Draft Assessment Report

Evaluation of Active Substances

Plant Protection Products

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

Cinmethylin (BAS 684 H)

Volume 3 – B.6 (AS) – Part II

Toxicology & Metabolism Data

Great Britain

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Version History

When	What
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Acetone Solubility

Studies to determine the solubility of cinmethylin in DMSO and acetone have been submitted and have been presented in detail in Volume 3 – B.2, section B.2.6. Cinmethylin was not soluble in DMSO from the lowest tested concentration of 12 g/L. However, as the study was not conducted to GLP, it is not considered acceptable. Cinmethylin was found to be readily soluble in acetone, as confirmed by a GLP study.

B.6.8.1. Toxicity studies on metabolites

The following metabolites were selected for potential inclusion in the plant and/or livestock residue definitions for risk assessment based on their significant occurrence in the plant and livestock metabolism studies:

- M684H001 (cinmethylin benzoate)
- M684H002 ([2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]hept-2-yl]oxy}methyl)phenyl]methanol)
- M684H005 ([2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl]oxy}methyl)phenyl]methyl beta-D-glucopyranoside)
- M684H006 ([2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl]oxy}methyl)phenyl]methyl 6-O-(carboxyacetyl)-beta-D-glucopyranoside)

- M684H009 (N-(2-methylbenzoyl)glycine)
- M684H010 (2-hydroxymethyl benzoate)
- M684H012 (sum of isomers) (cinmethylin benzyl alcohol glucuronide)
- M684H021 (sum of isomers) (chemical name not available)
- M684H022 (sum of isomers) (chemical name not available)
- M684H026 (2-hydroxypropyl 2-hydroxycineol)
- M684H039 (chemical name not available)
- M684H058 (1-O-(2-methylbenzoyl) hexopyranuronic acid)
- M684H059 (2-benzofuran-1(3H)-one)

To assess the toxicological properties of these metabolites, all the available data (including data relating to cinmethylin) were considered. These included presence of these metabolites in rat ADME and toxicity studies performed with the parent, structural similarity to the parent, *in silico* genotoxicity assessment, read across prediction of genotoxicity and data gathering from online sources (i.e. ECHA REACH and C&L databases).

Presence of selected metabolites in rat ADME and toxicity studies

Review of the ADME studies conducted with cinmethylin has revealed that some of the above metabolites have been detected in rats. It is generally accepted that the toxicity of a metabolite is covered by the toxicity data of the parent if that individual metabolite contributes to ≥ 10% of the administered dose found in excreta, plasma and tissues in ADME studies.

Two urinary metabolites, **M684H010** (2-hydroxymethyl benzoate) and M684H011 (2-hydroxypropyl cinmethylin benzoate)) were present above 10 % of the administered dose. Furthermore, M684H010 was detected in the 2-year rat and 18-month mouse studies in plasma at higher levels compared to the parent. Therefore, the toxicity of metabolites M684H010 and M684H011 can be considered covered by the toxicity data of the parent.

The most abundant (13 – 21 % of the administered dose) metabolite in bile was **M684H012** (M684H012a + M684H012b) abbreviated to cinmethylin benzyl alcohol glucuronide. The presence of this metabolite found in bile is considered evidence of systemic availability as, for cinmethylin, one of the most critical target organs of toxicity is the liver.

Metabolites **M684H001** and **M684H026** were also found in rat and mouse plasma, taken from the 2-year and 18-month studies, at levels that were similar to or greater than those of the parent. In addition, M684H001 was found in plasma in the mouse micronucleus test at levels similar or greater than those of the parent. Therefore, it is acceptable to use the toxicity data from the parent compound for these metabolites.

Overall, metabolites M684H001, M684H010, M684H011, M684H012 and M684H026 were detected in rat and mouse plasma at levels similar or greater than the parent and/or in rat excreta at > 10% of the administered dose. Therefore, the toxicity of these metabolites is covered by the toxicity data of the parent and the dietary reference value of the parent can be used in the risk assessment of these metabolites. Overall, these metabolites are considered to be of equivalent toxicity to the parent and toxicologically relevant. It should be noted that metabolite M684H011 was not identified at significant levels in the plant and/or livestock metabolism studies; hence, it does not need to be considered further for the purposes of the residue definitions

Read-across: structural similarity of the selected metabolites with the parent or other metabolites, including their metabolic pathway precursors

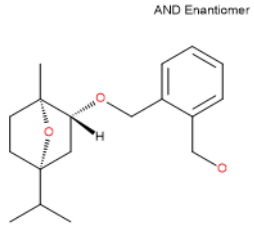
With regard to evaluation of chemical similarity, the general proposals given by the EFSA Scientific Opinion on Evaluation of the Toxicological Relevance of Pesticide Metabolites for Dietary Risk Assessment [EFSA Journal 2012;10(07):2799] were followed, taking into consideration:

- Metabolic steps that were identified to probably not cause additional toxicity of the metabolites:
  - Simple demethylation of the ring or side chain
  - Simple hydroxylation of the ring system without any cleavage of the ring
  - Hydroxylation of another ring position
  - Conjugation of metabolite with amino acid

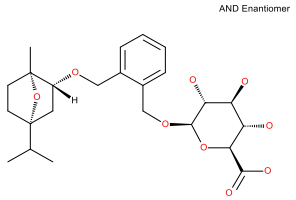
- Consideration of conjugated metabolites (O-glucuronides and sugar conjugates) being of similar or lower toxicity compared to their unconjugated products (due to cleavage in the human gastrointestinal tract)

Metabolite M684H002 is a direct, exclusive and stable precursor (see Figure B.6.1-4 in section B.6.1) of M684H012 (a major rat metabolite). Bile excretion of M684H012 is considered to be the major excretion path of the aglycon M684H002. As the liver is a primary target organ of toxicity of cinmethylin, it can be assumed that metabolite M684H012 and its respective aglycon M684H002 have contributed to the toxicity of cinmethylin and that metbaolite M684H002 can also be considered a major rat metabolite. In addition, M684H002 is the aglycon of M684H012 and it is most likely to be of similar toxicity to that of its glucuronide (M684H012). **Therefore, the toxicity of M684H002 is considered to be covered, on the basis of the metabolic path, and structural similarity, to of the major rat metabolite M684H012, by the toxicity data of the parent.**

M684H002 (the aglycon)

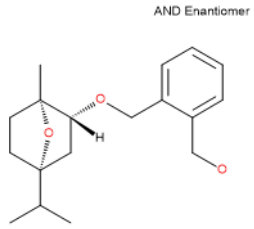


M684H012 (glucuronide of H002)

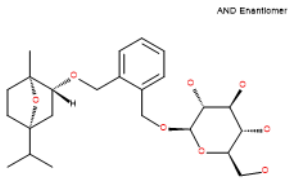


M684H002 is also similar in structure to the metabolites M684H005 and M684H006, which are both sugar conjugates of M684H002. These sugar conjugates are expected to be of similar or lower toxicity than M684H002 as they are unconjugated in the human gastro-intestinal tract. **Therefore, the toxicity of M684H005 and M684H006 are considered to be covered, on the basis of chemical similarity to M684H002, by the toxicity data of the parent.**

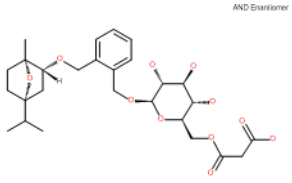
M684H002 (the aglycon)



M684H005 (the sugar conjugate)



M684H006 (the sugar conjugate)



***In silico* genotoxicity assessment of selected metabolites**

To predict the genotoxic potential (gene mutation and clastogenicity/aneugenicity) of the identified metabolites of cinmethylin, the presence for potential structural alerts was evaluated with three (Q)SAR models, one rule based and one statistical model. Models used were:

- CaseUltra, version 1.6.2.3, date 09/02/2018, 11/02/2018 and 14/02/2018, using the models GT1-_A7B, GT1_AT_ECOLI, GT_Expert and GT3_MNT_Mouse (trained)
- DEREK Nexus, version 6.0.1, knowledge base Derek KB 2018 1.1, date 23/04/2018.
- ToxTree *in vivo* MNT, version 2.6.13.

The CaseUltra model for Ames prediction was used as commercially available, whereas the MNT model was trained additionally with data from EFSA conclusions published in the period 2006 - 2016 (to widen the applicability domain). In addition, the Toxtree profiling module which codify structural alerts of importance for *in vivo* MNT were included.

QSAR Model	Type of model		Domain definition?	Training of QSAR model?
	Mutagenicity / Ames	Clastogenicity/aneugenicity / MNT		
Toxtree	-	Mechanistic	No	No
DEREK	Mechanistic	Mechanistic	(Yes)*	No
CaseUltra	Statistical & Mechanistic	Statistical	Yes	Yes

* DEREK has no classical domain definition, however, the model screens for unknown fragments. In case of a negative prediction, the evaluator is prompted to investigate, if an unknown fragment could cause a genotoxic event. In case of cinmethylin, the complete chemical space is covered by the underlying DEREK knowledgebase

The results of the (Q)SAR predictions are summarised below (Table 6.8-1).

Table 6.8-1. Prediction of genotoxicity for potentially relevant cinmethylin metabolites

Compound identifier	Ames mutagenicity					Genotoxicity by clastogenicity/aneugenicity		
	statistical-based		rule-based	hybrid-model	rule-based	statistical-based	rule-based	rule-based
	CaseUltra *Salmonella T.*[1]	CaseUltra *E.coli*[1]	CaseUltra Expert[1]	CaseUltra Konsolidator[2]	DEREK[5]	CaseUltra MNT mouse (trained)[3]	DEREK[5]	ToxTree MNT in rodents[4]
Parent (cinmethylin)	Negative	Negative	Negative	Negative	Negative	Negative	Negative	SA 34[a]: H-acceptor-path3-H-acceptor SA 35[b]: Oxolane LogP 4.5 [a + b]
H001	Negative	Negative	Negative	Negative	Negative	Negative	Negative	SA34 & SA35: covered by parent LogP 3.07 (calc.)
H002	Negative	Negative	Negative	Negative	Negative	Negative	Negative	SA34 & SA35: covered by parent LogP 3.0 (KCA 2.7)
H009	Negative	Negative	Negative	Negative	Negative	Negative	Negative	SA34: covered by parent
H010	Negative	Negative	Negative	Negative	Negative	Negative	Negative	No alerts

Compound identifier	Ames mutagenicity					Genotoxicity by clastogenicity/aneugenicity		
	statistical-based		rule-based	hybrid-model	rule-based	statistical-based	rule-based	rule-based
	CaseUltra *Salmonella T.*[1]	CaseUltra *E.coli*[1]	CaseUltra Expert[1]	CaseUltra Konsolidator[2]	DEREK[5]	CaseUltra MNT mouse (trained)[3]	DEREK[5]	ToxTree MNT in rodents[4]
H011	Negative	Negative	Negative	Negative	Negative	Negative	Negative	SA34 & SA35: covered by parent LogP 2.43 (calc.)
H021 All isomers	Negative	Negative	Negative	Negative	Negative	Negative	Negative	SA34 & SA35: covered by parent LogP 1.2 (calc.)
H026	Negative	Negative	Negative	Negative	Negative	Negative	Negative	SA34 & SA35: covered by parent LogP 0.92 (calc.)
H039 All isomers	Negative	Negative	Negative	Negative	Negative	Negative	Negative	SA34 & SA35: covered by parent LogP 1.8-3.0 (calc.)
H059	Negative	Negative	Negative	Negative	Negative	Negative	Negative	No alert

Compound identifier	Ames mutagenicity					Genotoxicity by clastogenicity/aneugenicity		
	statistical-based		rule-based	hybrid-model	rule-based	statistical-based	rule-based	rule-based
	CaseUltra *Salmonella T.*[1]	CaseUltra *E.coli*[1]	CaseUltra Expert[1]	CaseUltra Konsolidator[2]	DEREK[5]	CaseUltra MNT mouse (trained)[3]	DEREK[5]	ToxTree MNT in rodents[4]

SA: structural alert
1: For individual QPRF reports please refer to BASF Doc ID 2017/1158715
2: For individual QPRF reports please refer to BASF Doc ID 2017/1158716
3: For individual QPRF reports please refer to BASF Doc ID 2017/1158717
4: Summary of ToxTree results please refer to BASF Doc ID 2017/1158718
5: For individual QPRF reports please refer to BASF Doc ID 2018/1086608
Blue – within applicability domain (revealing high reliability). DEREK has no classical domain definition, rather it uses a 'predictive space' stored within a modified knowledge base. ToxTree has no domain definition.
Peach – low reliability, therefore these limited alerts were rejected.

