Draft Assessment Report

Evaluation of Active Substances

Plant Protection Products

Prepared according to **Regulation (EC) 1107/2009**
as it applies in Great Britain

Metalaxyl-M

Volume 3 – B.6 (AS)

Toxicology & Metabolism Data

GB Article 7 Amendment

Great Britain

January 2024

Version History

When	What
January 2024	Initial DAR

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B.6. TOXICOLOGY AND METABOLISM DATA

Metalaxyl-M is an active substance that was included in Annex I to Council Directive 91/414/EEC concerning the placing of plant protection products on the market, by Commission Directive 2002/64/EC. Metalaxyl-M is deemed to have been approved under Regulation (EC) No 1107/2009 and is listed in Part A of the Annex to Commission Implementing Regulation (EU) No 540/2011.

In accordance with the provisions of Article 5 of Directive 91/414/EEC, Syngenta Crop Protection AG notified to the Commission of their wish to renew the approval of the active substance metalaxyl-M in Annex I to the Directive with the submission of the Supplementary Dossier in 2012.

The Rapporteur Member State Belgium and the co-Rapporteur Member State Greece, designated by Commission Regulation (EU) No 1141/2010, submitted in November 2013 the relevant renewal assessment reports (dRAR) and recommendations to the European Food Safety Authority (EFSA).

EFSA sent to the Commission its conclusion on the risk assessment on 30 January 2015[1].

According to the provisions of Article 17 of Regulation (EU) No 1141/2010, the Commission referred a draft review report on the renewal of approval to the Standing Committee on Plants, Animals, Food and Feed, for examination on 14 July 2015. A modified draft was referred to the Committee on 16 July 2019. The draft review report on renewal of approval was finalised by the Standing Committee on 24 March 2020.

On the 3 April 2020 Member States gave a favourable opinion (by written procedure) to renew the approval of metalaxyl-M and on 5 May 2020 the “Commission Implementing Regulation (EU) 2020/617 renewing the approval of the active substance metalaxyl-M, and restricting the use of seeds treated with plant protection products containing it, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market, and amending the Annex to Commission Implementing Regulation (EU) No 540/2011” was published[2]. The Commission Implementing Regulation (EU) 2020/617 also stipulates that a number of impurities are of

[1] EFSA (European Food Safety Authority), 2015. Conclusion on the peer review of the pesticide risk assessment of the active substance metalaxyl-M. EFSA Journal 2015;13(3):3999, 105 pp. doi:10.2903/j.efsa.2015.3999

[2] Official Journal of the European Union, L 143/6, 2020. COMMISSION IMPLEMENTING REGULATION (EU) 2020/617 of 5 May 2020 renewing the approval of the active substance metalaxyl-M, and restricting the use of seeds treated with plant protection products containing it, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market, and amending the Annex to Commission Implementing Regulation (EU) No 540/2011. Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32020R0617&from=EN.

toxicological relevance. In particular, the relevant impurity 2-[(2,6-dimethyl-phenyl)-(2- methoxyacetyl)-amino]-propionic acid 1-methoxycarbonyl-ethyl ester (CGA226048) was given a maximum content of 0.18 g/kg due to genotoxicity concerns. The renewal of metalaxyl-M applies since 1 June 2020.

Syngenta has provided an application to amend the conditions of the approval of metalaxyl-M in accordance with Article 7 of Regulation (EU) 1107/2009, as retained in GB. This application presents additional information (an in vivo micronucleus study) on the metalaxyl-M impurity CGA226048 to show that the impurity is not genotoxic, no longer relevant, and can be specified at a maximum level in line with the 5-batch analysis and Quality Control data which was evaluated in the original EU RAR 2014.

B.6.1. ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION IN MAMMALS

No new data have been submitted. Please refer to original EU RAR 2014.

B.6.2. ACUTE TOXICITY

No new data have been submitted. Please refer to original EU RAR 2014.

B.6.3. SHORT-TERM TOXICITY

No new data have been submitted. Please refer to original EU RAR 2014.

B.6.4. GENOTOXICITY

No new data have been submitted. Please refer to original EU RAR 2014.

B.6.5. LONG-TERM TOXICITY AND CARCINOGENESIS

No new data have been submitted. Please refer to original EU RAR 2014.

B.6.6. REPRODUCTIVE TOXICITY

No new data have been submitted. Please refer to original EU RAR 2014.

