucleated cells was determined in a sample of 2000 binucleated cells, [c] Number of cells with micronuclei per 1000 cells; [d] Number of micronucleated cells was determined in a sample of 519 binucleated cells, * Significantly different from the corresponding solvent control (*P <0.05, **P <0.01, ***P <0.001), n.s. not scored, [1] DMSO 1.0% (v/v), MMC = Mitomycin C, CP = Cyclophosphamide

Conclusion

In conclusion, in this GLP and OECD test guideline compliant study, FK772 did not induce micronuclei in human lymphocytes under any experimental conditions. Overall, FK772 showed no clastogenic or aneugenic potential under the conditions of this study either in the presence or absence of metabolic activation, up to the limit of cytotoxicity.

Evaluation of the Mutagenic Activity of FK772 in an in vitro Mammalian Cell Gene

Mutation Test with L5178Y Mouse Lymphoma Cells (2020)

Reference:	Evaluation of the Mutagenic Activity of FK772 in an in vitro Mammalian Cell Gene Mutation Test with L5178Y Mouse Lymphoma Cells, (2020) Report No. AGR 6067 Test facility study no. 20213109
Date performed:	Completion date: 18th Feb 2020
Test facility:	
Guideline(s):	Yes; OECD 490 (2016)
Deviations:	A minor deviation was reported, which did not impact the results or reliability of the study. The mutation frequency of one of the solvent controls (3 hours without S9 mix) was outside of the acceptability range of ≥ 50 and ≤ 170 /10^6 cells. However, as the other solvent control in this experiment was within the range and clear negative results were observed in all experiments, this deviation had no effect on the reliability of the study.
GLP:	Yes
Test material:	FK772 Batch: ND19/GF_2357 Purity: 96.9% Vehicle: DMSO
Study acceptable:	Yes

Method

The potential of FK772 to induce gene mutations in the thymidine kinase (TK) locus of mammalian cells (L5178Y TK+/- mouse lymphoma cells) in vitro was investigated in a GLP

and OECD TG compliant study. A preliminary assay ('pre-test') was performed to determine the test substance concentrations for the main study. In the main study, cells were incubated with FK772 for 3 hours in the absence and presence of metabolic activation (S9 mix) at concentrations of 5 – 1000 µg/ml (Experiment IA) and 10 – 2000 µg/ml (Experiment IB) respectively. In the absence of S9 mix, no concentration levels showed appropriate cytotoxicity as the obtained RTG at each concentration were not within the range recommended in the OECD test guideline if the highest tested concentration is based on cytotoxicity (i.e. between 20 % and 10 % RTG). Therefore, Experiment IA was repeated with the following concentrations of FK772 in the absence of metabolic activation: 150, 300, 600, 700, 800, 850, 900, 920, 940, 960 and 980 µg/ml. In Experiment II, cells were treated with FK772 for 24 hours in the absence of metabolic activation (S9 mix) at concentrations of 12.5 - 1000 µg/ml. Concurrent solvent (DMSO) and positive controls (methyl methane sulfonate (MMS) without S9 and cyclophosphamide (CP) with S9) were included in each experiment. Duplicate cultures were included in each experimental group.

Results

All relevant experimental conditions were tested and an adequate number of cells and concentrations were analysed in accordance with the acceptability criteria of the OECD 490 test guideline.

Cytotoxicity and solubility

No precipitation of FK772 was observed in the pre-test or in the main study at a concentration of up to 2000 µg/ml (limit concentration).

Testing on FK772 was performed up to the upper limit of cytotoxicity (RTG of 10 – 20%) and the upper limit of cytotoxicity was not exceeded in the positive control cultures (i.e. RTG was ≥ 10% for all positive controls).

In the pre-test, an acceptable cytotoxicity level was observed after 3 hours treatment at 2000 µg/ml in the presence of S9 and from 1000 µg/ml in the absence of S9, and from a concentration of 1000 µg/ml following 24 hours exposure in the absence of S9. In Experiment IA, cytotoxicity was observed at a concentration of 980 and 1200 µg/ml in the absence and presence of metabolic activation respectively. In Experiment II, cytotoxicity was observed at a concentration of 600 µg/ml without metabolic activation.

Mutagenicity

No statistically significant or concentration-dependent increases in mutation frequency (MF) in the TK locus were observed at any of the tested concentrations compared to solvent controls, either in the presence or absence of metabolic activation. The global evaluation factor (GEF) threshold (126 over vehicle control MF) was not exceeded in any of the experiments.

The concurrent positive controls (methylmethanesulfonate and cyclophosphamide) had induced MF (IMF) values which fulfilled the acceptance criteria developed by the IWGT workgroup and were within the 95% control limits of the laboratory positive HCD, indicating

that the test conditions were adequate and metabolic activation system (S9 mix) functioned properly.

The MF of one of the solvent control cultures following 3h exposure without metabolic activation was recorded as 187 x 10-6 cells and the mean MF of the concurrent negative control for this experiment was 171.5 x 10-6, which was outside the upper 95% control limit of the laboratory negative HCD (40 – 160 x 10-6) and slightly above the acceptance criteria range recommended by the IWGT MLA Workgroup (50 - 170 x 10-6). However, as clear negative results (MF < MF threshold; absence of statistical significance across an acceptable concentration range and replicates) were observed in all experiments and no concentration-response relationship was observed under any experimental condition, this deviation from the acceptability criteria was not considered to affect the results of the study.

Table C2.8-36. Summary of results of the TK locus mammalian gene mutation assay with FK772

Exp	Test group		Total MF [per 10^6 cells]	Small MF [per 10^6 cells]	Large MF [per 10^6 cells]	Toxicity data RTG Rel.	RSG Rel.	Cloning efficiency (CE_2 – viability) Abs.	Cloning efficiency (CE_2 – viability) Rel.
Exposure period 3 hrs without S9 mix									
IA	Vehicle control 1		156	62	83	100.0	100.0	86	100.0
	Vehicle control 2		187	73	102			72	
	MF threshold[a]		*298*						
	HCD[b]	*Mean ± SD [range]*	*100 ± 31 [40 – 160]*						
	FK772 (µg/ml)	600	145	58	77	153	140	86	109
		700	160	54	97	95	101	75	94
		800	128	50	72	94	105	71	90
		900	160	82	69	67	75	71	90
		920	140	55	80	49	62	63	80
		940	204	70	119	61	63	77	97
		960	149	64	80	40	62	50	63
		980	176	78	89	16	22	60	76
	MMS	15 µg/ml	1195	584	431	59	89	52	66
		IMF[c]	*1024*	516.5					
	HCD[b]	*Mean ± SD [range]*	817 ± 304 [221 – 1412]						
Exposure period 3 hrs with S9 mix									
IB	Vehicle control 1		84	30	51	100	100	93	100
	Vehicle control 2		113	31	78			84	
	MF threshold[a]		*225*						

Exp	Test group		Total MF [per 10^6 cells]	Small MF [per 10^6 cells]	Large MF [per 10^6 cells]	Toxicity data RTG Rel.	Toxicity data RSG Rel.	Toxicity data Cloning efficiency (CE_2 – viability) Abs.	Toxicity data Cloning efficiency (CE_2 – viability) Rel.
	HCD[b]	*Mean ± SD [range]*	*102 ± 30 [44 – 160]*						
	FK772 (µg/ml)	10	121	46	67	108	82	110	124
		50	88	41	44	103	102	85	96
		100	94	40	51	114	103	93	105
		250	113	51	56	116	98	99	113
		500	83	37	44	81	85	79	90
		1000	76	40	34	67	68	83	94
		1200	144	65	71	19	21	76	86
	CP	5 µg/ml	911	406	356	85	97	74	83
		IMF[c]	*812.5*	375.5					
	HCD[b]	*Mean ± SD [range]*	*1461 ± 885 [-273 – 3196]*						
Exposure period 24 hrs without S9 mix									
II	Solvent control 1		100	34	61	100	100	95	100
	Solvent control 2		107	37	66			75	
	MF threshold[a]		*230*						
	HCD[b]	*Mean ± SD [range]*	*100 ± 31 [38 – 162]*						
	FK772 (µg/ml)	50	127	42	80	71	81	75	88
		100	98	39	55	78	84	79	93
		200	97	38	55	74	75	84	99
		300	124	37	83	55	68	69	82
		400	94	36	55	38	40	81	96
		500	94	48	42	31	29	90	106
		550	61	13	47	21	22	80	94
		600	84	31	51	16	18	77	90
	MMS	5 µg/ml	786	318	354	70	80	75	88
		IMF[c]	682.5	282.5					
	HCD[b]	*Mean ± SD [range]*	722 ± 216 [298 – 1147]						

MF = Mutant frequency; RTG = Relative total growth; RSG = Relative Suspension growth; IMF = Induced mutant frequency; Vehicle control = DMSO, Positive controls = MMS; methylmethanesulfonate, CP; cyclophosphamide [a] MF threshold = $MF_{vehicle\ control}$ + GEF (126 x 10^{-6}), rounded; [b] Historical control data obtained in the performing laboratory between Sep 2016 – Sep 2019; IMF[c] = induced MF, rounded

Conclusion

In conclusion, in this GLP and OECD test guideline compliant study, FK772 did not increase mutation frequency in the thymidine kinase (TK) locus of mouse lymphoma cells under any experimental conditions. Overall, FK772 showed no mutagenic potential under the conditions of this study either in the presence or absence of metabolic activation, up to the limit of cytotoxicity.

FK772 – Summary of toxicological data and derivation of dietary reference values

The table below summarises all the data available on metabolite FK772.

Table C2.8-37 All available data on FK772

Study Type	Species / test system	Result	Reference
Acute oral LD_{50}	Rat	> 2000 mg/kg bw	report no. 25983 (2010)
90-day comparative study	Rat (Wistar Han)	Lower toxicity than imazalil	[redacted] 2021 (20192866; AGR 5959)
Ames	*S. typhimurium* 31.6 to 5000 µg/plate	± S9: negative	[redacted] (2012)
In vitro chromosome aberration	Chinese hamster V79 lung cells	± S9: negative	[redacted] (2017)
Mammalian cell gene mutation	CHO K1 Chinese hamster ovary cells *hprt* locus	± S9: negative	[redacted] (2017)
In vitro micronucleus	Human lymphocytes	± S9: negative	[redacted] 2020c (20202366; AGR 5956)
In vitro mammalian gene cell mutation	L5178Y mouse lymphoma cells	± S9:negative	[redacted] 2020 (20213109; AGR 6067)

Based on a comprehensive genotoxicity package, it can be concluded that FK772 is not genotoxic. With regard to general toxicity, the acute oral LD50 in the rat was > 2000 mg/kg bw. Compared to the acute oral LD50 for imazalil of 227 mg/kg bw (EFSA, 2010), FK772 is less acutely toxic than imazalil. In a comparative 90-day study in the rat, FK772 showed lower toxicity compared to imazalil. On this basis, the dietary reference values of imazalil (ADI = 0.025 mg/kg bw/d; ARfD = 0.05 mg/kg bw) can be used in the risk assessment of FK772 if required.

C.2.9 Medical data and information

No data submitted or required.

C.3 Residue data

C.3.1 Nature and magnitude of residues in primary crops

C.3.1.1 Nature of residues

No studies submitted or required.

C.3.1.2 Magnitude of residues

These studies have been previously evaluated by NL (NL ER, 2018) and have been presented below as information. The studies have been reviewed to confirm that they are relevant to the MRLs proposed within this application.

It is noted that studies 46650, 60016, 41805, 57200, and 46192 present trials within separate study reports, but were performed as replicates. For these trials, the highest residue from each replicate trial has been selected and underlined within tables C.3.1.2.1 to C.3.1.2.6.

C.3.1.2.1 Citrus

Study 1

Reference: Determination of residues of Imazalil, its metabolite R14821 (=T824) and Pyrimethanil after a single postharvest application (dip and drench) of Philabuster 400 SC in mandarin and orange, Southern Europe 2011, [redacted] (2013a), S11-03184 / AGR 4726

GLP:	Yes	Sample storage conditions:	max. 154 days ≤ -18ºC
Crop/crop group:	Oranges, mandarins / citrus	Analytical method:	QuECHERS, HPLC-MS/MS, validated
Indoor/Outdoor:	Indoor	Limit of Quantification (mg/kg):	0.01
Formulation:	SC	Limit of Detection (mg/kg):	0.003
Content of active substance (g/kg or g/l):	200 g/L Imazalil 200 g/L Pyrimethanil	Residues calculated as:	Imazalil, R14821, Pyrimethanil

The objective of the study was to determine residue levels of Imazalil, its metabolite R14821 and pyrimethanil in the raw agricultural commodities mandarin and orange.

During the 2011-2012, 8 trials were conducted on harvested oranges and mandarins grown in Spain, Italy and Greece to determine the residue level of Imazalil (and its metabolite R14821) in raw agricultural commodities after one post harvest dipping application (4 trials) or one post harvest drenching application (4 trials) with the formulated product Philabuster 400SC (nominal 200 g/L Imazalil and 200g/L) at a target rate of 250 mL/hL (equivalent to 0.050 kg Imazalil/ hL). All applications were done within 28 hours after harvest of fruits (except trial -04:31.25h). In trials -01, -02, -05 and -06 specimens of

samples from the untreated fruits were taken just before application and after a storage period of 28 - 30, 57 - 61 and 85 - 86 DAA. Samples from the treated fruits were taken after treating solution was dried on fruits and after a storage period of 28 - 30, 57 - 61 and 86 - 85 DAA. In trial -02, the last sampling was not done because mandarins were affected completely with fungus. In trials -03, -04, -07 and -08, samples from the untreated fruits were taken just before application. Samples from the treated fruits were taken after the treating solution was dried on the fruits. The trials did not exceed the demonstrated storage period of Imazalil and R14821.