Parent (cinmethylin)
The CaseUltra analysis revealed negative predictions for the bacterial mutagenicity by the Ames and Expert modules. This was confirmed in the hybrid model Konsolidator; no structural alert was identified. Negative prediction for *in vivo* clastogenicity/aneugenicity was obtained by CaseUltra using the trained MNT mouse module. All CaseUltra predictions were within the applicability domain of each module, revealing a high reliability. The DEREK prediction revealed negative predictions for bacterial and mammalian models for genotoxicity and chromosome damage; no structural alerts or unknown fragments were identified.

The ToxTree analysis for *in vivo* clastogenicity/aneugenicity by MNT revealed two structural alerts SA_34 and SA_35 - 'H-acceptor-path3-H-acceptor' and 'Oxolane' respectively.

- Positive predictivity for these structural alerts was low – 34 % for SA_34: 'H-acceptor-path3-H-acceptor' and 43 % for SA_35 : 'Oxolane' (Benigni *et al*., 2010[1]). The 'H-acceptor-path3-H-acceptor' alert explores the possibility that a chemical interacts with DNA and/or proteins via non-covalent binding, such as DNA intercalation or groove-binding (Snyder *et al*., 2006[2]). In this publication by Snyder *et al*. (2006) several molecules were discussed as having chromosome aberration properties, however, the structures of the example molecules are considered not relevant/relevant with low reliability for cinmethylin and its metabolites. Therefore, this alert is rejected based on i) low positive predictivity and ii) the structures described in Snyder *et al*. (2006) possess a N-dialkyl moiety in contrast to cinmethylin or its metabolites.
- The 'Oxolane' alert arises presumably from the ability of the nucleoside analogues to inhibit DNA polymerase function and/or to be incorporated into DNA as fraudulent nucleosides. However, this structural alert suffers from low specificity ; the tetrahydrofuran moiety represents the chemical skeleton of biologically important aldopentoses, such as ribose, that may be erroneously identified as positive alerting substances. This alert is of no concern for substances with a $LogP_{o/w} > 1.5$ (Benign, 2009[3]), which is the case for cinmethylin with $LogP_{o/w}$ of 4.5.

The low reliability of both ToxTree alerts for cinmethylin is verified by experimental *in vivo* MNT and chromosome aberration data that were clearly negative. Additionally, HSE notes the in domain negative prediction of the CaseUltra and DEREK models. Therefore, these limited alerts are considered to be of no relevance for cinmethylin (and its metabolites) and are rejected.

Overall, based on a weight of evidence approach, cinmethylin is predicted to be of no genotoxic potential by the (Q)SAR models applied and the ToxTree alerts SA_34 and SA_35 are of no relevance for cinmethylin and its structurally related metabolites.

CaseUltra Ames predictions for the metabolites (detailed reports available BASF DocID 2017/1158715 and 2017/1158716)
The following metabolites were predicted negative based on the lack of any structural alerts in the statistical model for Ames (*Salmonella* and *E.coli* models), the mechanistic Expert model for Ames, as well as the hybrid Konsolidator model for Ames:

- M684H001,
- M684H002,
- M684H009,
- M684H010,
- M684H021 (all except Isomers 4, 8, 11, 13),
- M684H027 (all except Isomers 1, 7-9),
- M684H039 (all except Isomers 16-20),
- M684H043,
- M684H059,
- the aglycon Mass 290 (except Isomer 4),
- 2-Methylbenzoic acid.

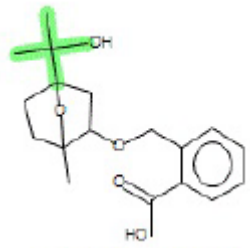
[1] Benigni, R., Bossa, C. and Worth, A. (2010), Structural analysis and predictive value of the rodent *in vivo* micronucleus assay results, *Mutagenesis* pp, 1 – 7, BASF DocID 2010/1233692.

[2] Snyder, R.D., Ewing, D. and Hendry, L.B. (2006), DNA intercalative potential of marketed drugs testing positive in *in vitro* cytogenetics assays, *Mutation Research* 609 (2006) 47 – 59, BASF DocID 2006/1051853.

[3] Benigni, R., Bossa, C., Tcheremenskaia, O. and Worth A. (2009), Development of structural alerts for the *in vivo* micronucleus assay in rodents, JRC Scientific and Technical Reports, EUR 23844 EN, BASF DocID 2017/1158725.

The Case Ultra predictions were within the applicability domain of each module, revealing a high reliability.

All mentioned exceptions shared the alert ID 1094 (Structural group of "2-Hydroxy-isopropyl") based on the hydroxylated isopropyl-group in position 2 (see below structure). This alert is identified also for metabolites M684H011 and M684H026. As M684H011 is a major rat metabolite and M684H026 is considered structurally similar, both these metabolites are considered covered by the toxicity data of the parent. Therefore, these metabolites are considered negative in the Ames assay (as for cinmethylin) and the reliability of the alert is considered low.



Positive Alert "2-Hydroxy-isopropyl" in M684H011

The probability of the positive prediction in the *Salmonella* model is limited to 34.4 % which is not sufficient for a positive call. No such structural alert was identified for bacterial mutagenicity by the *E.coli* model, nor by the rule based Expert module. The hybrid model Konsolidator dismissed the alert in all affected metabolites after being trained with the information that M684H011 is considered similar to parent based on its presence in the ADME study at > 10 % administered dose. Therefore, the CaseUltra predictions were negative within the applicability domain of each module, revealing a high reliability. This was confirmed in the hybrid model Konsolidator, since the structural alert was dismissed.

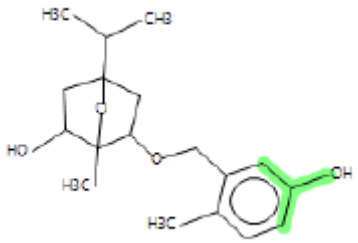
Overall, based on weight of evidence, all metabolites are predicted negative for Ames with two independent (Q)SAR models summarised in the Konsolidator model.

<u>CaseUltra MNT predictions for the metabolites (statistic model) (detailed reports available BASF DocID 2017/1158717)</u>

The following metabolites were predicted negative based on the lack of any structural alerts in the statistical trained model for *in vivo* MNT mouse:

- M684H001,
- M684H002,
- M684H009,
- M684H010,
- M684H011,
- M684H021 (all 16 Isomers),
- M684H026,
- M684H027 (all 15 Isomers),
- M684H039 (all except Isomers 3, 4, 8, 9, 13, 14, 18, 19, 23, 24, 28, 29),
- M684H043 (all isomers except Isomer 2, 3),
- M684H059,
- the aglycon Mass 290,
- 2-Methylbenzoic acid.

The Case Ultra predictions were negative for MNT and within the applicability domain of each module, revealing a high reliability. All mentioned exceptions shared the alert ID 112 (Structural group of "Substituted Phenol") based on the hydroxylated benzyl-part of cinmethylin (see below structure). No positive modulators for this alert were identified. However, a negative modulator (methyl group) was identified, therefore, the probability of the base prediction was increased from 15.2 % to 20.2 %. Although this positive alert (IDD 112) was identified, its contribution was not sufficient for a positive call (model probability threshold is 40 %). Therefore, based on weight of evidence, the CaseUltra prediction for MNT for all metabolites was negative and within the applicability domain, therefore revealing a high reliability of each module.



Positive Alert "substituted phenol" in M684H039 Isomer 4

DEREK Nexus analysis for mutagenesis and chromosome damage (rule-based expert model) (detailed reports available BASF DocID 2018/1086608)
The DEREK analysis for gene mutations in bacterial or mammalian cells, and chromosome damage in mammals indicated no structural alert. The complete chemical space for cinmethylin and its metabolite was covered by the analysis. No unknown fragment was identified. Overall, the DEREK nexus analysis for all metabolites was negative with high reliability.

ToxTree analysis for *in vivo* clastogenicity/aneugenicity by MNT (mechanistic model) (summary table available BASF DocID 2017/1158718)
The ToxTree analysis for *in vivo* clastogenicity/aneugenicity by MNT revealed the same structural alerts SA_34 and SA_35, 'H-acceptor-path3-H-acceptor' and 'Oxolane' (already discussed and rejected for the parent compound, see above), for all metabolites except for M684H009, which had only the structural alert SA_34 instead of both, and M684H010, M684H059 and 2-Methlbenzoic acid, which had no structural alert. The SA_35 alert is of no concern for substances with a LogPo/w > 1.5 (Benign, 2009), which is true for cinmethyin, M684H001, M684H002, M684H011, M684H039, M684H043 and Mass 290.

In summary, all metabolites shared those structural fragments which were also responsible for the occurrence of the SA_34 and SA_35 alert in the parent compound or had no alerts. No new alerts were identified. As the parent compound is negatively tested in the MNT *in vitro* and *in vivo*, and the metabolites are structural similar to the parent compound, these alerts are considered to be of low reliability in the metabolites, too, and are rejected.

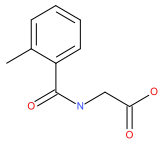
Overall, based on weight of evidence, the ToxTree analysis for clastogenicity/aneugenicity by MNT revealed no relevant alert and is considered negative for all metabolites.

Conclusion
(Q)SAR assessment did not identify a genotoxic potential of any investigated metabolite. All evaluated cinmethylin metabolites revealed a negative, in domain prediction for gene mutation in bacteria by Ames Test and for clastogenicity/aneugenicity by *in vivo* MNT. It should be noted that of all the selected metabolites for potential inclusion in the residue defintions, the applicant did not evaluate the genotoxic potential of metabolite M684H058 using (Q)SAR models.