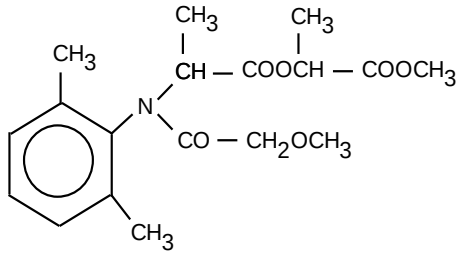
B.6.7. NEUROTOXICITY

No new data have been submitted. Please refer to original EU RAR 2014.

B.6.8. OTHER TOXICOLOGICAL STUDIES

B.6.8.1. Toxicity studies on metabolites and relevant impurities

Relevant impurity CGA226048



During the renewal evaluation on metalaxyl-M, an Ames test, a mammalian cell gene mutation assay and an in vitro chromosome aberration study on impurity CGA226048, were considered. Below, the evaluation of these studies from the EU RAR (2014) is re-presented.

Genotoxicity studies already available in the EU RAR (2014)

***Salmonella* and *Escherichia* microsome test, CGA 226048 (2-[(2,6-dimethyl-phenyl)-(2-methoxy-acetyl)-amino]-propionic acid 1-methoxycarbonyl-ethyl ester) ([redacted], 2000)**

[Study reference: Salmonella and Escherichia/mammalian-microsome mutagenicity test. Test number: 19 993 124; Test substance: CGA 226048 (By-product of CGA 329351). Final report issued february 29, 2000, Novartis Crop Protection Limited, Basel, Switzerland]

Guidelines:
Experimental protocol not fully in compliance with test method B.14, Annex V, directive 92/69/EEC or directive 84/449/EEC or OECD guideline 471 (1983).
Deviation from official protocol : A statistical analysis was not performed, because their use is not generally recommended concerning this particular test (minor point).

GLP: Yes (no attest of competent authority)

Material and methods:
CGA 226048 (99 % ; b.n°.BPS 659/2) dissolved in DMSO was tested for mutagenicity towards *S.typhimurium* TA 98, TA 100, TA 102, TA 1535, TA 1537 and *E.coli* WP2 uvrA with and without liver S9 (10% v:v) from Aroclor 1254 pretreated rats. A preliminary experiment on strains TA100 and WP2 uvrA, was conducted at 20.58, 61.73, 185.19, 555.56, 1666.67 and 5000 µg/plate. Five concentrations were used: 312.50, 625.00, 1250.00, 2500.00, 5000.00 µg/plate. The experiment was repeated. The standard plate incorporation assay was used in the first experiment, and the pre-incubation assay in the confirmatory experiment.

Indirect mutagens used as positive controls : 2-aminoanthracene (TA 98, TA 100, TA 102, TA 1535, TA 1537 and *E.coli* WP2 uvrA) , cyclophosphamide (TA 1535).
Direct mutagens used as positive controls : Sodium azide (TA1535, TA100), 4-nitroquinoline (WP2 uvrA), mitomycin-C (TA102), 2-nitrofluorene (TA98) and 9-aminoacridine (TA1537). An analytical control was performed in order to verify the stability of the test article in the solvent by GF/FID. It was observed that the measured levels were in line with the intended ones (105-111% of nominal). The study was performed in good experimental conditions; the acceptance and evaluation criteria were well defined and appropriate. Positive controls gave the expected results.
The study was acceptable.

Findings:
A range-finding study was performed with 6 concentrations, ranging from 20.6 to 5000 µg/plate tested with strain *Salmonella typhimurium* TA 100 and strain *Escherichia coli* WP2 uvrA to determine the highest concentration to be used in the mutagenicity test. Negative results were observed with both strains.
In the first experiment as well as in the confirmatory experiment, no increased incidence of mutants was observed. A reduction of the colony counts was noted at the top-concentartion, in the presence of metabolic activation, in the confirmatory assay (all strains except TA1535 and TA98).