Residues of Imazalil and its metabolite R14821 were analysed according to QuECHERS multi residue method and determination by HPLC with MS/MS detection (validated). Untreated and treated post harvest fruit samples were taken and analysed for residues of Imazalil, R14821 and pyrimethanil. Mean recovery efficiencies were found to lie within the required range of 70-110% at all levels of fortification and the relative standard deviation was less than 20%, thus demonstrating sufficient accuracy and precision of the method. In untreated samples, no residues of Imazalil were found above the limit of quantification (LOQ), except sample L11-03184-06-010R1 (0.014 mg/k) and L11-03184-06-011R1 (0.099 mg/kg). No reason was provided for this, however as these levels are insignificant compared to the residue levels found in the treated samples, this is not considered a major deviation and does not impact the results of the study.

In the treated mandarin whole fruit samples residues of Imazalil ranged from 1.03 to 3.34 mg/kg. For mandarin pulp samples, Imazalil ranged from 0.020 mg/kg to 0.477 mg/kg. In the treated mandarin whole fruit samples, residues of metabolite R014821 ranged from < LOQ to 0.127 mg/kg. For mandarin pulp samples, metabolite R014821 ranged from not detectable to 0.017 mg/kg.

In the treated orange whole fruit samples residues of Imazalil ranged from 0.524 to 2.81 mg/kg. For orange pulp samples Imazalil ranged from not detectable to 0.689 mg/kg . In the treated orange whole fruit samples residues of metabolite R014821 ranged from not detectable to 0.084 mg/kg. For orange pulp samples metabolite R014821 ranged from not detectable to 0.018 mg/kg.

Conclusion

The study supports one post harvest dipping application or one post harvest drenching application of mandarin and orange at a target rate of 0.05 kg Imazalil/hl with no waiting period. The study is valid to determine residue levels of Imazalil and metabolite R14821 in the raw agricultural commodities mandarin and orange.

A summary of the residue trials is given in Table C.3.1.2.1-1. When the residue level at a later WHP (with-holding period) is higher than at the recommended WHP, this highest value was selected.

In 19 samples residues of Imazalil where determined in whole fruit as well as pulp. From these samples the following individual transfer factors from whole fruit to pulp are calculated:

Mandarin: 0.009; 0.040; 0.131; 0.143; 0.067; 0.046; 0.049; 0.091; 0.208
Orange: 0.031; 0.012; 0.067; 0.261; 0.280; 0.057; 0.013; 0.009; 0.011; 0.042

Table C.3.1.2.1-1. Residue trial summary for citrus (Drenching application)

Trial No./ Location/ Year	Commodity/ Variety	Date of 1.Sowing or planting 2.Flowering 3. Harvest	App. rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analyzed	Residues (mg/kg)		WHP (days)	Remarks
			g a.s./ ha	Water (l/ha)	kg a.s./hl (IMZ/ PYR)				Imazalil (IMZ)	R14821		
S11-03184, S11-03184-02 46650, Canals, Valencia, Spain	mandarin / CIDRE / Clemenules	1) Oct 1996 2) Mar – Apr 2011 3) 20 Dec 2011	n.a.	n.a.	(0.05/ 0.05)	21 Dec 2011	85 - 89	whole fruits pulp whole fruits pulp whole fruits pulp	2.26 0.020 1.69 0.067 1.03 0.135	0.012 <0.01 0.065 <0.01 0.077 0.017	0 0 30 30 61 61	
S11-03184, S11-03184-04 40016, San Giorgio di Piano, Bologna, Italy	mandarin / CIDRE / Simeto	1) 2005 2) 04 Apr – 05 Jun 2011 3) 28 Nov 2011	n.a.	n.a.	(0.05/ 0.05)	29 Nov 2011	89	whole fruits pulp	3.34 0.477	0.020 <0.01	0 0	
S11-03184, S11-03184-06 46650, Canals, Valencia, Spain	orange / CIDSI / Navelina	1) Nov 1986 2) Mar – Apr 2011 3) 20 Dec 2011	n.a.	n.a.	(0.05/0.05)	21 Dec 2011	85 - 89	whole fruits pulp whole fruits pulp whole fruits pulp whole fruits pulp	2.81 0.087 1.47 0.017 0.524 0.035 0.564 0.147	<0.01 <0.01 0.043 <0.01 0.060 0.016 0.084 0.018	0 0 30 30 61 61 85[2] 85[2]	
S11-03184, S11-03184-08: 40016, San Giorgio di Piano, Bologna, Italy	orange / CIDSI / Navel	1) 1995 2) 05 May – 06 Jun 2011 3) 28 Nov 2011	n.a.	n.a.	(0.05/0.05)	29 Nov 2011	89	whole fruits pulp	2.46 0.689	<0.01 <0.01	0 0	

S11-03184, S11-03184-01 41805, Mairena del Aljarafe, Andalusia, Spain	mandarin / CIDRE / Oronules	) 2002 2) 19 Mar – 02 Apr 2011 3) 09 Nov 2011	n.a.	n.a.	(0.05/ 0.05)	09 Nov 2011	89	whole fruits pulp whole fruits pulp whole fruits pulp whole fruits pulp	2.66 0.177 1.53 0.070 1.68 0.082 1.34 0.122	0.018 n.d. 0.059 <0.01 0.096 <0.01 0.127 0.014	0 0 28 28 57 57 86[1] 86[1]	
S11-03184, S11-03184-03: 57200, Kolchiko, Thessaloniki, Greece	mandarin / CIDRE / Clementins	1) 1974 2) 01 Apr – 26 May 2011 3) 01 Mar 2012	n.a.	n.a.	(0.05/ 0.05)	02 Mar 2012	89	whole fruits pulp	1.77 0.368	<0.01 <0.01	0 0	
S11-03184, S11-03184-05 41805, Mairena del Aljarafe, Andalusia, Spain	orange / CIDSI / New Hall	1) 01 Jan 1999 2) 08 Mar – 11 Apr 2011 3) 16 Nov 2011	n.a.	n.a.	(0.05/ 0.05)	16 Nov 2011	89	whole fruits pulp whole fruits pulp whole fruits pulp whole fruits pulp	2.09 0.120 1.709 0.022 1.08 <0.01 0.908 <0.01	<0.01 <0.01 0.040 <0.01 0.061 <0.01 <0.01 <0.01	0 0 28 28 57 57 86[1] 86[1]	
S11-03184, S11-03184-07: 57200, Kolchiko, Thessaloniki, Greece	orange / CIDSI / Kyno	1) 1985 2) 01 Apr – 28 May 2011 3) 01 Mar 2012	n.a.	n.a.	(0.05/ 0.05)	02 Mar 2012	89	whole fruits pulp	1.56 0.066	<0.01 <0.01	0 0	

[1]At 85 DAA fruits were covered by fungus, no sampling done

[2] Residues (mg/kg) of Imazalil / pyrimethanil found in U1-samples 85 DAA: whole fruit: 0.014 / <LOQ; pulp: 0.099 / 0.127

Study 2

Reference:	Determination of residues of Imazalil and its metabolite R14821 (=T824) after a single postharvest application (dip and drench) of Fecundal 7.5 S in mandarin and orange, Southern Europe 2011, [redacted] (2013b), S11-03186 / AGR 4728		
GLP:	Yes	Sample storage conditions:	max. 155 days ≤ -18ºC
Crop/crop group:	Oranges, mandarins / citrus	Analytical method:	QuECHERS, HPLC-MS/MS, validated
Indoor/Outdoor:	Indoor	Limit of Quantification (mg/kg):	0.01
Formulation:	SL	Limit of Detection (mg/kg):	0.003
Content of active substance (g/kg or g/l):	75 g/L Imazalil	Residues calculated as:	Imazalil, R14821

The objective of the study was to determine residue levels of Imazalil, its metabolite R14821 in the raw agricultural commodities mandarin and orange.

During the 2011-2012, 8 trials were conducted on harvested oranges and mandarins grown in Spain, Italy and Greece to determine the residue level of Imazalil (and its metabolite R14821) in raw agricultural commodities after one post harvest dipping application (4 trials) or one post harvest drenching application (4 trials) with the formulated product Fecundal 7.5 S (syn. IMAZALIL 7.5 S) (75 g/L Imazalil) at a target rate of 600 mL/hL (equivalent to 0.045 kg Imazalil/ hL). Samples were taken 0 days after the last application, when the treating solution was dried on the fruits. The trials did not exceed the demonstrated storage period of Imazalil and R14821.

Residues of Imazalil and its metabolite R14821 were analysed according to QuECHERS multi residue method and determination by HPLC with MS/MS detection. Untreated and treated post harvest fruit samples were taken and analysed for residues of Imazalil and R14821. Mean recovery efficiencies were found to lie within the required range of 70-110% at all levels of fortification and the relative standard deviation was less than 20%, thus demonstrating sufficient accuracy and precision of the method.

In untreated samples, no residues were found above the limit of quantification (LOQ). In treated citrus samples, residue values 0 days after application by drenching (median: 1.98 mg/kg; highest residue: 2.76 mg/kg) were in the same range as residue values 0 days after application by dipping (median: 1.99 mg/kg; highest residue: 2.72 mg/kg).

Conclusion

The study supports one post harvest dipping application or one post harvest drenching application of mandarin and orange at a target rate of 0.045 kg Imazalil/hl. The study is valid to determine residue levels of Imazalil and R014821 in the raw agricultural commodities mandarin and orange.

A summary of the residue trials is given in Table C.3.1.2.2-1. When the residue level at a later WHP is higher than at the recommended WHP, this highest value was selected.

In 8 samples residues of Imazalil where determined in whole fruit as well as pulp. From these samples the following individual transfer factors from whole fruit to pulp are calculated:

Mandarin: 0.038; 0.253; 0.148; 0.158;
Orange: 0.049; 0.201; 0.115; 0.081.

Table C.3.1.2.2-1. Residue trial summary for citrus (Drenching application)

Trial No./ Location/ Year	Commodity/ Variety	Date of 1.Sowing or planting 2.Flowering 3. Harvest	App.	rate	per treatment	Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analyzed	Residues	(mg/kg)	WHP (days)	Remarks
			g a.s./ ha	Water (l/ha)	kg a.s./hl (IMZ/ PYR)				Imazalil (IMZ)	R14821		
S11-03186, S11-03186-02 46650, Canals, Valencia, Spain	mandarin / CIDRE / Clemenules	1) Oct 1996 2) Mar – Apr 2011 3) 20 Dec 2011	n.a.	n.a.	0.045	21 Dec 2011	85 - 89	whole fruits pulp	2.12 0.081	0.019 <0.01	0 0	
S11-03186, S11-03186-04 40016, San Giorgio di Piano, Bologna, Italy	mandarin / CIDRE / Simeto	1) 2005 2) 04 Apr – 05 Jun 2011 3) 05 Dec 2011	n.a.	n.a.	0.045	29 Nov 2011	89	whole fruits pulp	2.76 0.698	0.014 <0.01	0 0	
S11-03186, S11-03186-06 46650, Canals, Valencia, Spain	orange / CIDSI / Navelina	1) Nov 1986 2) Mar – Apr 2011 3) 20 Dec 2011	n.a.	n.a.	0.045	21 Dec 2011	85 - 89	whole fruits pulp	1.39 0.068	<0.01 <0.01	0 0	
S11-03186, S11-03186-08 40016, San Giorgio di Piano, Bologna, Italy	orange / CIDSI / Navel	1) 1995 2) 05 May – 06 Jun 2011 3) 05 Dec 2011	n.a.	n.a.	0.045	06 Dec 2011	89	whole fruits pulp	1.84 0.370	<0.01 <0.01	0 0	

S11-03186, S11-03186-01: 41805, Mairena del Aljarafe, Andalusia, Spain	mandarin / CIDRE / Oronules	1) 2002 2) 19 Mar – 02 Apr 2011 3) 09 Nov 201	n.a.	n.a.	0.045	09 Nov 2011	89	whole fruits pulp	1.13 0.167	<0.01 <0.01	0 0	
S11-03186, S11-03186-03: 57200, Kolchiko, Thessaloniki, Greece	mandarin / CIDRE / Clementines	1) 1977 2) 01 Apr – 26 May 2011 3) 01 Mar 2012	n.a.	n.a.	0.045	02 Mar 2012	89	whole fruits pulp	2.72 0.430	0.027 <0.01	0 0	
S11-03186, S11-03186-05: 41805, Mairena del Aljarafe, Andalusia, Spain	orange / CIDSI / New Hall	1) 01 Jan 1999 2) 08 Mar – 11 Apr 2011 3) 16 Nov 2011	n.a.	n.a.	0.045	16 Nov 2011	89	whole fruits pulp	1.39 0.160	<0.01 <0.01	0 0	
S11-03186. S11-03186-07: 57200, Kolchiko, Thessaloniki, Greece	orange / CIDSI / Kyno	1) 1985 2) 01 Apr – 28 May 2011 3) 01 Mar 2012	n.a.	n.a.	0.045	02 Mar 2012	89	whole fruits pulp	2.58 0.210	<0.01 <0.01	0 0	

Study 3

Reference:	Determination of residues of Imazalil and its metabolite R14821 (=T824) after a single postharvest application (dip and drench) of Fungaflor 500 EC in mandarin and orange, Southern Europe 2011, [redacted] (2013c), S11-03188 / AGR 4730

GLP:	Yes	Sample storage conditions:	max. 155 days ≤ -18ºC
Crop/crop group:	Oranges, mandarins / citrus	Analytical method:	QuECHERS, HPLC-MS/MS, validated
Indoor/Outdoor:	Indoor	Limit of Quantification (mg/kg):	0.01
Formulation:	EC	Limit of Detection (mg/kg):	0.003
Content of active substance (g/kg or g/l):	500 g/L Imazalil	Residues calculated as:	Imazalil, R14821

During 2011-2012, 8 trials were conducted on harvested oranges and mandarins grown in Spain, Italy and Greece to determine the residue level of Imazalil (and its metabolite R14821) in raw agricultural commodities after one post harvest dipping application (4 trials) or one post harvest drenching application (4 trials) with the formulated product Fungaflor 500 EC (syn. IMAZALIL 500 EC) (500 g/L Imazalil) at a target rate of 100 mL/hL (equivalent to 0.05 kg ai/hL). Samples were taken 0 days after the last application, when the treating solution was dried on the fruits. The trials did not exceed the demonstrated storage period of Imazalil and R014821.