The applicant also submitted a read across prediction/case for genotoxicity using the OECD Toolbox, however, HSE did not consider this necessary as the genotoxicity of the metabolites selected for potential inclusion in the residue definitions was sufficiently covered by the above (Q)SAR analysis and additional information. Further information is available in the applicant's MCA document, section 5.

Other toxicological information

M684H009
(Synonymes: 2-Methyl-hippuric acid; o-Toluric acid, N-(o-toluoyl)glycine; 2-Methylbenzoylglycine, CAS No. 42013-20-7)



M684H009, 2-Methyl-hippuric acid, is the glycine conjugate of the 2-Methylbenzoic acid (M684H061) metabolite. 2-Methylhippuric acid (see https://pubchem.ncbi.nlm.nih.gov/compound/Methylhippuric%20acid#section=Canonical-SMILES) is a minor metabolite of fatty acids and as this listed in the human metabolome database as naturally occurring human metabolite (see http://www.hmdb.ca/metabolites/HMDB0011723).

The European chemical agency (ECHA) has listed 2-Methyl-hippuric acid (M684H009) as a pre-registered substance. According to the notifications provided by companies to ECHA no hazards have been identified.

Furthermore, 2-methyl-hippuric acid is a metabolite of xylene and is used as an exposure biomarker. The metabolic pathway involves hydroxylation of one methyl-group of xylene to form 2-methylbenzalcohol, followed by oxidation via 2-Methylbenzaldehyde and 2-Methylbenzoic acid to subsequent conjugation with glycine to 2-methyl-hippuric acid (M684H009) or conjugation with glucuronic acid to form M684H058.

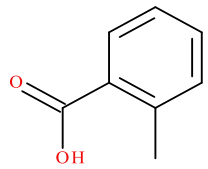
The literature search revealed a publication investigating the different xylene isomers for their ototoxic potential. It was shown that neither o-Xylene nor the respective metabolite 2-Methyl-hippuric acid is ototoxic (Maguin *et al.,* 2006[4]).

The MAK Commission (the Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area) adopted a Biological Tolerance Values (BAT) of 2 g 2-Methylhippuric acid / L urine as a biological marker for an 8-hour xylene exposure at the level of the currently valid MAK value for xylene (100 mL/m³). This is equivalent to a systemic dose of 57 mg/kg bw/day, if default values of 70 kg body weight and 2 L urine are considered. At that level there are no observations indicating the occurrence of adverse effects. The BAT Value Documentation for Xylene (all isomers) is available online at http://onlinelibrary.wiley.com/doi/ 10.1002/3527600418.bb133020e0005/pdf). The BAT value of 2 g/L urine is also included in the German technical rules for hazardous substances TRGS 903 and considered safe. (http://www.gaa.baden-wuerttemberg.de/servlet/is/16495/5_903.pdf).

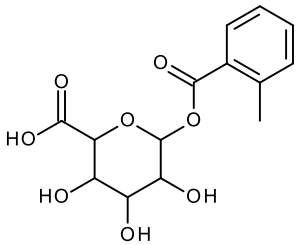
Overall, these data support the conclusion that M684H009 is not of toxicological concern compared to cinmethylin and should not be considered further (from a toxicological point view) for the purposes of the residue definitions.

M684H058
Metabolite **M684H058** is the glucuronide of 2-methylbenzoic acid (M684H061) (information presented below). It should also be noted that metabolited M684H009 (considered above) is the glycine conjugate of 2-methylbenzoic acid. Based on a number of considerations (see below information on 2-methylbenzoic acid), HSE has concluded that M684H009 is of no toxicological concern.

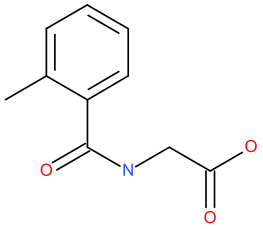
[4] Maguin, K., Lataye, R., Campo, P., Cossec, B., Burgart, M. and Waniusiow, D. (2006), Ototoxicity of the three xylene isomers in the rat, *Neurotoxicity and Teratology* 28 (2006) 648-656, BASF DocID 2006/1053413.



2-Methylbenzoic acid
(the aglycon)



M684H058
(conjugated with glucuronic acid)



M684H009
2-methylhippuric acid
(conjugated with glycine)

2-Methylbenzoic acid is pre-registered under REACH as 'intermediate' (https://echa.europa.eu/registration-dossier/-/registered-dossier/24928/2/1). On the REACH registered substance factsheet, no classification for human health hazards is proposed; however, data are listed as 'lacking' for all human health endpoints.

2-Methylbenzoic acid does not have harmonised classification. Notified classifications according to CLP criteria indicate that 2-methylbenzoic acid possesses irritating potential for skin (skin irrit. 2; H315), eye (eye irrit. 2; H319) and respiratory tract (STOT SE 3; H335) (https://echa.europa.eu/information-on-chemicals/cl-inventory-database/-/discli/details/46000). These notified classifications identify an increased hazard compared to the parent (cinmethylin); however, these local effects hazards are of no relevance to low level exposures of these plant/livestock metabolites through the diet.

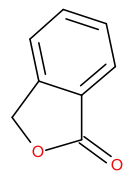
2-Methylbenzoic acid is listed in the Human Metabolome Database (http://www.hmdb.ca/metabolites/HMDB0002340) as a derivative of several compound classes belonging to the aromatic homo-monocyclic compounds. 2-Methylbenzoic acid can be found primarily in faeces, saliva, and urine. Within the cell, 2-methylbenzoic acid is primarily located in the membrane (predicted from logP).

2-Methylbenzoic acid has been negatively tested on *Salmonella typhimurium* strains TA1535, TA 97, TA98, TA100 within the National Toxicology Program in an Ames test with and without activation (Study ID 715175, 1993; https://manticore.niehs.nih.gov/cebssearch/test_article/118-90-1).

2-Methylbenzoic acid is expected to be one intermediate metabolite of xylene and of the flavouring substance o-methylbenzaldehyde (FL-no 05.026), which was evaluated by EFSA in the Scientific Opinion of Flavouring Group Evaluation 20, Revision 4 (EFSA Journal 2012; 10(12):2994). It is conjugated mainly with glycine to form 2-methylhippuric acid (see **M684H009)** or conjugated with glucuronic acid (see **M684H058**) at higher levels before it is eliminated via the urine. The EFSA Panel concluded that all the substances of this flavouring group, including 2-methylbenzylaldehyde as part of subgroup 1 "benzyl derivatives" do not give rise to safety concerns at their levels of dietary intake. The maximum use level for o-methylbenzaldehyde is found in confectionery products in which up to 111.2 mg/kg were provided by industry. The risk assessment performed was calculated against the TTC Cramer Class I, which considers an ADI of 1800 µg/person/day.

Overall, these data support the conclusion that 2-methylbenzoic acid and its glucuronide M684H058, like the glycine conjugate of 2-methybenzoic acid (M684H009), are of no toxicological concern (and of significantly lower toxicity than cinmethylin), as highlighted by the use of the TTC Cramer Class I value in the Scientific Opinion of Flavouring Group Evaluation 20. On this basis, M684H058 should not be considered further (from a toxicological point view) for the purposes of the residue definitions. However, if a risk assessment were to be required, the **TTC Cramer Class I value of 30 µg/kg bw/day** could be used as reference value.

M684H059
(Synonymes: Phthalide / FL-No 10.056 / CAS 87-41-2/Reg.No 18851)



M684H059 is the ring-closed M684H010 (2-hydroxy-methylbenzoic acid) and is a downstream metabolite of this major rat metabolite (Figure 6.1-2 in Section B.6.1.1).

M684H059 is registered under REACH as an 'intermediate' (https://echa.europa.eu/de/registration-dossier/-/registered-dossier/20112), however, no chemical safety assessment has been performed for this substance. No classification has been proposed for this substance either due to a lack of data or data which was conclusive but not sufficient for classification.

According to the information provided by companies to ECHA in REACH registrations no hazard classifications have been identified. However, the classification provided by companies to ECHA in CLP notifications identifies that this substance causes serious eye irritation.

According to the ECHA REACH information (lead registrant: BASF):
'Three Ames tests were performed covering *Salmonella typhimurium* as well as *Escherichia coli* strains with and without S9 metabolic activation: in none of the tested strains an increase of colonies at whatever dose could be found. The number of revertants was always similar to the control. In an *in vivo* micronucleus assay in mice, the test substance did not lead to an increase in the number of polychromatic erythrocytes containing either small or large micronuclei. Thus, phthalide has no chromosome-damaging (clastogenic) effect nor does it lead to any impairment of chromosome distribution in the course of mitosis (aneugenic activity) in bone marrow cells *in vivo*. There is no genotoxic effect for phthalide to be determined.'

No genotoxic potential was observed for phthalide in an Ames test (BASF 40M684H0360/054048, 2005) according to OECD guideline 471, with and without metabolic activation at 55-5500 µg/plate. No genotoxic potential was observed in an *in vivo* micronucleus test (BASF 26M684H0360/054030, 2005) in mice treated orally with 187.5-750 mg/kg bw according to OECD guideline 474.

Acute data indicate no need for classification for skin or eye irritation. No indication of a carcinogenic effect in rats and mice was observed up to limit dose in a reliable study (NCI, 1979) for phthalic anhydride.

The literature search revealed one study (Araki *et al.,* 2005[5]), in which Phthalid was screened for androgen receptor activity in an *in vitro* reporter gene assays using AR-Eco Screen™ cells. Although the method used in the publication/study seems to be reliable, no detailed data are given for Phthalide. Based on the information given, it can be assumed that Pthtalide, tested at a concentration of 10 µM in three separate measurements, neither acted as an androgen nor as an anti-androgen with an applied cut-off limit of 1.7-fold induction. However, no detailed data are given, therefore, this information is considered to be of limited reliability.

Phthalide (M684H059) is used as flavouring substance FL-no: 10.056, defining Flavouring Group 27. EFSA evaluated this flavouring substance as being classified into structural class III (EFSA Journal (2008) 806, 1-27) with a TTC level of 90 µg/person /day (equivalent to 1.5 µg/kg bw/day for a 60 kg person). The flavouring substance has been reported to occur naturally in food (tomato and wine). Quantitative data on the natural occurrence in food have been reported with up to 0.07 mg/kg in wine. According to the Flavour Industry the maximum use level for Phthalide is in the range of 10 - 100 mg/kg.

Overall, these data support the conclusion that M684H059 is not of genotoxic concern. EFSA assigned M684H059 to Cramer Class III with a TTC value of 1.5 µg/kg bw/day. If a dietary risk assessment were to be required for M684H059, the Cramer Class III TTC value should be used.

[5] Araki, N., Ohno, K., Nakai, M., Takeyoshi, M. and Iida, M. (2005), Screening for androgen receptor activities in 253 industrial chemicals by *in vitro* reporter gene assays using AR-EcoScreen™ cells, *Toxicology in Vitro* 19 (2005) 831-842, BASF DocID 2005/1045820.