Conclusion:
CGA 226048 did not induce point mutations in *S.typhimurium* and in *E.coli* neither in the presence nor in the absence of S9 up to the limit concentration for this test.
([redacted], 2000)

Mouse lymphoma cell line L5178Y/TK$^{+/-}$ CGA 226 048; CGA 226 048 (2-[(2,6-dimethyl-phenyl)-(2-methoxy-acetyl)-amino]-propionic acid 1-methoxycarbonyl-ethyl ester) ([redacted], 2004)

[Report: [redacted] (2004), Metalaxyl-M impurity CGA 226 048: L5178Y TK$^{+/-}$ Mouse Lymphoma Mutation Assay. Central Toxicology Laboratory, Alderley Park Macclesfield Chesire, UK. CTL Study NumberVV0319 No., issue date: 22 December 2004. Unpublished. Sponsor reference T004822-04]

Guidelines:
Experimental protocol fully in compliance with EC test method B17 of Dir. 2000/32/EC (2000) and In Vitro Mammalian Cell Gene Mutation Test OECD 476 (1997)

Material and methods:
Test material: CGA 226 048 (batch number: BPS 659/2; Purity: 99 % w/w).
The concentration *levels* used were determined in a pre-experiment for toxicity.
The main experiments were performed at 0 (solvent control); 250.0; 500.0; 1000; 2000 or 3514 µg/mL, both in the presence and in the absence of metabolic activation (rat-liver S9).

Cell treatment:

- Cells were exposed to test compound, negative/solvent or positive controls for 4 hours in both the presence and absence of S9 mix. Both, pH value and osmolarity was determined at the maximum concentration of the test substance and at the solvent control without metabolic activation.
- After washing, cells were cultured for 2 days (expression period) before cell selection.
- After expression, 2×10^3 cells/mL were dispensed, at 200 µL/well, into 96 well plates. The cells were cultured for 10-13 days in selection medium to determine numbers of mutants. Dilutions of the cultures to approximately 8 cells/mL were cultured for 10-13 days without selective agent to determine cloning efficiency. There were 2 cultures/experiment.

Cell growth in individual microwell plates was assessed after 10-13 days using a dissecting microscope. The survival plates and viability plates were scored for the number of wells containing no cell growth (negative wells). The mutation plates were scored so that each well contained either a small colony (considered to be associated with clastogenic effects), a large colony (considered to be associated with gene mutation effects), or no colony.

Statistical Methods:

A logistic regression was performed to assess a possible dose dependent increase of mutant frequencies. The number of mutant colonies obtained for the groups treated with the test item was compared to the solvent control groups. A trend is judged as significant whenever the p-value (probability value) is <0.05. However, both, biological relevance and statistical significance were considered together.

Evaluation Criteria:

Each well of the mutation plates (those containing TFT) was scored as containing either, a small colony, a large colony or no colony according to the following criteria:

Manually count colonies were classified into two groups, in accordance with their size. The colony size distribution was determined in the controls and at all concentrations of the test item. Criteria to determine colony size were the absolute size of the colony (more than $^1/_4$ of a well diameter for large colonies) and the density of the colonies (the density of the small colonies is generally higher than that of the large colonies).

The test medium was visually checked for precipitation at the end of the treatment period just before the test substance was removed.

Negative controls: The range of the solvent controls was 80-110 mutant colonies per 10^6 cells; the range of the groups treated with the test substance was 70-140 mutant colonies per 10^6 cells.

Positive controls: EMS (5 mg/mL) and Benzo[*a*]pyrene (1 µg/mL) were used as positive controls and showed a distinct increase in induced total mutant colonies at acceptable levels of toxicity with at least one of the concentrations of the controls.

The study was acceptable.

Historical control mutant frequency (MF)* values compiled (period not stated) were submitted and tabulated below:

	Negative control	Positive control	Negative control	Positive control

	+S9		–S9	
Mean	1.40	10.90	1.40	12.50
Standard deviation	0.50	8.20	0.60	11.00
Minimum	0.70	1.60	0.50	2.80
Maximum	3.30	69.20	3.90	65.10
N° assays	78	78	69	69

*expressed as incidence /10^4 cells

Findings:
The study was performed to investigate the potential of CGA226048 to induce mutations at the mouse lymphoma thymidine kinase locus of the cell line L5178Y.

Preliminary toxicity assay:
The pre-experiment was performed in the presence and absence of metabolic activation with a treatment time of 4h at the concentration-levels of 250-3514 µg/mL. No test article precipitation nor cytotoxic effects leading to RSG values < 50% were observed up to the top-concentration with and without metabolic activation. No relevant increase of the osmolarity or pH value was observed neither. Therefore, the maximum concentration of 3514 µg/mL (~10 mM) was also used in the main experiments. The lower concentrations of both main experiments were spaced by a factor of two and more than four concentrations were used to overcome problems with possible deviations in toxicity.