Residues of Imazalil and its metabolite R14821 were analysed according to QuECHERS multi residue method and determination by HPLC with MS/MS detection. Untreated and treated post harvest fruit samples were taken and analysed for residues of Imazalil and R14821. Mean recovery efficiencies were found to lie within the required range of 70-110% at all levels of fortification and the relative standard deviation was less than 20%, thus demonstrating sufficient accuracy and precision of the method.

In untreated samples, no residues were found above the limit of quantification (LOQ). In treated citrus samples, residue values for Imazalil 0 days after application by drenching were 1.48 to 2.69 mg/kg. In treated citrus samples, residue values for Imazalil 0 days after application by dipping were 1.89 to 2.44 mg/kg. Residue values for R14821 after application by drenching/dipping were negligible (minimum: <0.003 mg/kg; maximum: 0.02 mg/kg).

Conclusion

The study supports one post harvest dipping application or one post harvest drenching application at a target rate of 0.05 kg Imazalil/hl. The study is valid to determine residue levels of Imazalil and R014821 in the raw agricultural commodities mandarin and orange.

A summary of the residue trials is given in Table C.3.1.2.3-1. When the residue level at a later WHP is higher than at the recommended WHP, this highest value was selected.

In 8 samples residues of Imazalil where determined in whole fruit as well as pulp. From these samples the following individual transfer factors from whole fruit to pulp are calculated:

Mandarin: 0.009; 0.155; 0.077; 0.111;
Orange: 0.036; 0.045; 0.100; 0.066.

Table C.3.1.2.3-1. Residue trial summary for citrus (Drenching application)

Trial No./ Location/ Year	Commodity/ Variety	Date of 1.Sowing or planting 2.Flowering 3. Harvest	App.	rate	per treatment	Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analyzed	Residues	(mg/kg)	WHP (days)	Remarks
			g a.s./ ha	Water (l/ha)	kg a.s./hl (IMZ/ PYR)				Imazalil (IMZ)	R14821		
S11-03188, S11-03188-02 46650, Canals, Valencia, Spain	mandarin / CIDRE / Clemenules	1) Oct 1996 2) Mar – Apr 2011 3) 20 Dec 2011	n.a.	n.a.	0.05	21 Dec 2011	85 - 89	whole fruits pulp	1.48 0.014	0.010 <0.01	0 0	
S11-03188, S11-03188-04 40016, San Giorgio di Piano, Bologna, Italy	mandarin / CIDRE / Simeto	1) 2005 2) 04 Apr – 05 Jun 2011 3) 05 Dec 2011	n.a.	n.a.	0.05	06 Dec 2011	89	whole fruits pulp	2.55 0.395	0.010 <0.01	0 0	
S11-03188, S11-03188-06 46650, Canals, Valencia, Spain	orange / CIDSI / Navelina	1) Nov 1986 2) Mar – Apr 2011 3) 20 Dec 2011	n.a.	n.a.	0.05	21 Dec 2011	85 - 89	whole fruits pulp	2.17 0.078	<0.01 <0.01	0 0	
S11-03188, S11-03184-02 40016, San Giorgio di Piano, Bologna, Italy	orange / CIDSI / Navel	1) 1995 2) 05 May – 06 Jun 2011 3) 05 Dec 2011	n.a.	n.a.	0.05	06 Dec 2011	89	whole fruits pulp	2.69 0.122	<0.01 <0.01	0 0	

S11-03188, S11-03188-01: 41805, Mairena del Aljarafe, Andalusia, Spain	mandarin / CIDRE / Oronules	1) 2002 2) 19 Mar – 02 Apr 2011 3) 09 Nov 201	n.a.	n.a.	0.050	09 Nov 2011	89	whole fruits pulp	2.37 0.183	0.020 <0.01	0 0	
S11-03188, S11-03188-03: 57200, Kolchiko, Thessaloniki, Greece	mandarin / CIDRE / Clementines	1) 1977 2) 01 Apr – 26 May 2011 3) 01 Mar 2012	n.a.	n.a.	0.050	02 Mar 2012	89	whole fruits pulp	2.44 0.272	0.016 <0.01	0 0	
S11-03188, S11-03188-05: 41805, Mairena del Aljarafe, Andalusia, Spain	orange / CIDSI / New Hall	1) 01 Jan 1999 2) 08 Mar – 11 Apr 2011 3) 16 Nov 2011	n.a.	n.a.	0.050	16 Nov 2011	89	whole fruits pulp	1.89 0.189	<0.01 <0.01	0 0	
S11-03188. S11-03188-07: 57200, Kolchiko, Thessaloniki, Greece	orange / CIDSI / Kyno	1) 1985 2) 01 Apr – 28 May 2011 3) 01 Mar 2012	n.a.	n.a.	0.050	02 Mar 2012	89	whole fruits pulp	2.14 0.142	0.013 <0.01	0 0	

Study 4

Study 5

Reference:	Determination of residues of Imazalil after a postharvest drencher applications of Citrosol 500 EC in four orange trials in Southern Europe (Spain 2010), [redacted] 2010a), SX/10/001 / R-27198
	Determination of Residues of Imazalil Alcohol after a Postharvest Drencher Application of Citrosol 500 EC in Four Orange Trials in Southern Europe (Spain, 2010), [redacted] (2011a), SX/10/028 / R-27198 B

GLP:	Yes	Sample storage conditions:	max. 8 months ≤ -18ºC (Imazalil) max. 12 months ≤ -18ºC (R14821)
Crop/crop group:	Oranges / citrus	Analytical method:	QuECHERS, HPLC-MS/MS, validated
Indoor/Outdoor:	Indoor	Limit of Quantification (mg/kg):	0.01
Formulation:	EC	Limit of Detection (mg/kg):	0.003
Content of active substance (g/kg or g/l):	500 g/L Imazalil	Residues calculated as:	Imazalil, R14821

Imazalil residue levels were determined in orange pulp, peel and whole fruit after a post harvest drencher application of Citrosol 500 EC, from four decline trials conducted in Spain. The test product was applied to orange fruits (ripe for consumption, BBCH 86-89). Fruits were drenched without any prior rinsing, simulating a commercial drenching application. The nominal application rate was 50 g a.i./hl. Fruits were sampled on six occasions in each trial from 0 to 29 DALA. For the “peel and pulp” sample, peel/rind was separated from the flesh before deep-freezing. The application solution and storage conditions were the same for both trials.

The determination of Imazalil residues in orange samples was performed by liquid chromatography coupled with a tandem mass spectrometer (LC-MS/MS), according to the method validated in the Battelle UK Study No. SX/10/003, reported by G Andrews on November 25, 2010. The analytical procedure involved an extraction of the analyte containing a stable labeled internal standard from citrus fruit, peel and pulp using a QuEChERS extraction method and a dilution step with 1:1 methanol:water (v/v) followed by injection of the diluted solutions and analysis by LC-MS/MS.

The LOQ of the validated method was 0.01 mg/kg.

Mean recovery efficiencies were found to lie within the required range of 70-110% at all levels of fortification and the relative standard deviation was less than 20%, thus demonstrating sufficient accuracy and precision of the method.

No residue of Imazalil was detected above the LOQ (0.01 mg/kg) in any control samples.

In the treated whole fruit samples residues of Imazalil ranged from 0.54 to 1.83 mg/kg. For pulp samples, Imazalil ranged from <LOQ to 0.021 mg/kg. In the treated whole fruit samples, residues of metabolite R014821 ranged from < LOQ to 0.017 mg/kg. For pulp samples, metabolite R014821 ranged from not detectable to <LOQ.

Conclusion

A summary of the residue trials is given in Table C.3.1.2.4. When the residue level at a later WHP is higher than at the recommended WHP, this highest value was selected.

The study was not valid to determine residue levels of R14821, since the demonstrated storage stability of R14821 in citrus (high acid content) is exceeded. Therefore, no residues of R14821 are selected for MRL-calculation.

In 10 samples residues of Imazalil where determined in whole fruit as well as pulp and peel. From these samples the following individual transfer factors from whole fruit to pulp and to peel are calculated:

Orange whole fruit - pulp: <0.100; <0.01; 0.014; 0.016; 0.008; 0.007; <0.02; <0.01; <0.01; 0.016

Orange whole fruit – peel: 3.07; 2.92; 4.35; 2.72; 2.39; 1.74; 4.00; 2.02; 3.37; 5.26

Table C.3.1.2.4-1. Residue trial summary for citrus (Drenching application)

Trial No./ Location/ Year	Commodity/ Variety	Date of 1.Sowing or planting 2.Flowering 3. Harvest	App.	rate	per treatment	Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analyzed	Residues	(mg/kg)	WHP (days)	Remarks
			g a.s./ ha	Water (l/ha)	kg a.s./hl (IMZ/ PYR)				Imazalil (IMZ)	R14821		
SX/10/001, SX/10/001-1 46192, Monserrat, Valencia, Spain	oranges / valencia	1) n.a. 2) n.a. 3) 01 Mar 2010	n.a.	25	0.049	03 Mar 2010	87	whole fruits peel pulp whole fruits peel pulp whole fruits peel pulp whole fruits whole fruits	0.604 1.852 <0.01 0.740 2.164 <0.01 0.900 3.916 0.013 1.100 0.728	0.010 <0.01 <0.01 0.010 0.013 <0.01 0.011 0.019 <0.01 0.010 0.017	0 0 0 1 1 1 7 7 7 14 29	LOQ= 0.01 mg/kg Untreated = <0.01
SX/10/001, SX/10/001-2 46192, Monserrat, Valencia, Spain	oranges / Navel	1) n.a. 2) n.a. 3) 02 Mar 2010	n.a.	25	0.049	03 Mar 2010	89	whole fruits peel pulp whole fruits peel pulp whole fruits peel pulp whole fruits whole fruits	1.308 3.556 <u>0.021</u> 1.320 3.152 0.010 <u>1.828</u> 3.188 0.012 0.764 1.216	0.011 <0.01 <0.01 0.010 0.020 <0.01 0.011 0.020 <0.01 0.014 0.016	0 0 0 1 1 1 7 7 7 14 29	LOQ= 0.01 mg/kg Untreated = <0.01

SX/10/001, SX/10/001-3 46192, Monserrat, Valencia, Spain	oranges / Lane late	1) n.a. 2) n.a. 3) 02 Mar 2010	n.a.	25	0.049	03 Mar 2010	86	whole fruits Peel Pulp whole fruits Peel pulp	0.544 2.176 <0.01 0.932 1.884 <0.01	<0.01 0.014 <0.01 <0.01 0.014 <0.01	0 0 0 1 1 1	LOQ= 0.01 mg/kg Untreated = <0.01 n.d= not detectable (
SX/10/001, SX/10/001-4 46192, Monserrat, Valencia, Spain	oranges / Navel late	1) n.a. 2) n.a. 3) 02 Mar 2010	n.a.	25	0.049	03 Mar 2010	88	whole fruits Peel Pulp whole fruits Peel pulp	1.016 3.424 <0.01 0.836 4.400 0.013	<0.01 0.013 <0.01 <0.01 0.014 <0.01	0 0 0 1 1 1	 LOQ= 0.01 mg/kg Untreated = <0.01

Study 6

Study 7

Reference:	Determination of residues of Imazalil after a postharvest drencher applications of Citrosol 500 EC in four mandarin trials in Southern Europe (Spain 2010), [redacted] (2010b), SX/10/004 / R-27198A Determination of Residues of Imazalil Alcohol after a Postharvest Drencher Application of Citrosol 500 EC in Four Mandarin Trials in Southern Europe (Spain, 2010), [redacted] (2011b), SX/10/027 / R-27198 C

GLP:	Yes	Sample storage conditions:	max. 8 months ≤ -18ºC (Imazalil) max. 12 months ≤ -18ºC (R14821)
Crop/crop group:	Mandarins / citrus	Analytical method:	QuECHERS, HPLC-MS/MS, validated
Indoor/Outdoor:	Indoor	Limit of Quantification (mg/kg):	0.01
Formulation:	EC	Limit of Detection (mg/kg):	0.003
Content of active substance (g/kg or g/l):	500 g/L Imazalil	Residues calculated as:	Imazalil, R14821

Imazalil residue levels were determined in mandarin pulp, peel and whole fruit after a post harvest drencher application of Citrosol 500 EC, from four decline trials conducted in Spain. The test product was applied to mandarin fruits (ripe for consumption, BBCH 89). Fruits were drenched directly from the field without any prior rinsing, simulating a commercial drenching application. The application solution and storage conditions were the same for both trials. Distinction in variety alone is not sufficient to consider the trials as independent in a magnitude of residues study. Therefore, the study is concluded to consist of two replicate trials. The nominal application rate was 50 g a.i./hl. Fruits were sampled on six occasions in each trial from 0 to 30 DALA. For the “peel and pulp” sample, peel/rind was separated from the pulp before deep-freezing.

The determination of Imazalil residues in mandarin samples was performed by liquid chromatography coupled with a tandem mass spectrometer (LC-MS/MS), according to the

method validated in the Battelle UK Study No. SX/10/003, reported by G Andrews on November 25, 2010

The analytical procedure involved an extraction of the analyte containing a stable labeled internal standard from citrus fruit, peel and pulp using a QuEChERS extraction method and a dilution step with 1:1 methanol:water (v/v) followed by injection of the diluted solutions and analysis by LC-MS/MS.

The LOQ of the validated method was 0.01 mg/kg.

Mean recovery efficiencies were found to lie within the required range of 70-110% at all levels of fortification and the relative standard deviation was less than 20%, thus demonstrating sufficient accuracy and precision of the method.

No residue of Imazalil was detected above the LOQ (0.01 mg/kg) in any control samples.