Overall toxicological assessment of selected metabolites

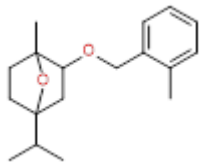
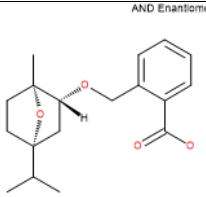
The table below summarises the available toxicological data for the selected metabolites (M684H001, M684H002, M684H005, M684H006, M684H009, M684H010, M684H012, M684H021, M684H022, M684H026, M684H039, M684H058 and M684H059).

For **M684H001, M684H002, M684H005, M684H006, M684H010, M684H012 and M684H026**, the toxicity of these metabolites is covered by the toxicity data of the parent and if a risk assessment were to be required, the **dietary reference values of cinmethylin** could be used. Therefore, these metabolites are considered to be of equivalent toxicity to the parent, toxicologically relevant and potential candidates for inclusion in the residue definitions for risk assessment.

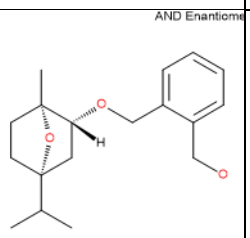
Metabolite **M684H009** (the gycine conjugate of 2-methylbenzoic acid) is a minor metabolite of fatty acids and is listed in the human metabolome database as a naturally occurring compound. This metabolite is considered to be of no toxicological concern and of significantly lower toxicity than cinmethylin. On this basis, it should not be considered further (from a toxicological point view) for the purposes of the residue definitions. However, if a dietary risk assessment were to be required, the **BAT (Biological Tolerance) value of 57 mg/kg bw/day** could be used as a reference value.

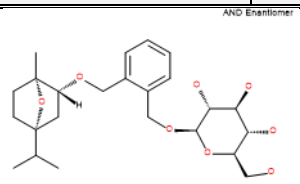
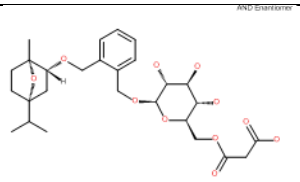
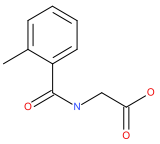
Metabolite **M684H058** (the glucuronide conjugate of 2-methybenzoic acid) is of no toxicological concern (and of significantly lower toxicity than cinmethylin), as highlighted by the use of the TTC Cramer Class I value in the Scientific Opinion of Flavouring Group Evaluation 20. On this basis, M684H058 should not be considered further (from a toxicological point view) for the purposes of the residue definitions. However, if a risk assessment were to be required, the chronic **TTC Cramer Class I value of 30 µg/kg bw/day** could be used as reference value.

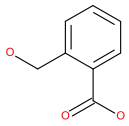
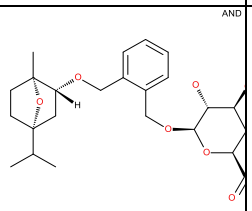
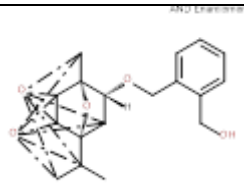
For **M684H021, M684H022, M684H039 and M684H059**, there are negative genotoxicity (Q)SAR predictions but no information on relative levels in rat and/or mouse plasma compared to the parent or on levels in rat excreta > 10 % of the administered dose. Therefore, the toxicity of these metabolites is not covered by the toxicity data of the parent and if a dietary risk assessment were to be required, the **Cramer class III TTC chronic value of 1.5 µg/kg bw/day and acute value of 5 µg/kg bw**[6] could be used in a first-tier assessment. In addition, due to the structural similarity of metabolites M684H021, M684H022 and M684H039, these metabolites should be combined in the assessment; M684H059 is not structurally similar to metabolites M684H021, M684H022 and M684H039 and does not need to be combined in the assessment. Overall, these metabolites are of potential higher toxicity than cinmethylin, toxicologically relevant and potential candidates for inclusion in the residue definitions for risk assessment.

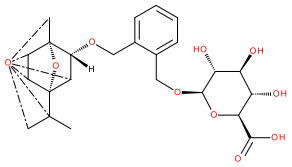
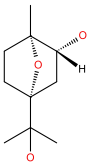
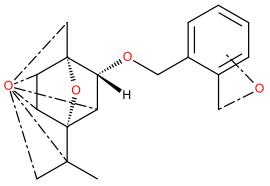
Metabolite No.	Structure	Name/code No. (synonyms)	Rat ADME coverage (% of administered dose)	Toxicological assessment
a.s.		**Cinmethylin**	No unchanged cinmethylin was detected urine and very little (<1 %) in bile.	Active substance Full data package available.
M684H001		Cinmethylin benzoate		No study data available. Found in rat plasma collected after 12-month dosing with parent, at higher concentrations (229 – 20743 ng/mL) than parent (<LOQ – 138 ng/mL)

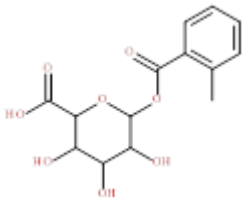
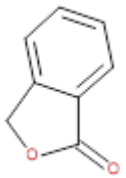
[6] EFSA (2012) Scientific Opinion on Evaluation of the Toxicological Relevance of Pesticide Metabolites for Dietary Risk Assessment, EFSA Journal 2012;10(07):2799

Metabolite No.	Structure	Name/code No. (synonyms)	Rat ADME coverage (% of administered dose)	Toxicological assessment
				([redacted] 2018). Found in mouse plasma collected after 63-days dosing with parent, at similar (<LOQ – 231 ng/mL) concentrations to parent (<LOQ – 174 ng/mL) ([redacted] 2018d). Found in mouse plasma in a negative *in vivo* MNT in mice, collected after 2 and 4 hr dosing with parent, at lower concentrations (<LOQ – 2.1 µg/mL) than parent (<LOQ – 26.7 µg/mL) ([redacted] 2018). Negative *in silico* genotoxicity predictions. **Overall, the toxicity of M684H001 is covered by the toxicity data of the parent (cinmethylin).**
M684H002				No study data available. The aglycon of M684H012 (glucuronide of M684H002). The direct, exclusive and stable precursor of M684H012 (a major rat metabolites). Therefore, toxicity is covered by the parent on the basis of the metabolic path of this major rat metabolite. Negative *in silico* genotoxicity predictions. **Overall, the toxicity of M684H002 is covered by the toxicity data of the parent (cinmethylin).**

Metabolite No.	Structure	Name/code No. (synonyms)	Rat ADME coverage (% of administered dose)	Toxicological assessment
M684H005	 [2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl]oxy}methyl)phenyl]methyl beta-D-glucopyranoside			No study data available. A sugar conjugate (glucoside) of M684H002 – hence of similar or lower toxicity compared to M684H002. **Overall, the toxicity of M684H005 is covered by the aglycon M684H002 and in turn by the toxicity data of the parent (cinmethylin).**
M684H006	 [2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl]oxy}methyl)phenyl]methyl 6-O-(carboxyacetyl)-beta-D-glucopyranoside			No study data available. A sugar conjugate (malonyl-glycoside) of M684H002 – hence of similar or lower toxicity compared to M684H002. **Overall, the toxicity of M684H006 is covered by the aglycon M684H002 and in turn by the toxicity data of the parent (cinmethylin).**
M684H009		2-methyl-hippuric acid, 2-Methylbenzoylglycine		Some open-access data available. Negative *in silico* genotoxicity predictions. Existing data raise no toxicological concern. A biological tolerance (BAT) value of 2 g/L, equivalent to 57 mg/kg bw/d was identified. **Overall, M684H009 is of no toxicological concern and of significantly lower toxicity compared to cinmethylin. However, the BAT value of 57 mg/kg bw/d could be used if required.**

Metabolite No.	Structure	Name/code No. (synonyms)	Rat ADME coverage (% of administered dose)	Toxicological assessment
M684H010		2-hydroxymethyl benzoate	A major rat metabolite – identified in the urine of rats above > 10 % of the administered dose (3.5 % - 19 %).	No study data available. Found in rat plasma collected after 12-month dosing with parent, at higher concentrations (<LOQ – 7816 ng/mL) than parent (<LOQ – 138 ng/mL) ([redacted] 2018) Found in mouse plasma collected after 63-days dosing with parent, at higher concentrations (<LOQ – 2381 ng/mL) than parent (<LOQ – 174 ng/mL) ([redacted] 2018d). Negative *in silico* genotoxicity predictions. **Overall, the toxicity of M684H010 is covered by the toxicity data of the parent (cinmethylin).**
M684H012		cinmethylin benzyl alcohol glucuronide	A major rat metabolite – identified in the bile from rats above > 10 % of the administered dose (13 % - 21 %).	No study data available. A conjugate of M684H002. **Overall, the toxicity of M684H012 is covered by the toxicity data of the parent (cinmethylin).**
M684H021		(chemical name not available)		No study data available. The aglycon of M684H022. A downstream metabolite of M684H039. Negative *in silico* genotoxicity predictions. **Overall, toxicity of H021 is not covered by the toxicity data of the parent (cinmethylin). Cramer Class III TTC values could be used if required.**

Metabolite No.	Structure	Name/code No. (synonyms)	Rat ADME coverage (% of administered dose)	Toxicological assessment
M684H022	AND Enantiomer  (chemical name not available)			No study data available. The glucuronide of M684H021. **Overall, toxicity of H022 is not covered by the toxicity data of the parent (cinmethylin). Cramer Class III TTC values could be used if required.**
M684H026	AND Enantiomer 	2-hydroxypropyl 2-hydroxycineol		No study data available. Some plasma kinetic data available from rat and mouse lifetime/carcinogenicity studies: - Found in rat plasma collected after 12-month dosing with parent, at higher concentrations (up to 13 and 16 µg/mL in M/F) than parent (up to 0.1 µg/mL) ([redacted] 2018) - Found in mouse plasma collected after 63-days dosing with parent, at higher concentrations (up to 0.9 µg/mL) than parent (up to 0.2 µg/mL) ([redacted] 2018d). Negative *in silico* genotoxicity predictions. **Overall, the toxicity of M684H026 is covered by the toxicity data of the parent (cinmethylin).**
M684H039	AND Enantiomer  (chemical name not available)			No study data available. A precursor of M684H021 and M684H022. Negative *in silico* genotoxicity predictions. **Overall, toxicity of H039 is not covered by the toxicity data of the parent (cinmethylin). Cramer Class III TTC values could be used if required.**

Metabolite No.	Structure	Name/code No. (synonyms)	Rat ADME coverage (% of administered dose)	Toxicological assessment
M684H058		1-O-(2-methylbenzoyl) hexopyranuronic acid) Glucuronid of 2-Methyl-benzoic acid		Some open-access data available. The glucuronide of 2-methylbenzoic acid, for which there is some open-access data available, including a negative Ames test. Existing data raise no toxicological concern as indicated by the Scientific Opinion of Flavouring Group Evaluation 20. **Overall, M684H58 is of no toxicological concern and of significantly lower toxicity compared to cinmethylin. However, the TTC Cramer Class I value of 30 µg/kg bw/day could be used if required.**
M684H059		Benzofuranone / Phthalide / 2-benzofuran-1(3H)-one		Some open-access data available. Downstream metabolite of M684H010. Negative *in silico* genotoxicity predictions. Existing data raise no genotoxic concern. **Overall, the toxicity of M684H009 is not covered by the toxicity data of the parent (cinmethylin). Cramer Class III TTC values could be used if required.**

B.6.8.2. Supplementary studies on the active substance

Two realatively old GLP-compliant mechanistic studies on liver enzyme activity induction were performed in rats and mice after single gavage and repeated dietary cinmethylin administration for 7 weeks. In addition, ToxCast data considering non-endocrine related endpoints are presented.