Mutation assay:
The assay was performed in two independent experiments, using two parallel cultures each and a treatment period of 4 hours. Experiments I and II were performed with and without liver microsomal activation. No relevant test substance related effect on pH or osmolarity, and no precipitation was noted. No relevant cytotoxic effect indicated by a relative cloning efficiency 1 (survival) or a relative total growth (RTG) of <50% in both parallel cultures occurred in the first and second experiment with and without metabolic activation.

No substantial and reproducible dose dependent increase of the mutation frequency was observed in any of the experimental parts with and without metabolic activation. EMS (5 mg/mL) and Benzo[*a*]pyrene (1 µg/mL) were used as positive controls and showed a distinct increase in induced total mutant colonies at acceptable levels of toxicity with at least one of the concentrations of the controls.

Conclusion:
In conclusion, the test substance CG226048 did not induce mutations in the mouse lymphoma thymidine kinase locus assay using the cell line L5178Y in the absence or presence of metabolic activation up to the limit concentration for this assay.

Table B.6.8.1-1: Mammalian gene mutation assay on CGA226048 ([redacted], 2004)

	Test compound	**Dose (µg/mL)**	**S9**	**RTG (%)**	**MF (×10^{-4})**	**S9**	**RTG (%)**	**MF (×10^{-4})**
Experime	**DMSO**	**0**	–	100	0.8	+	100	1.1

nt I								
	CGA 226048	**250**	–	99	1.0	+	93	1.0
		500	–	113	1.1	+	114	1.1
		1000	–	101	1.0	+	86	1.5
		2000	–	91	0.8	+	69	1.4
		3514	–	58	0.7	+	79	1.2
	EMS	***500***	–	*40*	*6.2***			
	BP	***1***				+	*44*	*7.3***
Experiment II	**DMSO**	**0**	–	100	0.8	+	100	1.1
	CGA 226048	**250**	–	82	1.1	+	99	1.0
		500	–	75	0.9	+	83	1.1
		1000	–	101	0.7	+	75	1.4
		2000	–	81	1.0	+	80	1.3
		3514	–	91	1.2		66	1.4
	EMS	***500***	–	*50*	*3.3***			
	BP	***1***				+	*63*	*4.0***

RTG: Relative Total Growth (relative suspension growth × viability)
MF: mutant colonies per 10^4 cells
Statistically significant deviation: **p ≤0.01

([redacted], 2004)

CGA226048, chromosome aberration assay, human lymphocytes ([redacted], 2005)

[Report: [redacted] (2005) Metalaxyl-M impurity CGA 226 048: In vitro cytogenetic assay in Human Lymphocytes. Central Toxicology Laboratory - Alderley Park Macclesfield, Chesire UK. Sponsor Reference T013126-04; CTL Test substance Reference number: Y13192/001; CTL Study number SV1276; Document number CTL/SV1276/REG/REPT; issue date: 14 April 2005. Unpublished.]

Guidelines:
Experimental method fully in compliance with test method Chromosome Aberration Test in Human Lymphocytes In Vitro, OECD 473 (1997).

GLP: yes

Material and methods:
Human lymphocytes from 2♂ or 2♀ donors (pooled) were incubated in RPMI-1640 medium (+10% FCS) 48h before treatment. Three experiments, and 2 cultures/experiment were set up. No preliminary range-finder was performed.
Test material CGA226048, batch number: BPS 659/02; Purity: 99 % dissolved in DMSO (final conc. 10µL/mL, i.e.1% v:v), was added in the duplicate cultures, at concentration-levels of:

- Experiment 1: 0, 1.0, 1.5 or 2.25 mg/mL (+S9) and 0, 1.0, 1.25 or 1.5 mg/mL (–

S9)
- Experiment 2: 0, 1.5, 2.0 or 2.5 mg/mL (+S9) and 0, 0.125, 0.500 or 0.750 mg/mL (–S9)
- Experiment 3: 0, 0.125, 0.250 or 0.500 mg/mL (–S9)