In the treated whole fruit samples residues of Imazalil ranged from 0.28 to 1.14 mg/kg. For pulp samples, Imazalil ranged from <LOQ to 0.034 mg/kg. In the treated whole fruit samples, residues of metabolite R014821 ranged from < LOQ to 0.030 mg/kg. For pulp samples, metabolite R014821 ranged from not detectable to <LOQ.

Conclusion

A summary of the residue trials is given in tables C.3.1.2.5-1. When the residue level at a later WHP is higher than at the recommended WHP, this highest value was selected.

The study was not valid to determine residue levels of R14821, since the demonstrated storage stability of R014821 in citrus (high acid content) is exceeded. Therefore, no residues of R014821 are selected for MRL-calculation.

In 10 samples residues of Imazalil where determined in whole fruit as well as pulp and peel. From these samples the following individual transfer factors from whole fruit to pulp and to peel are calculated:

Mandarin whole fruit - pulp: 0.020; 0.032; 0.021; <0.02; 0.014; 0.032; 0.031; <0.01; 0.038; 0.018

Mandarin whole fruit – peel: 3.79; 3.52; 3.67; 4.64; 2.73; 2.53; 2.22; 3.02; 4.28; 3.91;

Table C.3.1.2.5-1. Residue trial summary for citrus (Drenching application)

Trial No./ Location/ Year	Commodity/ Variety	Date of 1.Sowing or planting 2.Flowering 3. Harvest	App.	rate	per treatment	Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analyzed	Residues	(mg/kg)	WHP (days)	Remarks
			g a.s./ ha	Water (l/ha)	kg a.s./hl (IMZ/ PYR)				Imazalil (IMZ)	R14821		
SX/10/004, SX/10/004-1 46192, Monserrat, Valencia, Spain	Mandarins / ortanique	1) n.a. 2) n.a. 3) 24 Feb 2010	n.a.	25	0.049	25 Feb 2010	89	whole fruits peel pulp whole fruits peel pulp whole fruits peel pulp whole fruits whole fruits	1.056 4.000 0.021 0.928 3.264 0.030 0.840 3.080 0.018 0.936 0.282	<0.01 0.013 <0.01 <0.01 0.024 <0.01 <0.01 0.036 <0.01 0.010 0.010	0 0 0 1 1 1 7 7 7 14 29	Storage stability of R014821 exceeded
SX/10/004, SX/10/004-2 46192, Monserrat, Valencia, Spain	Mandarins / clemenvilla	1) n.a. 2) n.a. 3) 24 Feb 2010	n.a.	25	0.049	25 Feb 2010	89	whole fruits peel pulp whole fruits peel pulp whole fruits peel pulp whole fruits whole fruits	0.628 2.916 <0.01 1.020 2.788 0.014 1.012 2.564 0.032 <u>1.140</u> 1.064	0.010 0.020 <0.01 0.011 0.017 <0.01 0.012 0.039 <0.01 0.029 0.030	0 0 0 1 1 1 7 7 7 14 29	Storage stability of R014821 exceeded

SX/10/004, SX/10/004-3 46192, Monserrat, Valencia, Spain	Mandarins / Afourer	1) n.a. 2) n.a. 3) 24 Feb 2010	n.a.	25	0.049	25 Feb 2010	89	whole fruits Peel Pulp whole fruits Peel pulp	1.092 2.428 0.034 1.108 3.346 <0.01	0.026 0.075 <0.01 0.029 0.144 <0.01	0 0 0 1 1 1	Storage stability of R014821 exceeded
SX/10/004, SX/10/004-4 46192, Monserrat, Valencia, Spain	Mandarins / Fortune	1) n.a. 2) n.a. 3) 09 Mar 2010	n.a.	25	0.049	10 Mar 2010	89	whole fruits Peel Pulp whole fruits Peel pulp	0.444 1.900 0.017 0.684 2.672 0.012	0.016 0.026 <0.01 0.023 0.016 <0.01	0 0 0 1 1 1	Storage stability of R014821 exceeded

Study 8

Study 9

Reference:	Determination of Residues of Imazalil in Oranges and Processed Commodities after a Post Harvest Application of Magnate 50 ECNA in two Balance Trials in Southern Europe, [redacted] (2013a) R-29507 Determination of Residues of Imazalil Alcohol in Oranges and Processed Commodities after a Post Harvest Application of Magnate 50 ECNA in two Balance Trials in Southern Europe, [redacted] (2013b) R-29508

GLP:	Yes	Sample storage conditions:	max. 129 days ≤ -18ºC (Imazalil) Whole orange max. 151 days ≤ -18ºC (R14821) Whole orage
Crop/crop group:	Mandarins / citrus	Analytical method:	QuECHERS, HPLC-MS/MS, validated
Indoor/Outdoor:	Indoor	Limit of Quantification (mg/kg):	0.01
Formulation:	Magnate 50 ECNA	Limit of Detection (mg/kg):	0.003
Content of active substance (g/kg or g/l):	500 g/L Imazalil	Residues calculated as:	Imazalil, R14821

Oranges to be used for processing were generated from two trials. They were used in the balance studies to quantify the residues of Imazalil after processing into orange juice, marmalade, jam, jelly, essential oil, canned and dried orange. For processing data reference is made to C.3.2.2.5 and C.3.2.2.6.

In the treated samples, the test item Magnate 50 ECNA was applied at a nominal application rate of 50 g a.i./hL. A new test solution was prepared for each trial. Fruits from trial SX11014/1 and trial SX11014/2 were stored at two different storage facilities. After storage at for 31 days at approximately 5ºC, the oranges were transferred to the processing site. For trial SX11014/1 a whole fruit sample was taken after receipt at the processing site. For trial SX11014/2 orange specimens were stored in a cold room until the next morning. Whole orange specimens were deep-frozen (<-18ºC) for maximum 129 days before Imazalil analysis and 151 days before R-29508 analysis. This is within the demonstrated period of stability for Imazalil.

The determination of Imazalil and Imazalil Alcohol (R14821) in citrus processed commodities was performed using a QuEChERS extraction and dispersive SPE method followed by liquid chromatography with tandem mass spectrometry (LC-MS/MS) analysis. The limit of quantification of the validated methods was 0.01 mg/kg for Imazalil and 0.01 mg/kg for Imazalil Alcohol (R14821). Mean recovery efficiencies were found to lie within the required range of 70-110% at all levels of fortification and the relative standard deviation was less than 20%, thus demonstrating sufficient accuracy and precision of the method.

No residue of Imazalil was detected above the LOQ (0.01 mg/kg) in any control samples.

In the treated whole fruit samples residues of Imazalil were 0.636 and 0.972 mg/kg and residues of metabolite R014821 were 2x 0.024 mg/kg.

Conclusion

The study supports one post harvest drenching application at a target rate of 0.05 kg Imazalil/hl. A summary of the residue trials is given in tables C.3.1.2.6-1. However, residues were only determined at 31 days after the application and are not considered valid to determine residue levels of Imazalil in the raw agricultural commodities of orange directly after application. Therefore, no residues of Imazalil are selected for MRL-calculation. The study is however valid to quantify the residues of Imazalil after processing. For processing data reference is made to C.3.2.2.5 and C.3.2.2.6.

Table C.3.1.2.6-1. Residue trial summary for citrus (Drenching application)

Trial No./ Location/ Year	**Commodity/ Variety**	**Date of 1.Sowing or planting 2.Flowering 3. Harvest**	**App.**	**rate**	**per treatment**	**Dates of treatment or no. of treatments and last date**	**Growth stage at last treatment or date**	**Portion analyzed**	**Residues**	**(mg/kg)**	**WHP (days)**	**Remarks**
			g a.s./ ha	**Water (l/ha)**	**kg a.s./hl (IMZ/ PYR)**				Imazalil (IMZ)	R14821		
SX11014/1 46192, Monserrat, Valencia, Spain	Oranges / Navelina	1) n.a. 2) n.a. 3) 03 Feb 2012	n.a.	25	0.049	09-02-2012	89	whole fruits	0.972	0.024	31	
SX11014/2 46192, Monserrat, Valencia, Spain	Oranges / Lane Late	1) n.a. 2) n.a. 3) 03 Feb 2012	n.a.	25	0.049	09-02-2012	89	whole fruits	0.636	0.024	31	

Study 10

Reference:	Determination of residues of Imazalil, its metabolite R14821 (=T824) and pyrimethanil after a single postharvest application (low volume system) of WHPlabuster 400 SC in mandarin and orange, Southern Europe 2011, [redacted] (2013d), S11-03185 / AGR 4727

GLP:	Yes	Sample storage conditions:	max. 236 days ≤ -18ºC
Crop/crop group:	Oranges, mandarins / citrus	Analytical method:	QuECHERS, HPLC-MS/MS, validated
Indoor/Outdoor:	Indoor	Limit of Quantification (mg/kg):	0.01
Formulation:	SC	Limit of Detection (mg/kg):	0.003
Content of active substance (g/kg or g/l):	200 g/L Imazalil 200 g/L Pyrimethanil	Residues calculated as:	Imazalil, R14821, Pyrimethanil

The objective of the study was to determine residue levels of Imazalil, its metabolite R14821 and pyrimethanil in the raw agricultural commodities mandarin and orange.

Eight residue trials were conducted on mandarin and orange during 2011 - 2012 in Spain, Italy and Greece. In all trials -01 to -04 in mandarin and trials -05 to -08 in orange, one post harvest low volume application of WHPlabuster 400 SC (nominal 200 g/L Imazalil and 200 g/L pyrimethanil) was applied. All applications were done within 28 hours after harvest of fruits.

In all trials, samples from the untreated fruits were taken just before application. Samples from the treated fruits were taken after the treating solution was dried on the fruits.

Residues of Imazalil and its metabolite R14821 were analysed according to QuECHERS multi residue method and determination by HPLC with MS/MS detection The limit of quantification was 0.01 mg/kg and the limit of detection 0.003 mg/kg. Mean recovery efficiencies were found to lie within the required range of 70-110% at all levels of fortification and the relative standard deviation was less than 20%, thus demonstrating sufficient accuracy and precision of the method.

In the treated mandarin whole fruit samples residues of Imazalil ranged from 0.373 to 1.83 mg/kg. For mandarin pulp samples Imazalil ranged from < LOQ to 0.166 mg/kg. In the treated mandarin whole fruit samples residues of metabolite R014821 ranged from 0.012

mg/kg to 0.037 mg/kg. For mandarin pulp samples metabolite R014821 was not detectable.

Residues of Imazalil, its metabolite R014821 (=T824) and pyrimethanil in untreated orange whole fruit and orange pulp samples were all below the limit of detection (< 0.003 mg/kg). In the treated orange whole fruit samples residues of Imazalil ranged from 0.198 to 2.40 mg/kg. For orange pulp samples Imazalil ranged from not detectable to 0.049 mg/kg. In the treated orange whole fruit samples residues of metabolite R014821 ranged from not detectable to 0.018 mg/kg. For orange pulp samples metabolite R014821 was not detectable.

Conclusion

A summary of the residue trials is given in Table C.3.1.2.7-1. The study is valid to determine residue levels in the raw agricultural commodities mandarin and orange. When the residue level at a later WHP is higher than at the recommended WHP, this highest value was selected.

In 8 samples residues of Imazalil where determined in whole fruit as well as pulp. From these samples the following individual transfer factors from whole fruit to pulp are calculated:

Mandarin: 0.032; 0.019; <0.01; 0.091

Orange: 0.057; 0.091; <0.05; 0.018

Table C.3.1.2.7-1 Residue trial summary for citrus (Drenching application)

Trial No./ Location/ Year	**Commodity/ Variety**	**Date of 1.Sowing or planting 2.Flowering 3. Harvest**	**App.**	**rate**	**per treatment**	**Dates of treatment or no. of treatments and last date**	**Growth stage at last treatment or date**	**Portion analyzed**	**Residues**	**(mg/kg)**		**WHP (days)**	**Remarks**
			g a.s./ ha	**Water (l/ha)**	**kg a.s./hl (IMZ/ PYR)**				Imazalil (IMZ)	R14821	Pyrimethanil (PYR))		
S11-03185, S11-03185-01 25198, Lleida, Barcelona, Spain	mandarin / CIDRE / Satsuma	1) Oct 1999 2) Mar – Apr 2011 3) 15 Nov 2011	n.a.	n.a.	0.30 (0.15 / 0.15)	16 Nov 2011	85-89	whole fruits pulp	0.373 0.012	0.012 <0.01	0.089 <0.01	0 0	
S11-03185, S11-03185-02 25198, Lleida, Barcelona, Spain	mandarin / CIDRE / Orogrande	1) Nov 2000 2) Mar – Apr 2011 3) 15 Nov 2011	n.a.	n.a.	0.30 (0.15 / 0.15)	16 Nov 2011	85-89	whole fruits pulp	0.530 0.010	0.021 <0.01	0.190 <0.01	0 0	
S11-03185, S11-03185-03 95040, Belpasso, Sicily, Italy	mandarin / CIDRE / Avana	1) 2001 2) Mar – Apr 2011 3) 02 Jan 2012	n.a.	n.a.	0.30 (0.15 / 0.15)	03 Jan 2012	89	whole fruits pulp	0.709 <0.01	0.012 <0.01	0.623 <0.01	0 0	
S11-03185, S11-03185-04 40003, Agia, Larissa, Greece	mandarin / CIDRE / Ortanik	1) 1986 2) Apr – May 2011 3) 16 Mar 2012	n.a.	n.a.	0.30 (0.15 / 0.15)	17 Mar 2012	89	whole fruits pulp	1.83 0.166	0.037 <0.01	2.49 0.135	0 0	
S11-03185, S11-03185-05 25198, Lleida, Barcelona, Spain	orange / CIDSI / Navelina	1) Nov 1987 2) Mar –Apr 2011 3) 15 Nov 2011	n.a.	n.a.	0.30 (0.15 / 0.15)	16 Nov 2011	89	whole fruits pulp	0.866 0.049	<0.01 <0.01	0.299 0.089	0 0	
S11-03185, S11-03185-06 25198, Lleida, Barcelona, Spain	orange / CIDSI / New Hall	1) Nov 1994 2) Mar – Apr 2011 3) 15 Nov 2011	n.a.	n.a.	0.30 (0.15 / 0.15)	16 Nov 2011	85-89	whole fruits pulp	0.529 0.048	<0.01 <0.01	0.229 0.013	0 0	