Mechanistic studies

1) Hepatic enzyme activity in the rat

Author(s)	[redacted] ., [redacted] ., [redacted] and [redacted]
Study title	Biochemical studies of SD 95481 in the rat
Study reference	[redacted] 1983a BASF DocID : CI-440-001
Test facility	[redacted]
Date	19/04/1982 – 14/06/1982
Test substance	Cinmethylin BAS 684 H (SD 95481)
Batch no. **Purity (%)**	513D Purity not specified.
Test animals	Fisher F344 rats, Male and female.
Groups	5/sex/group
Dose/concentrations	Acute study: 0 and 316.2/365.2 mg/kg bw in males and females, respectively. Sub-chronic study: 0, 30, 100, 300 and 1000 ppm Equivalent to 0, 2.6, 8.7, 26.4 and 87 mg/kg bw/d for males and 0, 3.0, 9.8, 29.2 and 98.9 mg/kg bw/d for females
Route	Acute study: Single oral (gavage). Sub-chronic study: Administered daily via the diet for 7 weeks.
Vehicle	None.
GLP	Compliant.
Guideline	None.
Deviation	• Individual animal data were not included in the study report; raw data are limited to calculated mean values with corresponding SEM. • No methodological details were provided in the study report for GSH content determination. • No limit of quantification (LOQ) was specified for all individual bioanalytical assays conducted. • The method used for microsome preparation was limited with regard to the rate of yield (compared to modern/current methods). • Consequently, the protein content and CYP activities determined were low, resulting in a minimal fold-increase compared with the control and modern/current methods.
Impact of deviations	The deviations identified are considered to compromise the validity of the study.
Acceptable	Yes, regarded as supplemental information only.
Conclusion	Limited evidence of induction of hepatic CYP activities, preferentially in females from a dose of 29.2 mg/kg bw/d (300 ppm).

Methods

Acute study: Fisher F344 rats (5/sex/group) were administered cinmethylin at dose levels of 0 and 316.2/365.2 mg/kg bw in males and females, respectively, via gavage. Two hours after exposure, animals were sacrificed by CO_2 and liver homogenate was assayed for non-protein sulfhydryl (NPS) content, expressed as glutathione (GSH).

Sub-chronic study: Fisher F344 rats (5/sex/group) were administered cinmethylin at dietary concentrations of 0, 30, 100, 300 and 1000 ppm, equivalent to 0, 2.6, 8.7, 26.4 and 87 mg/kg bw/d for males and 0, 3.0, 9.8, 29.2 and 98.9 mg/kg bw/d for females, for a period of seven weeks. Animals were examined daily for clinical signs and mortalities. Food consumption and body weights were determined weekly. At termination, animals were sacrificed (by decapitation), their livers were removed, weighed, perfused to remove excess haemoglobin, homogenised and processed for the preparation of microsomes. The microsomes were then used for the

following analyses: protein content, total CYP content and hepatic CYP activities consisting of benzo[a]pyrene hydroxylase activity (CYP 1A1/2), aminopyrene demethylase activity (CYP 1A, 2A 2B, 2D and 3A) and aniline hydroxylase activity (CYP 2E1).

The study did not include any analytical determinations (see Volume 3 CA B5, section B.5.1.2).

Table 6.8-2. Average daily cinmethylin intake (sub-chronic study)

Dose level [ppm]	30	100	300	1000
Sex	**Average intake [mg/kg bw/day]**			
Males	2.61	8.74	26.43	86.98
Females	3.03	9.78	29.19	98.84

Results

Acute study: No modulation of hepatic non-protein sulfhydryl (NPS) (Table 6.8-3), indicative of depletion of glutathione (GSH), was observed in males and females treated with cinmethylin at 316/365 mg/kg bw/d.

Sub-chronic study: No mortality occurred and no clinical signs of toxicity were observed during the 7-week study period. There were no treatment-related effects on body weight (Table 6.8-4) and food consumption; no dose-response in these parameters was observed. At the top dose, liver weight (absolute and relative) was increased in both sexes (Table 6.8-5). Statistical-significance was seen for absolute (females only) and relative weight (both sexes) and change compared to controls was between 8.5 – 12.2 %. However, due to the level of increase (< 15 % change compared to control) this effect was not considered adverse. HSE notes that neither histopathology and/or clinical chemistry were performed.

Table 6.8-3. Acute study - Hepatic non-protein sulfhydryl (NPS) content [µmol GSH/g Liver]

Sex	**Males**				**Females**			
Dose [mg/kg bw/d. ♂/♀]	**0**		**316.2**		**0**		**365.2**	
	mean	**SEM**	**mean**	**SEM**	**mean**	**SEM**	**mean**	**SEM**
[µmol /g]	244.7	1.6	229.7	8.2	151.3	1.5	144.3	4.4
[fold of control]			0.9				1.0	

* = p<0.05; * = p<0.01; Dunnett test (one-sided). Δ% = difference to the respective control in percent
SEM - standard error of the mean.

Table 6.8-4. Body weight

Dose	**[ppm]**	**0**		**30**		**100**		**300**		**1000**	
	[mg/kg bw/d, ♂/♀]	**0**		**2.6 / 3.0**		**8.7 / 9.8**		**26.4 / 29.2**		**87.0 / 98.8**	
		mean	**SEM**	**mean**	**SEM**	**mean**	**SEM**	**mean**	**SEM**	**mean**	**SEM**
Males[#] (N = 5)											
Week 1	[g]	135.9	2.5	135.4	2.5	136.2	2.5	131.7	2.5	136.7	2.5
	[Δ% of control]			-0.4		+0.2		-3.1		+0.6	
Week 2	[g]	168.8	3.6	165.5	3.6	168.6	3.6	161.3	3.6	170.4	3.6
	[Δ% of control]			-2.0		-0.1		-4.4		+0.9	
Week 3	[g]	201.2	4.2	196.5	4.2	202.9	4.3	**188.9***	4.3	199.8	4.2
	[Δ% of control]			-2.3		+0.8		-6.1		-0.7	
Week 4	[g]	214.9	4.7	208.5	4.7	213.9	4.7	**202.0***	4.7	219.0	4.7
	[Δ% of control]			-3.0		-0.5		-6.0		+1.9	
Week 5	[g]	236.1	5.0	234.5	5.0	238.0	5.0	**224.2***	5.1	243.3	5.0
	[Δ% of control]			-0.7		+0.8		-5.0		+3.0	
Week 6	[g]	251.0	5.2	246.7	5.2	248.4	5.2	**235.4***	5.2	254.9	5.2
	[Δ% of control]			-1.7		-1.0		-6.2		+1.6	
Week 7	[g]	269.3	6.1	264.2	6.1	267.1	6.1	**250.3***	6.2	271.5	6.1

Dose	[ppm]	0		30		100		300		1000	
	[mg/kg bw/d, ♂/♀]	0		2.6 / 3.0		8.7 / 9.8		26.4 / 29.2		87.0 / 98.8	
		mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM
	[Δ% of control]			-1.9		-0.8		-7.1		+0.8	
Females[#] (N = 5)											
Week 1	[g]	104.6	1.0	105.8	1.0	**109.5***	1.0	103.5	1.1	106.1	1.0
	[Δ% of control]			+1.1		+4.7		-1.1		+1.4	
Week 2	[g]	121.7	1.6	121.5	1.6	**126.7***	1.6	119.3	1.6	120.5	1.6
	[Δ% of control]			-0.2		+4.1		-2.0		-1.0	
Week 3	[g]	128.4	1.8	126.8	1.8	133.3	1.8	125.9	1.8	127.5	1.8
	[Δ% of control]			-1.2		+3.8		-1.9		-0.7	
Week 4	[g]	143.2	1.9	141.6	1.9	147.5	1.9	138.6	1.9	141.3	1.9
	[Δ% of control]			-1.1		+3.0		-3.2		-1.3	
Week 5	[g]	150.03	2.3	148.3	2.3	155.9	2.3	146.1	2.3	149.5	2.3
	[Δ% of control]			-1.2		+3.9		-2.6		-0.4	
Week 6	[g]	159.0	2.8	156.6	2.8	164.6	2.8	153.0	2.8	156.3	2.8
	[Δ% of control]			-1.5		+3.5		-3.8		-1.7	
Week 7	[g]	167.0	3.0	163.9	3.0	173.2	3.0	159.7	3.0	163.9	3.0
	[Δ% of control]			-1.9		+3.7		-4.4		-1.9	

* = p<0.05; * = p<0.01; Dunnett test (one-sided), Δ% = difference to the respective control in percent
[#] = values were adjusted for differences in day 0 body weight
SEM - standard error of the mean.

Table 6.8-5. Liver weight

Sex	Males				Females			
Dose [ppm]	Absolute weight [g]	Δ%	Relative weight [% of bw]	Δ%	Absolute weight [g]	Δ%	Relative weight [% of bw]	Δ%
0	12.90		4.80		7.65		4.59	
30	12.70	-1.6	4.82	+0.4	7.80	+2.0	4.77	+3.9
100	13.60	+5.4	**5.12***	+6.7	7.95	+3.9	4.60	+0.2
300	12.20	-5.4	4.86	+1.3	7.59	-0.8	4.70	+2.4
1000	14.20	**+10.1**	**5.21***	+8.5	**8.37***	+9.4	**5.15***	+12.2

* p ≤ 0.05; ** p ≤ 0.01; Dunnett test (one-sided).
Δ% = percent difference compared to control.

Microsomal protein levels (Table 6.8-6) were statistically-significantly decreased in males at 8.7 (100 ppm) and 87.0 mg/kg bw/d (1000 ppm) and in females at 29.2 mg/kg bw/d (300 ppm). However, no dose-response relationship was evident in this parameter and fold change compared to the control was changed minimally for males. Statistically-significant changes in CYP content (total and/or specific CYP activities) were seen (Table 6.8-6). However, statistical-significance was inconsistent and a dose-response relationship was only observed for benzo[a]pyrene hydroxylase (CYP 1A1/2) and aminopyrine demethylase (CYP 1A, 2A 2B, 2D and 3A) in females from 300 ppm. The magnitude of change was very low across all CYP activities.

Overall, treatment-related changes in CYP activities - benzo[a]pyrene hydroxylase (CYP 1A1/2) and aminopyrine demethylase (CYP 1A, 2A 2B, 2D and 3A) were seen from 300 ppm (29.2 mg/kg bw/d) in females
.