Treatment durations (according to the study design in the report) in exp. 1: 3h (±S9) + 65h untreated, exp. 2: 3h (+S9) + 65h untreated or 20h (–S9) + 48h untreated; exp 3: 20h (–S9) + 48h untreated.
Compound concentrations were not checked analytically. Osmolality and pH were shown not to be altered meaningfully in the treated cultures compared with controls. Post-mitochondrial supernatant was obtained from phenobarbital/β-naphthoflavone induced ♂SD rat, and used for preparation of metabolic activation mixture.
Positive control was obtained by treating Mitomycin C (MMC, –S9) at 0.5 (exp.1,2) or 0.2 (exp.3) µg/mL or with Cyclophosphamide (CP,+S9) at the dose-level of 50 µg/mL (exp.1,2).
Negative control was obtained by treating with the vehicle (DMSO). Two hours before harvest, cells were treated with colcemid (0.4 µg/mL). Cells were harvested and were treated with 0.075M KCl for hypotonic shock, followed by fixation (methanol:acetic acid, 3:1). Mitotic Index (M.I.) was determined (on at least 1000 cells/concentration/culture), as well as structural aberrations (on at least 100 cells/concentration/culture). The occurrence of polyploidy was <u>not</u> assessed. For positive controls, both M.I. and % aberrant cells were calculated from results on one culture.
Criterium for determining positive response: if the number of cells with aberrations (gaps included) is increased above HC in a “substantial” way or above study control level (and >HC) in a statistically significant way. Data were evaluated for statistical significance using the one-sided Fisher Exact Probability Test ($p \leq 0.05$).
The study was acceptable.

Historical control data: % aberrant cells excluding gaps (68h sampling time), compiled from 241 (+S9) and 267 (-S9) assays dated 04/1995-12/2002 were submitted and tabulated below:

	Negative control +S9	Positive control	Negative control –S9	Positive control
Mean	1.0	29.3	1.0	36.5
Standard deviation	0.9	10.8	0.9	11.2
Minimum	0.0	7.0	0.0	9.0
Maximum	5.0	60.0	4.0	64.0

<u>Findings:</u>
Both in the absence as well as in the presence of S9 mix, treatment with CGA226048 reduced the mitotic index, when compared to the respective study solvent controls. Therefore, the tested concentrations were clearly cytotoxic, mostly in a concentration-dependent way. It was observed that the top-concentration levels had M.I. of about 36-58% of controls in the absence of S9, and about 34-75% of controls in the presence of S9.

In the first experiment, where cells were treated for 3h in the <u>absence of S9</u>, up to

1500 µg/mL, no increase of cells carrying aberrations above control level was observed.

In the second experiment, exposure of lymphocytes to the concentrations of 500 and 750 µg/mL during 20h, in the absence of S9, induced a significant increase of cells with aberrations (5%), when compared to the control. The historical control (HC) data indicated that the observed incidence was slightly outside the HC range of 0.0-4.0%, and above the average 1.0± 0.9%.

In the third experiment, meant to replicate the exposure conditions of experiment 2, but at slightly *lower* concentrations (up to and including 500 µg/mL), no biological significant increase increase was noted. In this third experiment, the top-concentration (500 µg/mL) was lower than the top-concentration tested in the second experiment (750 µg/mL), but the mitotic index was substantially reduced in each case (45-64% of control values), indicating that cell toxicity occurred. As the incidence of aberrant cells displayed no dose-dependency, the likelihood for a biologically significant effect was low. In addition, the lowest concentration induced a (non-statistically significant) increase as high as 6%. Therefore, the statistically significant increase seen at 500 µg/mL and 750 µg/mL in the second experiment was considered equivocal.

Lymphocytes, exposed to CGA226048 in the first experiment during 3h in the presence of S9, showed an increased incidence of aberrations when exposed to the top-concentration levels. The increase attained statistical significance (p≤0.01) in the first experiment (4% aberrant cells *vs.* 0.5% in concurrent controls) at the concentration of 2250 µg/mL.

In the second experiment, the top-concentration of 2500 µg/mL induced aberrations above controls (4.0% *vs.* 1.5%), however, *without* attaining statistical significance.

When comparing the observed incidences (4% aberrant cells) with those of the historical solvent controls (in the presence of S9), it appeared that they fell within the HCD range (0.0-5.5%). It was checked whether the observed effects could have been consecutive to an exceptionally low value of the study control but it appeared that the study controls were within the average ± 1 s.d. of the HCD. Study solvent controls were at the level of 0.50-1.50%, which is well in line with the HCD average of 1.0±0.9%, and could thus not be regarded exceptionally low.

The positive controls, MMC (0.2 or 0.5 µg/mL) and CP (50 µg/mL) showed distinct increases in cells with structural chromosome aberrations.