S11-03185, S11-03185-07 95040, Belpasso, Sicily, Italy	orange / CIDSI / Tarocco Sciara	1) 2003 2) Mar – Apr 2011 3) 09 Jan 2012	n.a.	n.a.	0.30 (0.15 / 0.15)	10 Jan 2012	89	whole fruits pulp	0.198 <0.01	<0.01 <0.01	0.135 <0.01	0 0	
S11-03185, S11-03185-08 40003, Agia, Larissa, Greece	orange / CIDSI / Merlin	1) 1988 2) Apr – May 2011 3) 16 Mar 2012	n.a.	n.a.	0.30 (0.15 / 0.15)	17 Mar 2012	89	whole fruits pulp	2.40 0.044	0.018 <0.01	2.87 0.072	0 0	

Study 11

Reference:	Determination of residues of Imazalil and its metabolite R14821 (=T824) after a single postharvest application (low volume system) of Fecundal 7.5 S in mandarin and orange, Southern Europe 2011, [redacted] (2013e), S11-03187 / AGR 4729

GLP:	Yes	Sample storage conditions:	max. 236 days ≤ -18ºC
Crop/crop group:	Oranges, mandarins / citrus	Analytical method:	QuECHERS, HPLC-MS/MS, validated
Indoor/Outdoor:	Indoor	Limit of Quantification (mg/kg):	0.01
Formulation:	SL	Limit of Detection (mg/kg):	0.003
Content of active substance (g/kg or g/l):	75 g/L Imazalil	Residues calculated as:	Imazalil, R14821,

During 2011-2012, 8 trials were conducted on harvested oranges and mandarins grown in Spain, Italy and Greece to determine the residue level of Imazalil (and its metabolite R14821) in raw agricultural commodities after one postharvest low volume application with the formulated product Fecundal 7.5 S (75 g/L Imazalil) at a target rate of 2 L/hL (0.15 kg Imazalil/ hL). Samples were taken 0 days after the last application, when the treating solution was dried on the fruits.

Residues of Imazalil and its metabolite R14821 were analysed according to QuECHERS multi residue method and determination by HPLC with MS/MS detection. Untreated and treated post harvest fruit samples were taken and analysed for residues of Imazalil and R14821. Mean recovery efficiencies were found to lie within the required range of 70-110% at all levels of fortification and the relative standard deviation was less than 20%, thus demonstrating sufficient accuracy and precision of the method. In untreated samples, no residues were found above the limit of quantification (LOQ).

In treated citrus samples, Imazalil residue values 0 days after application were 0.228 to 1.84 mg/kg.

Conclusion

A summary of the residue trials is given in table C.3.1.2.8-1. The study is valid to determine residue levels in the raw agricultural commodities mandarin and orange. When

the residue level at a later WHP is higher than at the recommended WHP, this highest value was selected.

In 8 samples residues of Imazalil where determined in whole fruit as well as pulp. From these samples the following individual transfer factors from whole fruit to pulp are calculated:

Mandarin: 0.040; 0.016; 0.015; 0.529

Orange: 0.118; 0.098; <0.01; 0.034

Table C.3.1.2.8-1. Residue trial summary for citrus (Low volume spray application)

Trial No./ Location/ Year	Commodity/ Variety	Date of 1.Sowing or planting 2.Flowering 3. Harvest	App.	rate	per treatment	Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analyzed	Residues	(mg/kg)	WHP (days)	Remarks
			g a.s./ ha	Water (l/ha)	kg a.s./hl (IMZ/ PYR)				Imazalil (IMZ)	R14821		
S11-03187, S11-03187-01: 25198, Lleida, Barcelona, Spain	mandarin / CIDRE / Satsuma	1) Oct 1999 2) Mar – Apr 2011 3) 15 Nov 2011	n.a.	n.a.	0.150	16 Nov 2011	85 - 89	whole fruits pulp	0.547 0.022	0.022 <0.01	0 0	
S11-03187, S11-03187-02 25198, Lleida, Barcelona, Spain	mandarin / CIDRE / Orogrande	1) Nov 2000 2) Mar – Apr 2011 3) 15 Nov 2011	n.a.	n.a.	0.150	16 Nov 2011	85 - 89	whole fruits pulp	1.16 0.019	0.032 <0.01	0 0	
S11-03187, S11-03187-03 95040, Belpasso, Sicily, Italy	mandarin / CIDRE / Avana	1) 2001 2) Mar – Apr 2011 3) 02 Jan 2012	n.a.	n.a.	0.150	03 Jan 2012	89	whole fruits pulp	1.05 0.016	0.019 <0.01	0 0	
S11-03187, S11-03187-04 40003, Agia, Larissa, Greece	mandarin / CIDRE / Ortanik	1) 1986 2) Apr – May 2011 3) 16 Mar 2012	n.a.	n.a.	0.150	17 Mar 2012	89	whole fruits pulp	1.84 0.974	0.036 0.019	0 0	
S11-03187, S11-03187-05 25198, Lleida, Barcelona, Spain	orange / CIDSI / Navelina	1) Nov 1987 2) Mar –Apr 2011 3) 15 Nov 2011	n.a.	n.a.	0.150	16 Nov 2011	85 - 89	whole fruits pulp	0.228 0.027	<0.01 <0.01	0 0	
S11-03187, S11-03187-06 46181, 25198, Lleida, Barcelona, Spain	orange / CIDSI / New Hall	1) Nov 1994 2) Mar – Apr 2011 3) 15 Nov 2011	n.a.	n.a.	0.150	16 Nov 2011	85 - 89	whole fruits pulp	0.328 0.032	<0.01 <0.01	0 0	
S11-03187, S11-03187-07	orange / CIDSI / Tarocco Sciara	1) 2003 2) Mar – Apr 2011 3) 09 Jan 2012	n.a.	n.a.	0.150	10 Jan 2012	89	whole fruits pulp	1.08 <0.01	<0.01 <0.01	0 0	

95040, Belpasso, Sicily, Italy												
S11-03187, S11-03187-08 40003, Agia, Larissa, Greece	orange / CIDSI / Merlin	1) 1988 2) Apr – May 2011 3) 16 Mar 2012	n.a.	n.a.	0.150	17 Mar 2012	89	whole fruits pulp	1.34 0.046	0.022 <0.01	0 0	

Study 12

Reference:	Determination of residues of Imazalil and its metabolite R14821 (=T824) after a single postharvest application (low volume system) of Fungaflor 500 EC in mandarin and orange, Southern Europe 2011, [redacted] (2013f), S11-03189 / AGR 4731

GLP:	Yes	Sample storage conditions:	max. 213 days ≤ -18ºC
Crop/crop group:	Oranges, mandarins / citrus	Analytical method:	QuECHERS, HPLC-MS/MS, validated
Indoor/Outdoor:	Indoor	Limit of Quantification (mg/kg):	0.01
Formulation:	EC	Limit of Detection (mg/kg):	0.003
Content of active substance (g/kg or g/l):	500 g/L Imazalil	Residues calculated as:	Imazalil, R14821,

During 2011-2012, 8 trials were conducted on harvested oranges and mandarins grown in Spain, Italy and Greece to determine the residue level of Imazalil (and its metabolite R14821) in raw agricultural commodities after one post harvest low volume application with the formulated product Fungaflor 500 EC (syn. IMAZALIL 500 EC) (500 g/L Imazalil) at a target rate of 300 mL/hL (0.15 kg Imazalil/ hL). Samples were taken 0 days after the last application, when the treating solution was dried on the fruits.

Residues of Imazalil and its metabolite R14821 were analysed according to QuECHERS multi residue method and determination by HPLC with MS/MS detection. Untreated and treated post harvest fruit samples were taken and analysed for residues of Imazalil and R14821. Mean recovery efficiencies were found to lie within the required range of 70-110% at all levels of fortification and the relative standard deviation was less than 20%, thus demonstrating sufficient accuracy and precision of the method. In untreated samples, no residues were found above the limit of quantification (LOQ).

In treated citrus samples, Imazalil residue values 0 days after application 0.212 to 2.106 mg/kg.

Conclusion

A summary of the residue trials is given in table C.3.1.2.9-1. The study is valid to determine residue levels in the raw agricultural commodities mandarin and orange. When the residue level at a later WHP is higher than at the recommended WHP, this highest value was selected.

In 8 samples residues of Imazalil where determined in whole fruit as well as pulp. From these samples the following individual transfer factors from whole fruit to pulp are calculated:

Mandarin: 0.052; <0.03; 0.016; 0.344

Orange: 0.107; 0.068; <0.01; 0.015

Table C.3.1.2.9-1. Residue trial summary for citrus (Low volume spray application)

Trial No./ Location/ Year	**Commodity/ Variety**	**Date of 1.Sowing or planting 2.Flowering 3. Harvest**	**App.**	**rate**	**per treatment**	**Dates of treatment or no. of treatments and last date**	**Growth stage at last treatment or date**	**Portion analyzed**	**Residues**	**(mg/kg)**	**WHP (days)**	**Remarks**
			g a.s./ ha	**Water (l/ha)**	**kg a.s./hl (IMZ/ PYR)**				Imazalil (IMZ)	R14821		
S11-03189, S11-03189-01 25198, Lleida, Barcelona, Spain	mandarin / CIDRE / Satsuma	1) Oct 1999 2) Mar – Apr 2011 3) 15 Nov 201	n.a.	n.a.	0.150	16 Nov 2011	85 - 89	whole fruits pulp	0.212 0.011	<0.01 <0.01	0 0	
S11-03189, S11-03189-02 25198, Lleida, Barcelona, Spain	mandarin / CIDRE / Orogrande	1) Nov 2000 2) Mar – Apr 2011 3) 15 Nov 2011	n.a.	n.a.	0.150	16 Nov 2011	85 - 89	whole fruits pulp	0.398 <0.01	0.017 <0.01	0 0	
S11-03189, S11-03189-03 95040, Belpasso, Sicily, Italy	mandarin / CIDRE / Avana	1) 2001 2) Mar – Apr 2011 3) 02 Jan 2012	n.a.	n.a.	0.150	03 Jan 2012	89	whole fruits pulp	0.996 0.013	0.016 <0.01	0 0	
S11-03189, S11-03189-04 40003, Agia, Larissa, Greece	mandarin / CIDRE / Ortanik	1) 1986 2) Apr – May 2011 3) 16 Mar 2012	n.a.	n.a.	0.150	17 Mar 2012	89	whole fruits pulp	2.067 0.711	0.032 <0.01	0 0	
S11-03189, S11-03189-05 25198, Lleida, Barcelona, Spain	orange / CIDSI / Navelina	1) Nov 1987 2) Mar –Apr 2011 3) 15 Nov 2011	n.a.	n.a.	0.150	16 Nov 2011	89	whole fruits pulp	0.514 0.055	<0.01 <0.01	0 0	
S11-03189, S11-03189-06 46181, 25198, Lleida, Barcelona, Spain	orange / CIDSI / New Hall	1) Nov 1994 2) Mar – Apr 2011 3) 15 Nov 2011	n.a.	n.a.	0.150	16 Nov 2011	85 - 89	whole fruits pulp	0.644 0.044	<0.01 <0.01	0 0	

S11-03189, S11-03189-07 95040, Belpasso, Sicily, Italy	orange / CIDSI / Tarocco Sciara	1) 2003 2) Mar – Apr 2011 3) 09 Jan 2012	n.a.	n.a.	0.150	10 Jan 2012	89	whole fruits pulp	0.962 <0.01	<0.01 <0.01	0 0	
S11-03189, S11-03189-08 40003, Agia, Larissa, Greece	orange / CIDSI / Merlin	1) 1988 2) Apr – May 2011 3) 16 Mar 2012	n.a.	n.a.	0.150	17 Mar 2012	89	whole fruits pulp	2.106 0.032	0.018 <0.01	0 0	

Study 13

Reference:	Determination of residues of Imazalil and its metabolite R14821 (=T824) after a single postharvest application (waxing system) of Fungaflor 500 EC in mandarin and orange, Southern Europe 2011, [redacted] (2013g), S11-03190 / AGR 4732

GLP:	Yes	Sample storage conditions:	max. 188 days ≤ -18ºC
Crop/crop group:	Oranges, mandarins / citrus	Analytical method:	QuECHERS, HPLC-MS/MS, validated
Indoor/Outdoor:	Indoor	Limit of Quantification (mg/kg):	0.01
Formulation:	EC	Limit of Detection (mg/kg):	0.003
Content of active substance (g/kg or g/l):	500 g/L Imazalil	Residues calculated as:	Imazalil, R14821,

During the 2011-2012, 6 trials were conducted on harvested oranges and mandarins grown in Spain, Italy and Greece to determine the residue level of Imazalil (and its metabolite R14821) in raw agricultural commodities after one post harvest waxing application with the formulated product Fungaflor 500 EC (syn. IMAZALIL 500 EC) (500 g/L Imazalil) at a target rate of 400 mL/hL (0.2 kg Imazalil/hL) of wax. Samples were taken 0 days after the last application, when the treating solution was dried on the fruits. The trials did not exceed the demonstrated storage period of Imazalil and R14821.

Residues of Imazalil and its metabolite R14821 were analysed according to QuECHERS multi residue method and determination by HPLC with MS/MS detection. Untreated and treated post harvest fruit samples were taken and analysed for residues of Imazalil and R14821. In untreated samples, no residues were found above the limit of quantification (LOQ). In treated citrus samples, Imazalil residue values 0 days after application by waxing were 0.197 to 0.518 mg/kg. R14821 residue values were not detectable (<0.003 mg/kg).

Conclusion

The study is valid to determine residue levels of Imazalil in the raw agricultural commodities mandarin and orange. The study supports one post harvest waxing application on mandarin and oranges at a rate of 0.2 kg Imazalil/hL. A summary of the

residue trials is given in table C.3.1.2.10-1. It is however noted that the critical GAP for waxing is 0.3 kg Imazalil/hL. The trials therefore do not support the critical GAP.