Table 6.8-6. Hepatic biochemical parameters

Dose [ppm]	0		30		100		300		1000	
Dose [mg/kg bw/d ♂/♀]	0		2.6 / 3.0		8.7 / 9.8		26.4 / 29.2		87.0 / 98.8	
	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM
Males (N = 5)										
Microsomal protein content										
[mg/g liver]	11.64	0.83	10.04	0.30	**9.40***	0.47	10.00	0.57	**9.80***	0.50
[fold of control]			0.9		0.8		0.9		0.8	
Total CYP content										
[nmol/mg protein]	0.440	0.030	0.511	0.014	**0.526***	0.029	**0.517***	0.021	0.504	0.028
[fold of control]			1.16		1.20		1.18		1.15	
Benzo[a]pyrene hydroxylase (CYP 1A1/2), [quinine sulphate units/min/mg protein]										
[U/min/mg]	25.48	2.42	**31.14***	0.85	28.80	0.98	**29.44***	0.94	**31.52***	0.51
[fold of control]			1.22		1.13		1.16		1.24	
Aminopyrine demethylase (CYP 1A, 2A 2B, 2D and 3A), [nmol formaldehyde/min/mg protein]										
[nmol/min/mg]	2.05	0.18	2.22	0.09	2.45	0.11	2.13	0.12	2.39	0.17
[fold of control]			1.1		1.2		1.0		1.2	
Aniline hydroxylase (CYP 2E1), [µg p-aminophenol/min/mg protein]										
[µg/min/mg]	0.055	0.002	**0.046***	0.002	**0.039***	0.002	**0.029***	0.004	**0.032***	0.001
[fold of control]			0.8		0.7		0.5		0.6	
Females (N = 5)										
Microsomal protein content										
[mg/g liver]	7.74	0.24	7.14	0.38	7.06	0.23	**6.64***	0.29	7.24	0.35
[fold of control]			0.9		0.9		0.9		0.9	
Total CYP content										
[nmol/mg protein]	0.448	0.021	0.448	0.021	0.429	0.021	**0.511***	0.014	0.460	0.021
[fold of control]			1.0		1.0		1.1		1.0	
Benzo[a]pyrene hydroxylase (CYP 1A1/2), [quinine sulphate units/min/mg protein]										
[U/min/mg]	3.35	0.17	3.35	0.12	3.31	0.15	**4.20***	0.10	**4.93***	0.20
[fold of control]			1.0		1.0		1.3		1.5	
Aminopyrine demethylase (CYP 1A, 2A 2B, 2D and 3A), [nmol formaldehyde/min/mg protein]										
[nmol/min/mg]	0.476	0.030	0.488	0.034	0.592	0.064	**0.644***	0.052	**0.787***	0.017
[fold of control]			1.0		1.2		1.4		1.7	
Aniline hydroxylase (CYP 2E1), [µg p-aminophenol/min/mg protein]										
[µg/min/mg]	0.041	0.001	0.040	0.004	0.044	0.001	**0.048***	0.002	0.044	0.001
[fold of control]			1.0		1.1		1.2		1.1	

* = p<0.05; * = p<0.01; Dunnett test (one-sided). Δ% = difference to the respective control in percent
SEM - standard error of the mean.

Conclusion

In conclusion, under the conditions of this relatively old, GLP compliant, mechanistic rat study, dietary administration of cinmethylin for 7-weeks, did not result in adverse increases in liver weight up to the top dose of 1000 ppm (87.0/98.8 mg/kg bw/d in males and females, respectively). Total CYP content was not convincingly increased by treatment, however increases in certain specific CYP activities (benzo[a]pyrene hydroxylase (CYP 1A1/2) and aminopyrine demethylase (CYP 1A, 2A 2B, 2D and 3A)) were seen from 300 ppm (29.2 mg/kg bw/d) in females. Administration of a single oral gavage dose of cinmethylin did not result in a change in hepatic glutathione levels (GSH). Therefore, there is limited evidence from this study in rats of induction of hepatic CYP activities, preferentially in females from a dose of 29.2 mg/kg bw/d.

([redacted] 1983a)

2) Hepatic enzyme activity in the mouse

Author(s)	[redacted] and [redacted]
Study title	Biochemical studies of SD 95481 in the mouse
Study reference	[redacted] 1983b BASF DocID : CI-440-002
Test facility	[redacted]
Date	03/05/1982 – 28/06/1982
Test substance	Cinmethylin BAS 684 H (SD 95481)
Batch no. **Purity (%)**	513D Purity not specified.
Test animals	B6C3F1 mice, Male and female.
Groups	5/sex/group
Dose/concentrations	Acute study: 0 and 578.1/482.8 mg/kg bw in males and females, respectively. Sub-chronic study: 0, 30, 100, 300 and 1000 ppm Equivalent to 0, 3.4, 12.3, 38.9 and 129.8 mg/kg bw/d for males and 0, 4.7, 15.8, 40.7 and 134.0 mg/kg bw/d for females
Route	Acute study: Single oral (gavage). Sub-chronic study: Administered daily via the diet for 7 weeks.
Vehicle	None.
GLP	Compliant.
Guideline	None.
Deviation	• Individual animal data were not included in the study report; raw data are limited to calculated mean values with corresponding SEM. • No methodological details were provided in the study report for GSH content determination. • No limit of quantification (LOQ) was specified for all individual bioanalytical assays conducted. • The method used for microsome preparation was limited with regard to the rate of yield (compared to modern/current methods). Consequently, the protein content and CYP activities determined were low, resulting in a minimal fold-increase compared with the control and modern/current methods.
Impact of deviations	The deviations identified are considered to compromise the validity of the study.
Acceptable	Yes, regarded as supplemental information only.
Conclusion	Limited evidence of induction of hepatic CYP activities in both sexes from 300 ppm (38.9/40.7 mg/kg bw/d).

Methods

Acute study: B6C3F1 mice (5/sex/group) were administered cinmethylin at dose levels of 0 and 578.1/482.8 mg/kg bw in males and females, respectively, via gavage. Two hours after exposure, animals were sacrificed by CO_2 and liver homogenate was assayed for non-protein sulfhydryl (NPS) content, expressed as glutathione (GSH).

Sub-chronic study: B6C3F1 mice (5/sex/group) were administered cinmethylin at dietary concentrations of 0, 30, 100, 300 and 1000 ppm, equivalent to 0, 3.4, 12.3, 38.9 and 129.8 mg/kg bw/d for males and 0, 4.7, 15.8, 40.7 and 134.0 mg/kg bw/d for females, for a period of seven weeks. Animals were examined daily for clinical signs and mortalities. Food consumption and body weights were determined weekly. At termination, animals were sacrificed (by decapitation), their livers were removed, weighed, perfused to remove excess haemoglobin, homogenised and processed for the preparation of microsomes. The microsomes were then used for the following analyses: protein content, total CYP content and hepatic CYP activities consisting of benzo[a]pyrene hydroxylase activity (CYP 1A1/2), aminopyrene demethylase activity (CYP 1A, 2A 2B, 2D and 3A) and aniline hydroxylase activity (CYP 2E1).

The study did not include any analytical determinations (see Volume 3 CA B5, section B.5.1.2).

Table 6.8-7. Average daily cinmethylin intake (sub-chronic study)

Dose level [ppm]	30	100	300	1000
Sex	**Average intake [mg/kg bw/day]**			
Males	3.35	12.27	38.88	129.77
Females	4.65	15.84	40.70	133.95

Results

Acute study: No modulation of hepatic non-protein sulfhydryl (NPS) (Table 6.8-8), indicative of depletion of glutathione (GSH), was observed in males and females treated with cinmethylin at 578/483 mg/kg bw/d.

Sub-chronic study: No mortality occurred and no clinical signs of toxicity were observed during the 7-week study period. There were no treatment-related effects on body weight (Table 6.8-9) and food consumption; no dose-response in these parameters was observed. Absolute and relative liver weight was not affected by the treatment up to the highest dose of 1000 ppm (129.8/134.0 mg/kg bw/d in males and females respectively) (Table 6.8-10)

Table 6.8-8. Acute study - Hepatic non-protein sulfhydryl (NPS) content [µmol GSH/g Liver]

Sex	**Males**				**Females**			
Dose [mg/kg bw/d. ♂/♀]	**0**		**578.1**		**0**		**482.8**	
	mean	**SEM**	**mean**	**SEM**	**mean**	**SEM**	**mean**	**SEM**
[µmol /g]	7.02	0.39	6.20	1.00	6.02	0.12	5.63	0.25
[fold of control]			0.9				0.9	

* = p<0.05; * = p<0.01; Dunnett test (one-sided). Δ% = difference to the respective control in percent
SEM - standard error of the mean.

Table 6.8-9. Body weight

Dose	**[ppm]**	**0**		**30**		**100**		**300**		**1000**	
	[mg/kg bw/d, ♂/♀]	**0**		**3.4 / 4.7**		**12.3 / 15.8**		**38.9 / 40.7**		**129.8 / 134.0**	
		mean	**SEM**	**mean**	**SEM**	**mean**	**SEM**	**mean**	**SEM**	**mean**	**SEM**
Males[#] (N = 5)											
Week 1	[g]	20.67	0.56	**22.62***	0.46	**23.76***	0.47	**22.99***	0.47	**23.16***	0.49
	[Δ% of control]			+9.4		+14.9		+11.2		+12.0	
Week 2	[g]	22.48	0.76	**24.46***	0.63	**24.71***	0.63	24.01	0.64	**24.65***	0.67
	[Δ% of control]			+8.8		+9.9		+6.8		+9.7	
Week 3	[g]	24.11	0.49	24.87	0.40	24.93	0.41	24.73	0.41	24.12	0.43
	[Δ% of control]			+3.2		+3.4		+2.6		±0.0	
Week 4	[g]	24.97	0.72	25.57	0.59	25.97	0.60	25.01	0.60	25.76	0.63
	[Δ% of control]			+2.4		+4.0		+0.2		+3.2	
Week 5	[g]	25.49	0.77	25.74	0.64	24.36	0.64	25.51	0.65	25.50	0.68
	[Δ% of control]			+1.0		-4.4		+0.1		±0.0	
Week 6	[g]	25.78	0.60	26.73	0.50	27.06	0.50	26.71	0.51	**27.79***	0.53
	[Δ% of control]			+3.7		+5.0		+3.6		+7.8	
Week 7	[g]	26.60	0.67	28.00	0.56	27.75	0.56	27.76	0.57	28.37	0.59
	[Δ% of control]			+5.3		+4.3		+4.4		+6.7	
Females[#] (N = 5)											
Week 1	[g]	17.86	0.41	17.38	0.41	18.36	0.41	18.41	0.41	17.78	0.41
	[Δ% of control]			-2.7		+2.8		+3.1		-0.4	
Week 2	[g]	18.75	0.44	18.85	0.44	18.88	0.44	18.10	0.44	17.60	0.44
	[Δ% of control]			+0.5		+0.7		-3.5		-6.1	

Dose	[ppm]	0		30		100		300		1000	
	[mg/kg bw/d, ♂/♀]	0		3.4 / 4.7		12.3 / 15.8		38.9 / 40.7		129.8 / 134.0	
		mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM
Week 3	[g]	19.99	0.33	**18.74***	0.33	19.32	0.33	**18.52***	0.33	19.32	0.33
	[Δ% of control]			-6.3		-3.4		-7.4		-3.4	
Week 4	[g]	19.34	0.42	19.91	0.42	20.14	0.42	19.39	0.42	20.08	0.42
	[Δ% of control]			+2.9		+4.1		+0.3		+3.8	
Week 5	[g]	20.50	0.54	21.06	0.54	20.84	0.54	19.77	0.54	20.78	0.54
	[Δ% of control]			+2.7		+1.7		-3.6		+1.4	
Week 6	[g]	20.40	0.39	**21.60***	0.39	21.46	0.39	21.21	0.39	21.03	0.39
	[Δ% of control]			+5.9		+5.2		+4.0		+3.1	
Week 7	[g]	22.49	0.37	22.97	0.37	22.82	0.37	22.15	0.37	22.99	0.37
	[Δ% of control]			+2.1		+1.5		-1.5		+2.2	

* = p<0.05; * = p<0.01; Dunnett test (one-sided), Δ% = difference to the respective control in percent
# = values were adjusted for differences in day 0 body weight
SEM - standard error of the mean.