Table B.6.8.1-2: summary of chromosome aberration assay (CGA226048) on human lymphocytes ([redacted], 2005)

	exposure period	**S9**	**test article**	**Concentration**	**M.I.**	**Aberrant cells**
						excl. gaps
				µg/mL	**%**	**%**
I	3h	–	DMSO	0	9.3	3.5

		–	CGA 226048	1000	8.7	6.0
		–		1250	6.4	3.0
		–		1500	5.4	2.5
		–	***MMC***	***0.5***	***8.5***	***56.00****
II	20h	–	DMSO	0	7.2	0.50
		–	CGA 226048	125	6.1	0.50
		–		500	4.6	**5.00****
		–		750	2.6	**5.00****
		–	***MMC***	***0.2***	***5.4***	***22.00****
III	20h	–	DMSO	0	7.6	2.5
		–	CGA 226048	125	7.5	6.00
		–		250	4.5	3.50
		–		500	4.2	4.00
		–	***MMC***	***0.2***	***3.1***	***28.57****
I	3h	+	DMSO	0	5.1	0.50
		+	CGA 226048	1000	5.7	1.00
		+		1500	4.1	3.00
		+		2250	3.8	**4.00****
		+	***CP***	***50***	***2.4***	***44.00****
II	3h	+	DMSO	0	11.6	1.50
		+	CGA 226048	1500	9.5	2.00
		+		2000	6.9	3.50
		+		2500	4.0	**4.00**
		+	***CP***	***50***	***7.6***	***36.67****

*Statistically significant deviation (*p≤0.05, **p≤0.01, Fisher exact test); positive controls Mitomycin C (MMC) or cyclophospamide (CP); M.I.: mitotic index, average % for two cultures; three experiments: exp I, II and III performed.

Conclusion

Overall, in a guideline in vitro chromosome aberration assay, CGA226048 was equivocally positive in the absence of S9 and weakly positive in the presence of S9 at cytotoxic concentrations.

([redacted], 2005)

Overall conclusion on the toxicological relevance assessment of impurity CGA228048 from the EU RAR (2014)

CGA226048 was present at 1 g/kg in batch OP.4 used in several toxicological studies: acute inhalation toxicity, three month oral, three month dietary and teratology studies. CGA226048 was also present at <1g/kg in batch KGL4634/6, used in: acute oral/dermal, skin and eye irritation, skin sensitisation, 28-day toxicity, Ames and cytogenetic studies. Additionally, CGA226048 was also present at 7 g/kg in batch EN603107 used in a micronucleus assay and a 28-day toxicity study in the rat. Overall, CGA226048 was not present in the toxicity batches at levels sufficient to

support a specification of 15 g/kg.

CGA226048, as pure material, was negative in an Ames test and a mouse lymphoma assay, but was weakly/equivocally positive for clastogenicity in vitro.

Although a SAR analysis (DEREK) for CGA226048 did not reveal the presence of any genotoxicity alerts, the potential in vitro clastogenicity could not be discarded.

Therefore, CGA226048 was regarded a **relevant impurity**, the proposed specification limit of 15 g/kg was not supported, and a lower specification at the <1 g/kg was warranted.

A consumer risk assessment was performed to determine the level of the impurity which would not pose a genotoxic risk for the consumer, according to the TTC approach ([3])

It was considered (see *EFSA Reasoned Opinion on the review of the existing EU MRLs – EFSA Journal 2011, 9(12):2494*) that the highest TMDI of metalaxyl-M represented maximally 17.6% of the proposed ADI of 0.08 mg/kg bw/d, i.e. 0.01408 mg/kg bw/d metalaxyl-M.

If a specification of 15 g impurity CGA226048 /kg techn. a.s. would be considered, the cited intake of a.s. would represent a concomitant intake of 0.2112 µg CGA226048/kg bw/d, which is clearly above the "genotoxicity TTC" of 0.0025 µg /kg bw/d.

Taking into account a content of 1 g/ impurity CGA226048/kg techn. a.s., the intake of impurity CGA226048 would be 0.01408 µg/kg bw/d, which is still about 5.6× above the genotoxicity TTC. Therefore, it is proposed to reduce the maximum amount of this impurity to <**0.18** (1 g/kg/5.6) g impurity CGA226048/kg metalaxyl-M.