In 6 samples residues of Imazalil where determined in whole fruit as well as pulp. From these samples the following individual transfer factors from whole fruit to pulp are calculated:

Mandarin: 0.025; 0.130; 0.066;
Orange: 0.019; 0.020; 0.026.

Table C.3.1.2.10-1. Residue trial summary for citrus (waxing application)

Trial No./ Location/ Year	**Commodity/ Variety**	**Date of 1.Sowing or planting 2.Flowering 3. Harvest**	**App.**	**rate**	**per treatment**	**Dates of treatment or no. of treatments and last date**	**Growth stage at last treatment or date**	**Portion analyzed**	**Residues**	**(mg/kg)**	**WHP (days)**	**Remarks**
			g a.s./ ha	**Water (l/ha)**	**kg a.s./hl (IMZ/ PYR)**				Imazalil (IMZ)	R14821		
S11-03190, S11-03190-02 95040, Belpasso, Sicily, Italy	mandarin / CIDRE / Avana	1) 2001 2) Mar – Apr 2011 3) 02 Jan 2012	n.a.	n.a.	0.2	03 Jan 2012	89	whole fruits pulp	0.404 <0.01	<0.01 <0.01	0 0	
S11-03190, S11-03190-03 40003, Agia, Larissa, Greece	mandarin / CIDRE / Ortanik	1) 1986 2) Apr – May 2011 3) 16 Mar 2012	n.a.	n.a.	0.2	17 Mar 2012	89	whole fruits pulp	0.299 0.039	<0.01 <0.01	0 0	
S11-03190, S11-03190-05 95040, Belpasso, Sicily, Italy	orange / CIDSI / Tarocco Sciara	1) 2003 2) Mar – Apr 2011 3) 09 Jan 2012	n.a.	n.a.	0.2	10 Jan 2012	89-99	whole fruits pulp	0.518 <0.01	<0.01 <0.01	0 0	
S11-03190, S11-03190-06 40003, Agia, Larissa, Greece	orange / CIDSI / Merlin	1) 1988 2) Apr – May 2011 3) 16 Mar 2012	n.a.	n.a.	0.2	17 Mar 2012	89	whole fruits pulp	0.492 <0.01	<0.01 <0.01	0 0	
S11-03190, S11-03190-07 25198, Lleida, Barcelona, Spain	mandarin / CIDRE / Fortuna	1) 2004 2) Mar – Apr 2011 3) 07 Feb 2012	n.a.	n.a.	0.2	08 Feb 2012	85-89	whole fruits pulp	0.197 0.013	<0.01 <0.01	0 0	
S11-03190, S11-03190-08 25198, Lleida, Barcelona, Spain	orange / CIDSI / Navelina	1) 1987 2) Mar – Apr 2011 3) 07 Feb 2012	n.a.	n.a.	0.2	08 Feb 2012	85-89	whole fruits pulp	0.388 <0.01	<0.01 <0.01	0 0	

C.3.1.2.2 Cucumber

Reference:	Determination of residues of Imazalil and its metabolite R14821 after four applications of Fungaflash in cucumber (indoor) at four sites in Southern Europe 2019, [redacted] 2020, S19-00816
Guideline(s):	OECD (2009) Guidance Document on Overview of Residue Chemistry Studies (Series on Testing and Assessment No. 64 and Series on Pesticides No. 32) OECD Test Guideline 509: Crop field trials OECD (2016) Guidance Document on Crop Field Trials (Series on Testing and Assessment No. 164 and Series on Pesticides No. 66) EC (1997) Guidance Document 7029/VI/95 rev. 5 general recommendations for the design, preparation and realization of residue trials European Community Guideline SANCO 7525/VI/95, Rev. 10.3, 13/06/17: Comparability, extrapolation, group tolerances and data requirements for setting MRLs. EU Guidance Document SANCO/3029/99 rev. 4 for generating and reporting methods of analysis in support of pre-registration data requirements
Deviations:	No
GLP:	Yes
Validity of the study:	Valid

Materials and methods

4 trials on cucumber were conducted in Bulgaria (S19-00816-01), one in Southern France (S19-00816-02), one in Italy (S19-00816-03) and one in Spain (S19-00816-04) (indoor in greenhouses) using ‘Fungaflash’, an EC formulation containing 100 g/L imazalil, in the 2019 growing season.

‘Fungaflash’ was applied as a foliar spray at a rate of 75 g/ha imazalil in 1000 - 1250 L/ha of water. 4 applications were made with the final application 1 day before harvest.

Weather data were reported for the trials and no exceptional events were noted. The trials were at harvest trials and samples the fruit were taken by hand 1 day after last application. Samples containing at least 2 kg from 12 or more units were collected from both treated and control plots. Samples were frozen within 4 hours, and stored at ≤-18 ^{o}C until analysis. The maximum storage interval between harvest and analysis was 117 days. Data was considered in the DAR which demonstrates stability of imazalil for this period of time in high water crops when stored at ≤-18 ^{o}C.

Analysis was using LC-MS/MS method GAB-08103V. The analytical method is based on the multi residue method DFG S 19. The extraction with acetone/water according to the extraction module E1 was used.

Results and discussions

The analytical method was satisfactorily validated in accordance with SANCO 3029/99 rev. 4. The limit of quantitation (LOQ) of the method is 0.01 mg/kg and limit of detection (LOD) of the method is 0.003 mg/kg. Procedural recovery data for imazalil (and metabolite R01482), was provided, at LOQ and 10x LOQ, which showed acceptable mean recovery rates and %RSD. However, there only 3 procedural recoveries at each fortification level. As this method has been previously fully validated it is considered that these recoveries are fit for regulatory purpose.

No residues above 30% LOQ were detected in control samples for all trials.

A summary of the residues trials is given in Table C.3.1.2.2-1.

Conclusion

4 indoor trials on cucumber were conducted in Bulgaria, Southern France, Italy and Spain, and the trials were the proposed critical GAP.

Residues of imazalil in cucumber at 1 day after last application were between <0.01 mg/kg and 0.02 mg/kg mg/kg.

Residues of metabolite R01482 in cucumber at 1 day after last application were <0.01 mg/kg.

Table C.3.1.2.2-1 Residue trials on cucumber

Reference: Determination of residues of Imazalil and its metabolite R014821 after four applications of Fungaflash in cucumber (indoor) at four sites in Southern Europe 2019 [redacted]
Report No.: S19-00816

GLP:	Yes	Sample storage conditions:	Up to 117 days
Crop/crop group:	Cucumber (fruit crops)	Analytical method:	DFG S19 E1, validated
Indoor/outdoor:	Indoor	Limit of Quantification (mg/kg):	0.01
Formulation:	EC	Residues calculated as:	Imazalil and R014821
Content of active substance:	100 g/L Imazalil		

Trial No./ Location/ Year	Commodity / Variety	Date of 1.Sowing or planting 2.Flowering 3. Harvest	App.	rate	per treatment	Dates of treatment or no. of treatments and last date	Interval between applications (days)	Growth stage at last treatment	Portion analyzed	Residues	(mg/kg)		WHP (days)	Remarks

			g a.s./ ha	**Water (l/ha)**	**kg a.s./hl (IMZ/ PYR)**					Imazalil (IMZ)	R14821	Sum (a		
S19-00816-01 4417 Ognyanovo, Pazardzhik, Bulgaria	Cucumber / CUMA / Columbia F1	1. 03May/2019 2. 22/May/2019 to 27/May/2019 3. 09/June/2019	73.8 77.4 78.2 77.0	1082 1136 1147 1130	6.8 6.8 6.8 6.8	24/May/2019 29/May/2019 03/June/2019 08/June/2019	7	71	Fruit	<0.01	<0.01	<0.02	1	-
S19-00816-02 66200 Elne, Pyrénées-Orientales, Southern France	Cucumber / CUMSA / Diapason	1. 27/Mar/2019 2. 05/May/2019 to 22/May/2019 3. 22/May/2019	73.4 73.1 73.9 74.2	979 975 985 990	7.5 7.5 7.5 7.5	06/May/2019 11/May/2019 16/May/2019 21/May/2019	7	87-89	Fruit	0.02	<0.01	<0.03	1	-

S19-00816-03 97019 Vittoria, Ragusa, Italy	Cucumber / CUMSA / Audax	1. 15/Jun/2019 2. 25/Jun2019 to 15/Aug/2019 3. 07/Aug/2019	77.5 77.8 78.3 77.3	1047 1038 1044 1044	7.4 7.5 7.5 7.4	22/Jul/2019 27/Jul/2019 01/Aug/2019 06/Aug/2019	7	83	Fruit	<u><0.01</u>	<0.01	<0.02	1	-
S19-00816-04 02500 Tobarra, Albacete, Spain	Cucumber / CUMSA / Slida	1. 20/Apr/2019 2. 15/May/2019 to 03/Jul/2019 3. 03/Jul/2019	77.7 68.9 74.8 72.5	1029 1056 1197 1208	7.5 6.5 6.2 6.0	17/Jun/2019 22/Jun/2019 27/Jun/2019 02/Jul/2019	7	73-74	Fruit	<u><0.01</u>	<0.01	<0.02	1	-

(a)Sum calculated as imazalil and R014821 expressed as imazalil (R014821 x 1.15). For a foliar yse the RD-RA is imazalil only.

Table C.3.1.2.1-2 Residue trials on cucumber accepted in the MRL review (EFSA, 2015)

Trial No./ Location/ Year	Commodity / Variety	Date of 1.Sowing or planting 2.Flowering 3. Harvest	App.	rate	per treatment	Dates of treatment or no. of treatments and last date	Interval between applications (days)	Growth stage at last treatment	Portion analyzed	Residues	(mg/kg)		WHP (days)

			g a.s./ ha	**Water (l/ha)**	**kg a.s./hl (IMZ/ PYR)**				Imazalil (IMZ)	R14821	Sum (a	
S13-04508 S13-04508-01 76698 Ubstadt-Weiher, Baden-Württemberg, Germany	Cucumber / Khassib	1) 29 May 2013 2) 10 Jul-15 Oct 2013 3) 28 Sep 2013	0.081 0.082 0.077 0.075	1079 1099 1020 1004	0.008 0.007 0.008 0.007	12 Sep 2013 17 Sep 2013 22 Sep 2013 27 Sep 2013	79	fruits	0.02 0.02 0.01 < 0.01	<0.01 <0.01 <0.01 <0.01	<0.03 <0.03 <0.02 <0.02	0 1 3 7	LOQ= 0.01 mg/kg
S13-04508 S13-04508-02 6662 PK Elst, Gelderland, The Netherlands	Cucumber / Cratos	1) 08 Oct 2013 2) 21 Oct-06 Dec 2013 3) 29 Nov 2013	0.077 0.076 0.077 0.074	1020 1009 1028 980	0.008 0.008 0.007 0.008	13 Nov 2013 18 Nov 2013 23 Nov 2013 28 Nov 2013	60-79	fruits	0.04 0.05 0.03 0.02	<0.01 < 0.01 <0.01 < 0.01	<0.05 <0.06 <0.04 <0.03	0 1 3 7	LOQ= 0.01 mg/kg

(a)Sum calculated as imazalil and R014821 expressed as imazalil (R014821 x 1.15)

C.3.2 Nature and magnitude of residues in processed commodities

C.3.2.1 Nature of residues (Standard hydrolysis study)

Not submitted or required.

C.3.2.2 Magnitude of residues (Processing studies)

Not submitted or required.

C.3.3 Nature and magnitude of residues in rotational crops

Not submitted or required.

C.3.4 Nature and magnitude of residues in livestock

Not submitted or required.

C.3.5 Residues in honey

Not submitted or required.

C.3.6 Storage stability

C.3.6.1 Storage stability of residues in plant products

All studies presented below were evaluated by NL in the framework of the EU review and have been reviewed by HSE as part of this assessment.

Study 1

Reference:	Deep-Freeze Storage Stability of Imazalil in Citrus Processed Commodities for a Period of 12 Months, [redacted] (2014a), R-29509
Guideline(s):	Document 7032/VI/95 Rev.5, July 22, 1997 EU Regulations 544/2011 and 545/2011
Deviations:	No
GLP:	Yes
Validity of the study:	Yes

Materials and methods

Citrus processing fractions (marmalade and essential oil) samples were fortified with 0.51 mg/kg Imazalil. All samples were stored at ≤18°C for 0, 3, 6 and 12 months. Samples for time point 0 months were analysed immediately. The untreated control samples were used to produce control and recovery samples at each time point.

Levels of Imazalil in stored samples were determined using validated methods. For the marmalade samples, this consisted of the addition of acetonitrile/water (2:1, v:v) to the samples before adding a pre-package of QuEChERS extraction salts (MgSO4, NaCl, sodium citrate, and disodium citrate sesquihydrate). The samples were shaken and centrifuged before an aliquot was transferred to a QuEChERS dispersive SPE tube for clean-up. After clean-up, the samples were diluted with methanol/water (1:1, v/v) before final determination by LC-MS/MS. For the essential oil samples, the method consisted of the addition of water followed by vortex mixing. Acetonitrile and hexane were then added to the samples which were vortexed again, before the addition of a pre-package of QuEChERS extraction salts. After shaking and centrifugation, the upper hexane and oil layer was discarded from the sample using a glass pipette. An aliquot was then transferred to a QuEChERS dispersive SPE tube for clean-up. After clean-up, the samples were diluted with methanol/water (1:1, v/v) before final determination by LC-MS/MS. The limit of quantitation (LOQ) of the test method for Imazalil was 0.01 mg/kg.

Results and discussions

Stored fortified and stored control samples of citrus marmalade and essential oil were analyzed at day 0, after 3, 6 and 12 months of storage at ≤18°C. Imazalil levels remained stable in all matrices with 95 and 92% of the residue recovered after storage for 12 months for marmalade and essential oil respectively. Analysis was performed after each storage period together with one freshly fortified sample.