Table 6.8-10. Liver weight

Sex	Males				Females			
Dose [ppm]	Absolute weight [g]	Δ%	Relative weight [% of bw]	Δ%	Absolute weight [g]	Δ%	Relative weight [% of bw]	Δ%
0	1.81		6.83		1.59		7.13	
30	1.95	7.7	6.97	2.0	1.62	1.9	7.05	-1.1
100	1.88	3.9	6.79	-0.6	1.46	-8.2	6.42	-10.0
300	1.86	2.8	6.70	-1.9	**1.43***	-10.1	6.51	-8.7
1000	1.95	7.7	6.87	0.6	1.63	2.5	7.03	-1.4

* p ≤ 0.05; ** p ≤ 0.01; Dunnett test (one-sided).
Δ% = percent difference compared to control.

Microsomal protein levels were not affected by treatment with cinmethylin (Table 6.8-11). Total hepatic CYP content was statistically-significantly increased in males and females from 300 ppm and a dose-response was evident in this parameter (Table 6.8-11). Statistically-significant increases in specific CYP activities were seen at all treatment doses. Benzo[a]pyrene hydroxylase (representative for CYP 1A1/2) activity (in males) and aminopyrine demethylase (representative for CYP 1A, 2A 2B, 2D and 3A) activity (in females) were statistically-significant increased, with some indication of a dose-response from 300 ppm. For all other specific CYP acitities, statistical-significance was inconsistent and a clear dose-response relationship was lacking. The magnitude of change was very low across all CYP activities.

Overall, treatment-related changes in CYP activities - benzo[a]pyrene hydroxylase (CYP 1A1/2) and aminopyrine demethylase (CYP 1A, 2A 2B, 2D and 3A) were seen from 300 ppm (38.9/40.7 mg/kg bw/d in males and females respectively).

Table 6.8-11. Hepatic biochemical parameters

Dose [ppm]	0		30		100		300		1000	
Dose [mg/kg bw/d ♂/♀]	0		3.4 / 4.7		12.3 / 15.8		38.9 / 40.7		129.8 / 134.0	
	mean	**SEM**	**mean**	**SEM**	**mean**	**SEM**	**mean**	**SEM**	**mean**	**SEM**
Males (N = 5)										
Microsomal protein content										
[mg/g liver]	6.32	0.39	5.40	0.23	6.88	0.58	7.40	0.65	7.08	0.29
[fold of control]			0.9		1.1		1.2		1.1	
Total CYP content										
[nmol/mg protein]	0.33	0.06	0.41	0.04	0.41	0.06	**0.63***	0.02	**0.74***	0.05
[fold of control]			1.2		1.2		1.9		2.2	
Benzo[a]pyrene hydroxylase (CYP 1A1/2), [quinine sulphate units/min/mg protein]										
[U/min/mg]	10.84	0.80	**13.72***	1.34	12.38	0.62	**14.20***	0.56	**18.70***	0.90
[fold of control]			1.3		1.1		1.3		1.7	
Aminopyrine demethylase (CYP 1A, 2A 2B, 2D and 3A), [nmol formaldehyde/min/mg protein]										
[nmol/min/mg]	2.67	0.06	3.17	0.32	3.08	0.32	2.24	0.08	2.87	0.12
[fold of control]			1.2		1.2		0.8		1.1	
Aniline hydroxylase (CYP 2E1), [µg p-aminophenol/min/mg protein]										
[µg/min/mg]	0.130	0.006	**0.176***	0.011	**0.154***	0.009	**0.153***	0.006	**0.159***	0.004
[fold of control]			1.4		1.2		1.2		1.2	
Females (N = 5)										
Microsomal protein content										
[mg/g liver]	9.12	0.49	8.68	0.46	10.60	0.59	8.96	0.69	10.24	0.16
[fold of control]			1.0		1.2		1.0		1.1	
Total CYP content										
[nmol/mg protein]	0.39	0.03	0.44	0.01	0.45	0.02	**0.57***	0.03	**0.67***	0.03
[fold of control]			1.1		1.2		1.5		1.7	
Benzo[a]pyrene hydroxylase (CYP 1A1/2), [quinine sulphate units/min/mg protein]										
[U/min/mg]	14.06	0.74	16.28	0.63	16.00	0.46	**22.02***	1.33	**22.60***	1.13
[fold of control]			1.2		1.1		1.6		1.6	
Aminopyrine demethylase (CYP 1A, 2A 2B, 2D and 3A), [nmol formaldehyde/min/mg protein]										
[nmol/min/mg]	2.85	0.13	2.78	0.13	2.26	0.11	**3.57***	0.40	**4.12***	0.20
[fold of control]			1.0		0.8		1.3		1.4	
Aniline hydroxylase (CYP 2E1), [µg p-aminophenol/min/mg protein]										
[µg/min/mg]	0.121	0.004	0.134	0.003	0.109	0.001	0.126	0.013	**0.157***	0.010
[fold of control]			1.1		0.9		1.0		1.3	

* = p<0.05; * = p<0.01; Dunnett test (one-sided). Δ% = difference to the respective control in percent
SEM - standard error of the mean.

Conclusion

In conclusion, under the conditions of this relatively old, GLP compliant, mechanistic mouse study, dietary administration of cinmethylin for 7-weeks, did not result in adverse increases in liver weight up to the top dose of 1000 ppm (129.8/134.0 mg/kg bw/d in males and females, respectively). Total CYP content was increased by treatment from 300 ppm (38.9/40.7 mg/kg bw/d in males and females, respectively). Increases in certain specific CYP activities (benzo[a]pyrene hydroxylase (CYP 1A1/2) and aminopyrine demethylase (CYP 1A, 2A 2B, 2D and 3A)) were seen from 300 ppm. Administration of a single oral gavage dose of cinmethylin did not result in a change in hepatic glutathione levels (GSH). Therefore, there is limited evidence from this study in mice of induction of hepatic CYP activities in both sexes from a dose of 300 ppm (38.9/40.7 mg/kg bw/d).

([redacted] 1983b)

ToxCast Data (excluding endocrine related parameters)

Collection and evaluation of ToxCast data was conducted by the applicant. The HSE has not re-conducted the collection of these data using the ToxCast database. The HSE has however, re-presented (see below) critically evaluated and summarised the information submitted by the applicant. All ToxCast data were retrieved in October 2015; the current/latest release was May 2019.

Introduction

Cinmethylin was tested in 689 assay endpoints of the 1192 available assay endpoints in the ToxCast and Tox21 initiative of *in vitro* high-throughput screening and was "active" in 43 of these assay endpoints. All data included in this chapter reflect concentration-response screening (ranging from 4 to 15 testing concentrations) with concentration ranges of cinmethylin from as low as 0.001 µM and up to 200 µM. Solubility in DMSO was verified up to the concentration used for stock solution generation (20 mM) (Richard *et al*., 2016; 2016/1352489). The solubility issue of cinmethylin in DMSO detected by BASF at concentrations of ≥ 43.7 mM has most probably not affected these assays (Rueckel, 2018; 2018/1000729).

Cinmethylin (CASRN 87818-31-3, mixture of stereoisomers) was procured from an undisclosed commercial source (Richard *et al*., 2016; 2016/1352489) with a structure quality control rating in the US EPA NCCT's Distributed Structure Searchable Toxicity Database (DSSTox_QC) of high. As of the latest release of chemical purity quality control from the Tox21 program (October 2015; https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data), cinmethylin is listed as having a "QC_Grade" not determined and a status of "Analysis in Progress".

All ToxCast/Tox21 data were retrieved from the last public release issued by the US EPA NCCT in October 2015. The data were accessed by MySQL database download of "invitrodb_v2" (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data). This database contains all data as analyzed using the US EPA NCCT's custom built ToxCast Pipeline (Filer *et al*., 2017; 2017/1227067), an R package for processing and modeling chemical screening data (version 1.3 which was also used to generate some of the figures herein). A comprehensive spreadsheet containing analysis and curve metrics, assay parameters, and assay annotation are included in the supplementary excel file (Anonymous, 2018; 2018/1090846). Furthermore, the concentration-response curves for all 689 assay endpoints are provided in the supplementary PDF file (Anonymous, 2018; 2018/1090847).

The ToxCast concepts of data analysis: Procedures for ToxCast/Tox21 Assay Data Evaluation

Details for how data are evaluated can be found in Filer *et al.*, 2017 (2017/1227067). "tcpl: the ToxCast pipeline for high-throughput screening data". Briefly, tcpl is an R package that is publicly available for download. This R package functions by linking to a MySQL database containing the ToxCast and Tox21 data (freely downloadable at https://www.epa.gov/chemical-research/toxicity-forecaster- toxcasttm-data). The versions used for analyses conducted herein were tcpl v1.3 and the MySQL database "invitrodb_v2". The analyses herein summarize only concentration-response data.

Maximum of median responses (*max_med*)

When referring to the concentration series the "mean" and "median" values are defined as the mean or median of the percent response values at every concentration. In other words, the maximum median (***max_med***) is the measured substance specific maximum response defined over all median values across the concentration series.

Modelling prerequisites for multiple concentration assays

- Concentration series must have at least four concentrations to enter the fitting algorithm.
- By default, concentration series must additionally have at least one median value > ***3-bmad*** to enter the fitting algorithm.
- Activity is modelled only in the positive direction. Therefore, signal data in the negative direction must be transformed to the positive direction during normalization. Negative direction data are inverted by multiplying the final response values by −1.
- Normalisation to control responses can be done either as "fold-change" or "percent of control". Since the modelling requires that data is zero-centered, fold-change data requires log-transformation.