New genotoxicity studies submitted with the Art 7 application to withdraw relevance and increase the maximum content of impurity CGA226048.

To address the genotoxicity concern identified for impurity CGA226048 in the in vitro chromosome aberration assay ([redacted] , 2005), an in vivo micronucleus study was submitted by the applicant. This study has been evaluated below by HSE. The applicant also submitted a modern in vivo micronucleus study on the active substance itself ([redacted], 2015a). As a valid micronucleus study was already available for the purposes of renewal with no genotoxicity concern identified in the whole active substance dataset, the [redacted] (2015a) study has not been evaluated.

[3] *Reference: EFSA Panel on Plant Protection Products and their Residues (PPR); Scientific Opinion on Evaluation ofthe Toxicological Relevance of Pesticide Metabolites for Dietary Risk Assessment. EFSA Journal 2012;10(07): 2799. [187pp.] doi:10.2903/j.efsa.2012.2799*

In vivo micronucleus study ([redacted], 2017)

Study	CGA226048- Oral (Gavage) Mouse Micronucleus Test
Reference	[redacted] 2017
Test facility	[redacted]
Report reference	Report Number: [redacted] Study Number: [redacted] Task Number: TK0325252
Guideline(s)	OECD Guideline 474 (2016)
Deviations from the most up-to-date guideline	None
Impact of deviations on acceptability and reliability of study	None
GLP	Yes
Test material	CGA226048 Batch: BPS 659/2 Purity: 99% w/w
Validated method of analysis	The method is not fully validated in accordance with SANCO/3029/99 rev.4 as only 4 determinations were made at each level and the lack of chromatograms. Chromatograms were not provided; however, LC-MS/MS monitoring two mass transitions is considered to be a highly specific technique. Additionally, the method was only used for the purpose of detecting the presence of CGA 226048 in the samples – not quantification of the levels found. Additionally, the method utilised a reference standard for CGA 226048 and matrix matched standards; therefore, the method is considered sufficiently specific to detect the presence of CGA 226048 in the samples, despite lack of chromatograms. However, the method is suitable for the determination of CGA 226048 in blood/plasma samples with an LOQ of 25 ng/mL. Additionally, the method was only used in the range finding phase of the study to detect the presence of the analyte, the method is considered sufficiently validated for this purpose.
Study acceptable	Yes

Methods and Materials

In a GLP and OECD 474 (2016) compliant study, the potential genotoxicity of CGA226048 (impurity of metalaxyl-M) was assessed using an in vivo bone marrow micronucleus test in CD-1 mice. In a range finding study to determine the maximum tolerated dose (MTD) in male and female mice, the test substance (in 0.5 % hydroxypropyl methylcellulose HPMC) was administered twice orally to three animals

per sex at 2000 mg/kg bw. Blood samples were taken for proof of exposure analysis from all animals at 15 minutes, 1, 4 and 24 hours after the second dose. Concentrations of CGA226048 were determined using a validated bioanalytical method. Based on the results of the range finding study, the limit dose of 2000 mg/kg bw was tolerated in males and females.

Based on the findings of the range finding study, three dose levels were chosen for the main study: 500, 1000 and 2000 mg/kg bw i.e. the test was conducted up to the limit dose. For each test group and the negative control group, six males were dosed by oral gavage with the test item or negative control (0.5 % HPMC) twice, at 24 hour intervals. The positive control group was given a single dose of 1 mg/kg bw Mitomycin C (MMC) by intraperitoneal injection. The animals of all dose groups, except the positive control, were observed periodically for signs of toxicity for up to 48 hours post second dose.

All animals were terminated 24hr after the final scheduled dose and blood samples prepared. Blood samples were diluted, fixed, stored, labelled, and analysed by flow cytometry. A minimum of 4000 and a maximum of approximately 20000 reticulocytes were scored for the presence of micronuclei for each animal, in accordance with the criteria of the 2016 test guideline.

Results

Range finding study
Clinical signs
There were no clinical signs observed following administration of CGA226048 at 2000 mg/kg bw.

Based on the results of this phase, the main test was conducted up to the limit dose of 2000 mg/kg bw. As there was no sex difference in toxicity, the main study was conducted in males only.

Proof of exposure
Proof of exposure to CGA226048 was demonstrated by detection of the test item by LC-MS/MS chromatography in blood and plasma samples taken 15 minutes, 1 and 4 hours after the second dose in the range-finding study.