Table C.3.6.1-1. Recovery of Imazalil from citrus marmalade and essential oil

Processing matrix	**Storage periods [months]**	**Recoveries uncorrected**		**Procedural recoveries**
		Single values [%]	**Mean [%]**	**Single value [%]**
Marmalade	0	99, 95	97	98
	3	94, 108	101	87
	6	96, 90	93	90
	12	97, 96	97	103
Essential oil	0	92, 89	91	90
	3	95, 96	95	90
	6	93, 94	94	85
	12	85, 89	87	94

Conclusion

Imazalil was shown to be stable in citrus processing fractions (marmalade and essential oil) for a period up to 12 months when stored under frozen conditions.

Study 2

Reference:	Deep-Freeze Storage Stability of Imazalil Alcohol in Citrus Processed Commodities for a Period of 12 Months, [redacted] (2014b), R-29510
Guideline(s):	Document 7032/VI/95 Rev.5, July 22, 1997 EU Regulations 544/2011 and 545/2011
Deviations:	No
GLP:	Yes
Validity of the study:	Yes

Materials and methods

Citrus processing fractions (marmalade and essential oil) samples were fortified with 0.5 mg/kg Imazalil alcohol (R014821). All samples were stored at ≤18°C for 0, 3, 6 and 12 months. Samples for time point 0 months were analysed immediately. The untreated control samples were used to produce control and recovery samples at each time point.

Levels of Imazalil alcohol in stored samples were determined using validated methods. For the marmalade samples, this consisted of the addition of acetonitrile/water (2:1, v:v) to the samples before adding a pre-package of QuEChERS extraction salts (MgSO4, NaCl, sodium citrate, and disodium citrate sesquihydrate). The samples were shaken and centrifuged before an aliquot was transferred to a QuEChERS dispersive SPE tube for clean-up. After clean-up, the samples were diluted with methanol/water (1:1, v/v) before final determination by LC-MS/MS. For the essential oil samples, the method consisted of the addition of water followed by vortex mixing. Acetonitrile and hexane were then added to the samples which were vortexed again, before the addition of a pre-package of QuEChERS extraction salts. After shaking and centrifugation, the upper hexane and oil layer was discarded from the sample using a glass pipette. An aliquot was then transferred to a QuEChERS dispersive SPE tube for clean-up. After clean-up, the samples were diluted with methanol/water (1:1, v/v) before final determination by LC-MS/MS. The limit of quantitation (LOQ) of the test method for Imazalil alcohol was 0.01 mg/kg.

Results and discussions

Stored fortified and stored control samples of citrus marmalade and essential oil were analyzed at day 0, after 3, 6 and 12 months of storage at ≤18°C. Imazalil alcohol levels remained stable in all matrices with 93% of the residue recovered after storage for 12 months for both marmalade and essential oil samples. Analysis was performed after each storage period together with one freshly fortified sample. The mean recovery values of the stored samples are corrected for the freshly prepared fortification samples (procedural recovery) to compensate for matrix induced variations during the storage periods.

Table C.3.6.1-2. Recovery of Imazalil alcohol from citrus marmalade and essential oil

Processing matrix	**Storage periods [months]**	**Uncorrected recoveries**		**Procedural recoveries**
		Single values [%]	**Mean [%]**	**Single value [%]**
Marmalade	0	83, 77	80	76
	3	98,109	104	97
	6	89,84	87	91
	12	99, 88	93	88
Essential oil	0	82, 82	82	84
	3	77, 76	77	86
	6	78, 77	78	77
	12	78, 72	75	82

Conclusion

Imazalil alcohol was shown to be stable in citrus processing fractions (marmalade and essential oil) for a period up to 12 months when stored under frozen conditions.

Study 3

Reference: Storage stability of Imazalil-derived residues in citrus fruit and citrus by-products. [redacted] (1993), Report number R 23979/AGR

Guideline(s): EPA Pesticide Assessment Guidelines, Subdivision O, 174-4 (e)

Deviations: No

GLP: Yes

Validity of the study: Yes

Materials and methods

Orange fruits and processing fractions (dried peel, chopped peel, oil, molasses, and juice) samples were fortified at two different concentrations either with Imazalil or metabolite R 14821. Samples were stored at -20°C for 12, 24 and 34-35 weeks. Samples for time point 0 weeks were analysed within 24 hours.

Levels of Imazalil and R 14821 in stored samples were determined using the validated method GC-ECD.

Results and discussions

Imazalil and R 14821 residue levels as found in the triplicate samples taken for each commodity and fortification level after each storage period are summarized in Table 3.5.1.10-1 and Table 3.5.1.10-2, respectively. Procedural recoveries ranged from 87-110% and 93-112% for Imazalil and R 14821, respectively. RSD values were all <7.6%. The method is considered acceptable.

Residue recoveries ranged from 87.2-120% and 73.1-111% for Imazalil and R 14821, respectively.

Table 3.6.1-3 Stability of Imazalil residues following storage at -20°C.

Commodity	**Level** (µg/g or µg/mL)	**Storage interval** (months)	**Fresh recovery** (mean, %)	**Residues after storage** (mg/kg)		**Recovery** (% day 0)	
				Individual values	mean		
Whole fruit	0.250	0 3 6 8	97.1 97.1 99.3 91.3	0.248, 0.230, 0.250 0.246, 0.239, 0.243 0.254, 0.246, 0.245 0.238, 0.217, 0.230	0.243 0.243 0.248 0.228	100 100 102 94.1	
	10.0	0 3 6 8	110 108 104 95.5	11.2, 10.9, 10.8 10.9, 10.8, 10.8 10.5, 10.5, 10.2 9.42, 9.85, 9.39	11.0 10.8 10.4 9.55	100 98.7 95.3 87.2	

Commodity	**Level** (µg/g or µg/mL)	**Storage interval** (months)	**Fresh recovery** (mean, %)	**Residues after storage** (mg/kg)		**Recovery** (% day 0)	
				Individual values	mean		
Dried peel	2.50	0 3 6 8	87.0 91.2 92.1 84.4	2.17, 2.08, 2.27 2.23, 2.35, 2.26 2.31, 2.26, 2.34 2.22, 2.07, 2.04	2.17 2.28 2.30 2.11	100 105 106 97.1	
	25.0	0 3 6 8	103 96.6 107 94.4	25.8, 25.2, 26.1 24.3, 24.6, 23.5 28.9, 25.9, 25.2 24.4, 23.6, 22.7	25.7 24.1 26.7 23.6	100 94.1 104 91.9	

Commodity	**Level** (µg/g or µg/mL)	**Storage interval** (months)	**Fresh recovery** (mean, %)	**Residues after storage** (mg/kg)		**Recovery** (% day 0)	
				Individual values	mean		
Chopped peel	2.50	0	92.0	2.23, 2.46, 2.21	2.30	100	
		3	102	2.66, 2.52, 2.48	2.55	111	
		6	97.0	2.51, 2.34, 2.43	2.42	105	
		8	91.4	2.23, 2.32, 2.30	2.29	99.4	
	25.0	0	105	25.3, 26.6, 26.4	26.1	100	
		3	103	25.8, 26.3, 25.2	25.7	98.5	
		6	107	27.9, 26.3, 26.0	26.7	102	
		8	93.1	23.4, 23.5, 22.9	23.3	89.0	

Commodity	**Level** (µg/g or µg/mL)	**Storage interval** (months)	**Fresh recovery** (mean, %)	**Residues after storage** (mg/kg)		**Recovery** (% day 0)	
				Individual values	mean		
Oil	0.250	0 3 6 8	102 110 96.4 97.1	0.280, 0.255, 0.227 0.264, 0.268, 0.291 0.230, 0.222, 0.271 0.253, 0.240, 0.235	0.254 0.274 0.241 0.243	100 108 94.9 95.5	
	25.0	0 3 6 8	101 98.0 98.4 89.3	25.1, 26.6, 24.0 23.8, 24.9, 24.8 25.2, 25.5, 23.2 22.2, 22.9, 21.8	25.2 24.5 24.6 22.3	100 97.1 97.5 88.5	

Commodity	**Level** (µg/g or µg/mL)	**Storage interval** (months)	**Fresh recovery** (mean, %)	**Residues after storage** (mg/kg)		**Recovery** (% day 0)	
				Individual values	mean		
Molasses	1.00	0	102	1.03, 1.02, 1.03	1.02	100	
		3	107	1.03, 1.19, 0.996	1.07	105	
		6	96.6	0.990, 0.940, 0.969	0.966	94.4	
		8	95.7	0.942, 0.957, 0.973	0.957	93.5	
	10.0	0	109	10.9, 10.9, 11.0	10.9	100	
		3	104	10.3, 10.5, 10.4	10.4	95.5	
		6	105	10.2, 10.3, 10.9	10.5	96.0	
		8	105	10.4, 10.6, 10.4	10.5	95.8	

Commodity	**Level** (µg/g or µg/mL)	**Storage interval** (months)	**Fresh recovery** (mean, %)	**Residues after storage** (mg/kg)		**Recovery** (% day 0)	
				Individual values	mean		
Juice	0.250	0 3 6 8	87.6 101 105 95.7	0.225, 0.219, 0.213 0.256, 0.237, 0.266 0.259, 0.270, 0.262 0.236, 0.241, 0.241	0.219 0.253 0.264 0.239	100 116 120 109	
	1.00	0 3 6 8	101 103 106 98.0	1.03, 0.983, 1.01 1.05, 1.06, 0.979 1.04, 1.07, 1.07 1.02, 0.990, 0.929	1.01 1.03 1.06 0.980	100 102 105 97.2	

Table 3.6.1-4 Stability of R 14821 residues following storage at -20°C.

Commodity	**Level** (µg/g or µg/mL)	**Storage interval** (weeks)	**Fresh recovery** (mean, %)	**residues after storage** (mg/kg)		**Recovery** (% day 0)	
				Individual values	mean		
Whole fruit	0.250	0	98.7	0.247, 0.243, 0.250	0.247	100	
		3	98.7		0.247	100	
		6	104	0.250, 0.243, 0.247	0.259	105	
		8	97.1	0.265, 0.256, 0.256	0.243	98.4	
				0.239, 0.248, 0.241			
	1.00	0	106	1.10, 1.07, 0.999	1.06	100	
		3	109		1.09	103	
		6	98.9	1.09, 1.05, 1.14	0.989	93.7	
		8	94.7	1.00, 0.971, 0.993	0.947	89.7	
				0.954, 0.973, 0.913			
Dried peel	0.250	0	95.9	0.231, 0.238, 0.250	0.240	100	
		3	98.0		0.245	102	
		6	95.5	0.252, 0.254, 0.229	0.239	99.6	
		8	96.1	0.238, 0.231, 0.247	0.240	100	
				0.247, 0.231, 0.243			

Commodity	**Level** (µg/g or µg/mL)	**Storage interval** (weeks)	**Fresh recovery** (mean, %)	**residues after storage** (mg/kg)		**Recovery** (% day 0)	
				Individual values	mean		
	2.50	0	93.4	2.34, 2.33, 2.34	2.34	100	
		3	95.4	2.36, 2.41, 2.39	2.38	102	
		6	104	2.79, 2.51, 2.47	2.59	111	
		8	91.1	2.30, 2.30, 2.23	2.28	97.5	
Chopped peel	0.250	0	103	0.262, 0.271, 0.242	0.258	100	
		3	100	0.258, 0.238, 0.254	0.250	96.8	
		6	94.5	0.248, 0.238, 0.223	0.236	91.5	
		8	89.1	0.216, 0.219, 0.233	0.223	86.2	
	2.50	0	95.9	2.29, 2.45, 2.45	2.40	100	
		3	101	2.53, 2.58, 2.43	2.51	105	
		6	101	2.60, 2.54, 2.42	2.52	105	
		8	93.6	2.38, 2.38, 2.26	2.34	97.7	

Commodity	**Level** (µg/g or µg/mL)	**Storage interval** (weeks)	**Fresh recovery** (mean, %)	**residues after storage** (mg/kg)		**Recovery** (% day 0)	
				Individual values	mean		
Oil	0.250	0	100	0.255, 0.252, 0.245	0.251	100	
		3	96.1	0.249, 0.227, 0.245	0.240	95.9	
		6	92.5	0.235, 0.211, 0.248	0.231	92.5	
		8	88.5	0.237, 0.219, 0.208	0.221	88.5	
	2.50	0	106	2.65, 2.75, 2.55	2.65	100	
		3	98.2	2.37, 2.49, 2.51	2.45	92.6	
		6	91.3	2.45, 2.32, 2.07	2.28	86.1	
		8	77.5	1.82, 1.88, 2.11	1.94	73.1	
Molasses	0.250	0	98.9	0.249, 0.231, 0.262	0.247	100	
		3	108	0.257, 0.297, 0.258	0.271	109	
		6	102	0.257, 0.258, 0.253	0.256	104	
		8	99.2	0.250, 0.245, 0.249	0.248	100	

Commodity	**Level** (µg/g or µg/mL)	**Storage interval** (weeks)	**Fresh recovery** (mean, %)	**residues after storage** (mg/kg)		**Recovery** (% day 0)	
				Individual values	mean		
	1.00	0 3 6 8	103 99.2 94.6 98.7	1.05, 1.00, 1.05 0.996, 0.979, 1.00 0.953, 0.966, 0.919 1.00, 0.968, 0.992	1.05 0.996 0.953 1.00	100 96.0 91.5 95.5	
Juice	0.025	0 3 6 8	112 108 94.7 101	0.029, 0.028, 0.027 0.027, 0.025, 0.029 0.025, 0.023, 0.023 0.025, 0.026, 0.025	0.028 0.027 0.024 0.025	100 96.4 84.5 90.5	
	0.250	0 3 6 8	103 102 99.6 101	0.260, 0.257, 0.252 0.256, 0.254, 0.253 0.253, 0.251, 0.243 0.251, 0.251, 0.258	0.256 0.254 0.249 0.253	100 99.2 97.1 98.8	

Conclusion

Imazalil and metabolite R-14821 were shown to be stable in oranges and its processed fractions (dried peel, chopped peel, oil, molasses, and juice) for a period up to 34-35 weeks, i.e. 8 months, when stored under frozen conditions (-20°C).