Micronucleus assay

Clinical signs
There were no clinical signs following administration of CGA226048 to male mice at any dose level.

Cytotoxicity
There was no evidence of a statistically significant reduction in the % RET in male mice given CGA226048, indicating a lack of toxicity of CGA226048 to the bone marrow. However, proof of exposure to the test substance was demonstrated in blood samples taken from the range finding study.

Mutagenicity
There were no statistically significant increases in micronucleus frequency at any dose level of CGA226048, when compared with the negative control group. There was no dose-related increase in the frequency of micronucleated cells. The frequency of micronucleated cells at the high dose (2000 mg/kg bw) was not greater than the concurrent negative control and was within the laboratory historical control data range.

MMC administered by intraperitoneal injection produced a statistically significant increase of induced micronucleus frequency, demonstrating adequate performance of the study. There was a statistically significant decrease in the PCE/NCE ratio in the positive control group, indicating toxicity to the bone marrow. One animal showed no increase in induced micronucleus frequency and no decrease in the % RET, indicating that there was no apparent effect of the positive control. It was concluded that this animal had been dosed incorrectly and the data from this animal were not included in the statistical analysis.

Table B.6.8.1-3: Micronuclei induction in bone marrow cells ([redacted], 2017):

Sampling time	**Treatment groups**	**Dose (mg/kg Bw)**	**Micronucleated RET (in 20000 RET/animal)**			**% RET**	**Historical Control data**	
			total [N] (%)	**range [N]**				
			mean	**min**	**max**			
24 hours	Vehicle control (1% CMC)	0	45.50 (0.23)	35	69	2.04	Mean MN-RET ± SD	0.20 ± 0.05
							Range of mean group value	0.13 – 0.33
							95 % Control limit (mean +/- 2SD)	0.09 – 0.30
	CGA226048	500	48.83 (0.24)	41	53	2.01	No. of Experiments	45
		1000	44.50 (0.22)	34	62	2.53		
		2000	44.50 (0.22)	31	70	2.42		
	Positive control (MMC)	1	**415.40* (2.01*)**	171	546	0.52	Mean MN-RET ± SD	2.65 ± 0.77
							Range of mean group value	1.06 – 4.24
							95 % Control limit (mean +/- 2SD)	1.10– 4.19
							No. of Experiments	30

Statistical analysis: ***:** p ≤ 0.001 (Wilcoxon's); N: number.
RET: Reticulocyte; MN-RET: Micronucleated Reticulocytes
CMC: Carboxymethyl cellulose
MMC: Mitomycin C

Conclusion

In an GLP and OECD compliant study, the test item did not induce micronuclei up to the limit dose of 2000 mg/kg bw as determined by the mammalian bone marrow micronucleus test in the mouse. Exposure of the bone marrow was demonstrated by the presence of the test item in blood. Therefore, CGA226048 is non-genotoxic in this bone marrow micronucleus assay.

([redacted], 2017)

Overall conclusion on the toxicological relevance assessment of impurity CGA226048 and its maximum content in the technical material

The genotoxic potential of impurity CGA226048 was investigated in vitro in an Ames test, a mouse lymphoma assay and a chromomosme aberration study. The impurity was negative in the Ames test and mouse lymphoma assay, but was weakly/equivocally positive in the chromosome aberration study. However, in a modern in vivo micronucleus study recently submitted by the applicant for the purposes of amending the conditions of approval of the active substance metalaxyl-M, the clastogenicity of the impurity observed in vitro was not confirmed in vivo. Overall, impurity CGA226048 is not genotoxic and no longer toxicologically relevant. Therefore, the previously specified limit of 0.18 g/kg is no longer needed and the impurity can be specified in the technical material at a maximum level of 15 g/kg, in line with the 5-batch analysis and Quality Control data.

B.6.9. MEDICAL DATA AND INFORMATION

No new data have been submitted. Please refer to original EU RAR 2014.

B.6.10. REFERENCES RELIED ON

Data Point	Author (s)	Year	Title Company Report No. Source (where different from company) GLP or GEP	Vertebrate Study Y/N	Data Protection Claimed Y/N	Justification if Data Protection is claimed	Owner	Previous evaluation

			status Published or not					
B.6.8.1	[redacted]	2017	CGA226048- Oral (Gavage) Mouse Micronucleus Test Report Number: [redacted] GLP Not published	Y	Y	To support this Art 7 application	Syngenta	No