C.3.6.2 Storage stability of residues in animal products

Not submitted or required.

C.3.6.3 Storage stability in honey

Not submitted or required.

C.3.7 Unit to unit variability

A study has been submitted to replace the default VF for citrus used in the acute risk assessment. The study submitted has been evaluated fully below, and variability factors calculated from this have been used within the acute risk assessments (see Section 7.1).

C.3.7.1 Citrus

Reference:	Determination of variability factor of residues of imazalil and its metabolite R014821 following a post-harvest application by drencher of Fecundal 500 EC (Imazalil 50% w/v) on oranges (citrus sinensis) in southern Europe 2019, [redacted] 2020b, report No. 168SRES19R04, AGR 6003, SRES19-616-168FR

GLP:	Yes	Sample storage conditions:	max. 49 days ≤ -18ºC
Crop/crop group:	Oranges	Analytical method:	LC-MS/MS, validated in Section C.1.3
Indoor/Outdoor:	Indoor (Post-harvest)	Limit of Quantification (mg/kg):	0.01
Formulation:	EC	Residues calculated as:	Imazalil, R014821
Content of active substance (g/kg or g/l):	500 g/L Imazalil		

The objective of the study was to determine residue levels of Imazalil and its metabolite R014821 in a large sample group of oranges (n = 122). The variability of residues in this sample size has been used to generate variability factors for both analytes in whole fruit, which can be used to refine the acute dietary risk assessment.

Fecundal 500 EC (Imazalil 50% w/v) was applied via drencher once with an application rate of 0.1 L product per hectoliter (50 g Imazalil/hL) to fruits collected from a field located in Algemesi (Valenica, Spain).

Samples were collected at 0 Days Before Application (DBA) for untreated fruits and at 0 Days After Application (DAA) for treated fruits once application deposit was dry.

Before application the sampling of untreated fruits (1 or 6 whole fruits) was undertaken. Untreated samples were taken and introduced into double plastic bags, properly labelled and kept deep frozen until their shipment to the lab. Effort was made to ensure fruits were of the same category in order to avoid large differences in sizes.

After application, the fruits were allowed to dry at ambient temperature, according to GAP, in the same plastic boxes where they were kept during application (neither direct sunlight nor wiping off with a tissue was allowed). Once the spray solution was dry, sampling 0 DAA was undertaken. Treated fruits were collected with a metal clamp randomly from the 3 boxes and from all parts, top and bottom, exposed and covered by other fruits. Both large and small fruits were collected, but very small, damaged, diseased or abnormal fruits were disregarded.

Disposable gloves were used and changed between collecting samples at different plots. Treated and untreated samples were separated. Samples were obtained and introduced into double plastic bags, labelled and kept deep frozen until their shipment to the lab.

Residues of Imazalil and its metabolite R014821 were analysed according to LC with MS/MS detection (satisfactorily validated in this assessment, see section 1.3). Untreated and treated post-harvest whole fruit samples were taken and analysed for residues of Imazalil, and R014821. In untreated samples, no residues of Imazalil or R014821 were found above the limit of quantification (LOQ).

Results

In the treated orange whole fruit samples residues of Imazalil ranged from 1.04 to 3.81 mg/kg.

In the treated orange whole fruit samples residues of metabolite R014821 ranged from 0.015 to 0.048 mg/kg.

The Vf is calculated with the following formula:

$$Vf = \frac{97.5th\ percentile}{mean}$$

The percentile function in Excel can be used to calculate the 97.5th percentile from the data set. There are two statistical methods for calculating this value within excel (INC and EXC), however both lead to very similar values (1.56 and 1.57 for imazalil using INC and EXC respectively).

The Hamilton method uses cumulative probability contributions to estimate the percentiles and is recommended by JMPR and OECD 509. The variability factor produced using this method is 1.69 for imazalil and has been taken forward within this assessment.

The data are for treated oranges at a WHP of 0 days. This is inline with the GAP and is acceptable for imazalil. For R014821, residues may increase at longer WHP, so the data has not been used to calculate a Vf. The default Vf has been used in the acute risk assessment for the metabolite.

For completeness and transparency, the 122 values used for the calculation of the variability factor have been listed below. The residue levels for the metabolite are also given, but as stated have not been used to calculate a specific Vf.

Table C.3.7.1-1 Values used in variability factor calculation

Imazalil	R014821
3.81	0.046
2.94	0.038
2.92	0.023
2.91	0.033
2.82	0.035
2.8	0.041
2.78	0.032
2.72	0.048
2.7	0.032
2.62	0.037
2.6	0.027
2.57	0.029
2.52	0.03
2.51	0.033
2.48	0.023
2.38	0.034
2.37	0.029
2.37	0.022
2.32	0.036
2.31	0.033
2.29	0.028
2.21	0.022
2.2	0.035
2.18	0.028
2.15	0.03
2.14	0.027

2.13	0.045
2.13	0.021
2.11	0.029
2.08	0.029
2.06	0.037
2.04	0.031
2.03	0.021
2.02	0.022
2.01	0.033
2	0.024
1.99	0.029
1.99	0.025
1.97	0.021
1.96	0.037
1.96	0.031
1.95	0.025
1.94	0.03
1.91	0.031
1.91	0.028
1.9	0.033
1.9	0.027
1.9	0.025
1.89	0.038
1.89	0.029
1.88	0.038
1.88	0.024
1.86	0.027
1.86	0.026
1.84	0.025
1.84	0.019
1.83	0.033
1.83	0.032
1.83	0.028
1.81	0.03
1.81	0.024
1.81	0.024
1.8	0.031
1.79	0.032
1.78	0.023

1.76	0.023
1.75	0.026
1.75	0.026
1.74	0.028
1.74	0.023
1.74	0.018
1.73	0.028
1.71	0.036
1.71	0.035
1.7	0.025
1.69	0.025
1.68	0.029
1.68	0.028
1.68	0.023
1.66	0.023
1.63	0.023
1.62	0.024
1.61	0.03
1.6	0.026
1.59	0.027
1.56	0.029
1.56	0.027
1.56	0.023
1.55	0.025
1.54	0.027
1.54	0.027
1.54	0.025
1.52	0.038
1.52	0.021
1.51	0.033
1.48	0.028
1.46	0.03
1.45	0.032
1.45	0.03
1.45	0.025
1.44	0.024
1.42	0.016
1.4	0.03
1.39	0.02

1.39	0.017
1.38	0.023
1.37	0.023
1.37	0.018
1.36	0.019
1.35	0.025
1.33	0.033
1.32	0.032
1.31	0.024
1.31	0.024
1.28	0.02
1.27	0.042
1.25	0.03
1.25	0.027
1.24	0.017
1.12	0.023
1.04	0.021
1.04	0.015

Appendix D – List of endpoints

The endpoints outlined in Table D.1 to D.3 have been derived as a result of this assessment. All other endpoints remained unchanged. The full list of endpoints is outlined in EFSA, 2018.

Table D.1 Mammalian toxicology

	Value (mg/kg bw (per day))	**Study**	**Uncertainty factor**
Imazalil			
Acceptable Daily Intake (ADI)	0.025*	1-year dog study	100
Acute Reference Dose (ARfD)	0.05*	Rabbit developmental tox study	100
R014821			
Acceptable Daily Intake (ADI)	0.014	90-day rat study	1000
Acute Reference Dose (ARfD)	0.03	OECD 422 study in rats	1000

*The imazalil's ADI and ARfD apply also to metabolites FK772 and FK284

Table D.2 Plant residue definitions and methods for monitoring/enforcement

Plant residue definition for enforcement (RD-Enf) (for compliance with MRLs)	Imazalil (any ratio of constituent isomers)
Plant residue definition for risk assessment (RD-RA) (for estimation of dietary exposure)	1) Imazalil (any ratio of constituent isomers) 2) R014821

Table D.3 Animal residue definitions and methods for monitoring/enforcement

Animal residue definition for enforcement (RD-Enf) (for compliance with MRLs)	Sum of imazalil and FK-772 (any ratio of constituent isomers), expressed as imazalil.

Animal residue definition for risk assessment (RD-RA) (for estimation of dietary exposure)	Sum of imazalil, FK-284 and FK-772 (any ratio of constituent isomers), expressed as imazalil.

Appendix E – Import Tolerances

Sufficient information has not been provided to confirm that the GAPs outlined in Appendix A have been authorised in the EU.

It is noted that the MRL proposed in the exporting country is tabulated in Table E.1.

Table E.1 MRLs in force in the exporting country

Commodity	**MRL in force in the exporting country (mg/kg)**
Enforcement residue definition for products of plant origin: Imazalil (any ratio of constituent isomers)	
Citrus	7

Once this MRL comes into force and proof of authorisation of the GAP outlined in Appendix A is provided, the MRL can be adopted in GB.

Appendix F – Compound codes

No new metabolism studies were evaluated during this assessment and hence this section has not been completed.

Appendix G – Abbreviations

ADI	Acceptable daily intake
ADME	Absorption, distribution, metabolism and excretion
Animal model 2017	EFSA model used to calculate the dietary burden of livestock using the OECD feeding tables
ARfD	Acute reference dose
a.s.	Active substance
BBCH	Growth stages of mono- and dicotyledonous plants
bw	Body weight
CF	Conversion factor
cGAP	Critical GAP
CXL	Codex maximum residue limit
DAR	Draft assessment report
DAT	Days after treatment
DT_{90}	Period required for 90% dissipation (define method of estimation)
DT_{50}	Period required for 50% dissipation (define method of estimation)
EFSA	European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
GAP	Good Agricultural Practice
GB	Great Britain
GC-MS	Gas chromatography with mass spectrometry

GLP	Good laboratory practice
HPLC-MS/MS (LC-MS/MS)	High-performance liquid chromatography with tandem mass spectrometry
HR	Highest residue
HSE	Health and Safety Executive
IEDI	International estimate of daily intake
IESTI	International estimate of short-term intake
ILV	Independent laboratory validation
ISO	International Organisation for Standardization
LC_{50}	Lethal concentration, median
LD_{50}	Lethal dose, median
LOAEL	Lowest observed adverse effect level
LOD	Limit of detection or limit of determination (should be defined)
LoEP	List of endpoints
LOQ	Limit of quantification **NB** the limit of quantification and limit of determination are the same. Assimilated Regulation (EC) No 396/2005 refers to the limit of determination Assimilated Regulation (EC) No 1107/2009 refers to the limit of quantification MRLs marked with an asterisk (e.g. 0.01* mg/kg) are MRLs set at the limit of determination/quantification
MRL	Maximum residue level
NEDI	National estimate of dietary intake
NESTI	National estimate of short-term intake

NOAEL	No observed adverse effect level
NRL	National reference laboratory
OECD	Organisation for Economic Co-operation and Development
PBI	Plant-back interval
PES	Post-extraction solids
Pf	Processing factor
PHI	Preharvest interval
PRIMo	(EFSA) Pesticide Residue Intake Model
QuEChERS	Quick, Easy, Cheap, Effective, Rugged, and Safe (analytical method)
RA	Risk assessment
RD	Residue definition
RD-Enf	Residue definition for enforcement (also referred to as RD-Mo i.e. residue definition for monitoring)
RD-RA	Residue definition for risk assessment
RTI	Re-treatment interval
STMR	Supervised trials median residue
TRR	Total radioactive residue
TTC	Threshold of toxicological concern
WG	Water dispersible granule
WHP	With-holding period

Additional studies relied upon

Author(s)	Year	Title/Testing Facility/Report No/GLP or GEP Status/Published or not	Submitter
[redacted]	2020	Determination of variability factor of residues of imazalil and its metabolite R014821 following a post-harvest application by drencher of Fecundal 500 EC (Imazalil 50% w/v) on oranges (citrus sinensis) in southern Europe 2019, report No. 168SRES19R04, AGR 6003, SRES19-616-168FR	Janssen PMP
	2020	Cross validation of analytical methodology for Imazalil and its metabolite R014821 in mandarin samples. Report no. AGR6053, 358-20	Janssen PMP
	2021	A 90-Day Study of Imazalil, FK772, FK284 and R014821 by Dietary Administration in Wistar Han Rats. Report no. AGR5959. GLP. Unpublished	Janssen PMP
	2018	Combined 28-Day Repeated Dose Toxicity Study with the Reproduction/ Developmental Toxicity Screening Test of JNJ-123955-AAA (T000824) in Rats by Oral Gavage. Report no. 517992. GLP. Unpublished	Janssen PMP
	2022	A 90-Day Study of R014821 by Dietary Administration in Wistar Han Rats. Report no. AGR6320. GLP. Unpublished	Janssen PMP
	2019	An in vitro Micronucleus Assay with R014821 in Cultured Peripheral Human Lymphocytes. Report no. AGR5957. GLP. Unpublished	Janssen PMP
	2020a	The Alkaline in vivo Comet Assay with R014821 in Cultured Liver, Duodenum and Stomach of Wistar Han rats. Report no. AGR5964. GLP. Unpublished	Janssen PMP
	2020b	An in vitro Micronucleus Assay with FK284 in Cultured Peripheral Human Lymphocytes. Report no. AGR5955. GLP. Unpublished	Janssen PMP
	2020c	An in vitro Micronucleus Assay with FK772 in Cultured Peripheral Human Lymphocytes. Report no. AGR5956. GLP. Unpublished	Janssen PMP

Author(s)	Year	Title/Testing Facility/Report No/GLP or GEP Status/Published or not	Submitter
[redacted]	2020d	An in vivo Micronucleus Assay with FK284. Report no. 20247349. GLP. Unpublished	Janssen PMP
	2020	Evaluation of the Mutagenic Activity of FK772 in an in vitro Mammalian Cell Gene Mutation Test with L5178Y Mouse Lymphoma Cells. Report no. AGR6067. GLP. Unpublished	Janssen PMP