Data Models

The data from each chemical assay pair is fit to 3 models: a constant model, a Hill model, and a Gain-Loss model. The latter allows the curve to rise from zero to a plateau, and then fall off again. This curve shape allowed to account for non-specific assay interference, such as cytotoxicity occurring at high concentrations. The model with the best fit (lowest Akaike Information Criterion, AIC) is selected as the winning model (modl), and is used to determine the activity or hit-call for the concentration series. If two models have equal AIC values, the simpler model (the model with fewer parameters) wins the tie.

Prerequisites for prediction of a positive response "active hit-call" in ToxCast

For a sample to get an active hit-call in the single concentration assays, the ***max_med*** must be greater than an efficacy cutoff. The efficacy cutoff is predefined for each assay by the level 2 methods.

For a concentration series to get an active hit-call, either the Hill or gain-loss must be selected as the winning model. If the Hill or gain-loss model wins, and the modeled top parameter for the winning model (***modl_tp***) and the maximum median value (***max med***) are both greater than or equal to the efficacy cutoff (***coff***), the concentration series is considered active and the hit-call (***hitc***) is set to 1.

The hit-call (***hitc***) can be 1, 0, or -1. A hit-call of 1 or 0 indicates the concentration series is active or inactive, respectively, according to the analysis; a hit-call of -1 indicates the concentration series had less than four concentrations. In the latter case, a dose-response cannot be modelled in ToxCast.

Point-of-departure model estimates (***ACB*** and ***ACC*** and ***AC50***)

The response cutoff was selected per assay as being the maximum of 3-bmad, 20% above baseline, or an assay-specific cutoff, *e.g.*, 6-bmad or 10-bmad. For active concentration series, additional point-of-departure estimates are calculated for the winning model: (1) the AC50 (***modl_ga***) reflecting the concentration at which 50% of the substance specific maximum activity (***max_med***) is achieved, (2) the activity concentration at baseline (ACB or ***modl_acb***) and (3) the concentration at response cutoff exceedance (ACC or ***modl_acc***). The ACB and ACC are defined as the concentration where the estimated model value equals 3-bmad and the cutoff, respectively. The point-of-departure estimates are summarized in the following Figure 6.8-1.

To provide insight on efficacy the median across the replicates at the concentration where highest response is observed is computed. This term (***max_med***) provides an estimate of the highest efficacy achieved by the data points in the concentration-response curve. Additionally, the curve that best fits the data is also assessed for the highest response, and the top of the curve is also reported (***modl_tp***). It is important to review the response units (resp_unit) for all assays as different assay endpoints can vary in output data type (i.e., fold change, percent inhibition, percent of control, log2 (fold change), etc.). As a further important parameter, cytotoxicity information is critical for interpreting the observed effect.

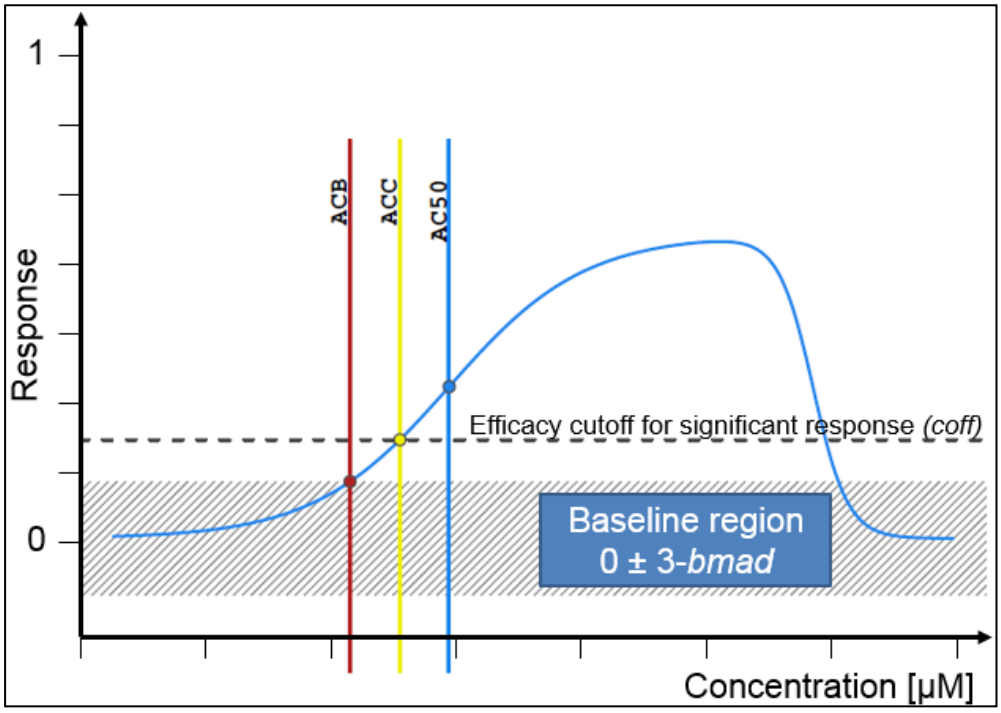


Figure 6.8-1. Point of departure estimates in ToxCasts/Tox21 assays.

Source: Filer *et al.*, 2017, 2017/1227067, modified. The point-of-departure estimates. The shaded rectangle represents the baseline region, 0 ± 3x bmad. The dark striped line represents the efficacy cutoff (*coff*) of the study. The vertical lines show where the point-of-departure estimates are defined: the red line shows the ACB, the yellow line shows the ACC, and the blue line shows the AC_{50}.

Summary of Cinmethylin data by biological target

Most ToxCast/Tox21 assay endpoints have been annotated to biological target processes. These assay annotations are available for public download (https://www.epa.gov/chemical- research/toxicity-forecaster-toxcasttm-data). Using these biological target terms, a summary of the 689 assay endpoints in which Cinmethylin was evaluated is provided in Figure 6.8-2. This breakdown reveals that cinmethylin was evaluated in assay endpoints mapping to 22 target terms (plus one called "null" to capture assays that did not have targets annotated). Active assay endpoints were related to 10 of these terms:

- Oxidoreductase (active in 1 of 3 assay endpoints evaluated)
- Esterase (active in 1 of 2 assay endpoints evaluated)
- Transferase (active in 2 of 9 assay endpoints evaluated)
- Background measurement (active in 2 of 88 assay endpoints evaluated)
- DNA binding (active in 3 of 84 assay endpoints evaluated)
- Transporter (active in 4 of 17 assay endpoints evaluated)
- Steroid hormone (active in 5 of 20 assay endpoints evaluated)
- Cell cycle (active in 5 of 80 assay endpoints evaluated)
- Cyp (active in 6 of 21 assay endpoints evaluated)
- Nuclear receptor (active in 13 of 105 assay endpoints evaluated)

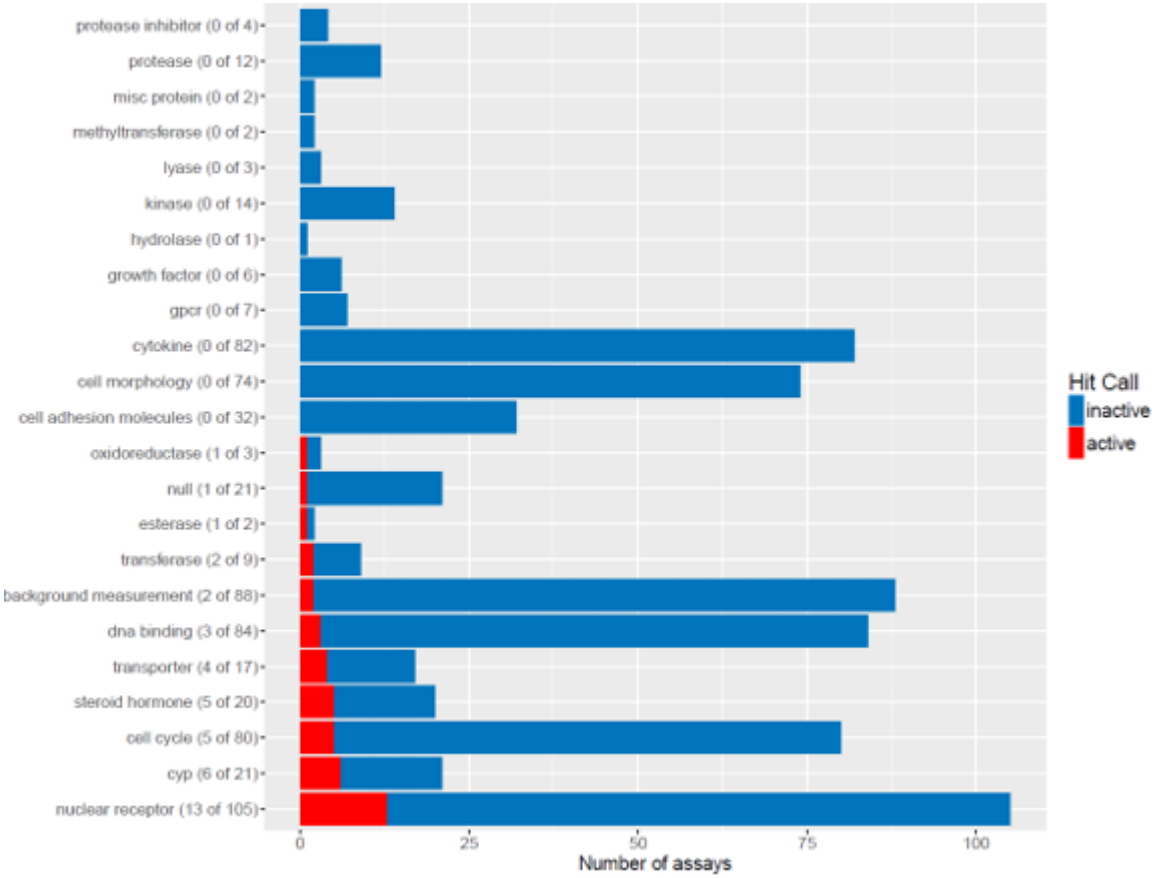


Figure 6.8-2. Summary of biological processes targeted by the assays in which cinmethylin was tested

Source: iCSS ToxCast Dashboard; *https://actor.epa.gov/dashboard/#chemical/87818-31-3*. The terms labeled on the y-axis are the "intended_target_family" terms provided in the downloaded ToxCast assay annotation files. The values following those terms represent how many of the assays Cinmethylin was active in, out of how many assays Cinmethylin was tested in (to facilitate interpretation along the x-axis). These values are not a sum of all possible assays mapping to the term, but rather they only encompass assays in which Cinmethylin was evaluated. Of the assays in which Cinmethylin was evaluated, 21 were not associated with a target and are thus summarized under the term "null".

To further investigate the bioactivity associated with biological targets, the relative potency and scaled efficacy (maximum effect / assay-specific activity cutoff threshold) was plotted (Figure 6.8-3). This plot demonstrates that most hits elicited by cinmethylin is centered around a potency of 10-100 µM with varying efficacy, and some activity was found in the concentration range of 1-10 µM. No bioactivity was seen in the sub-micromolar range.

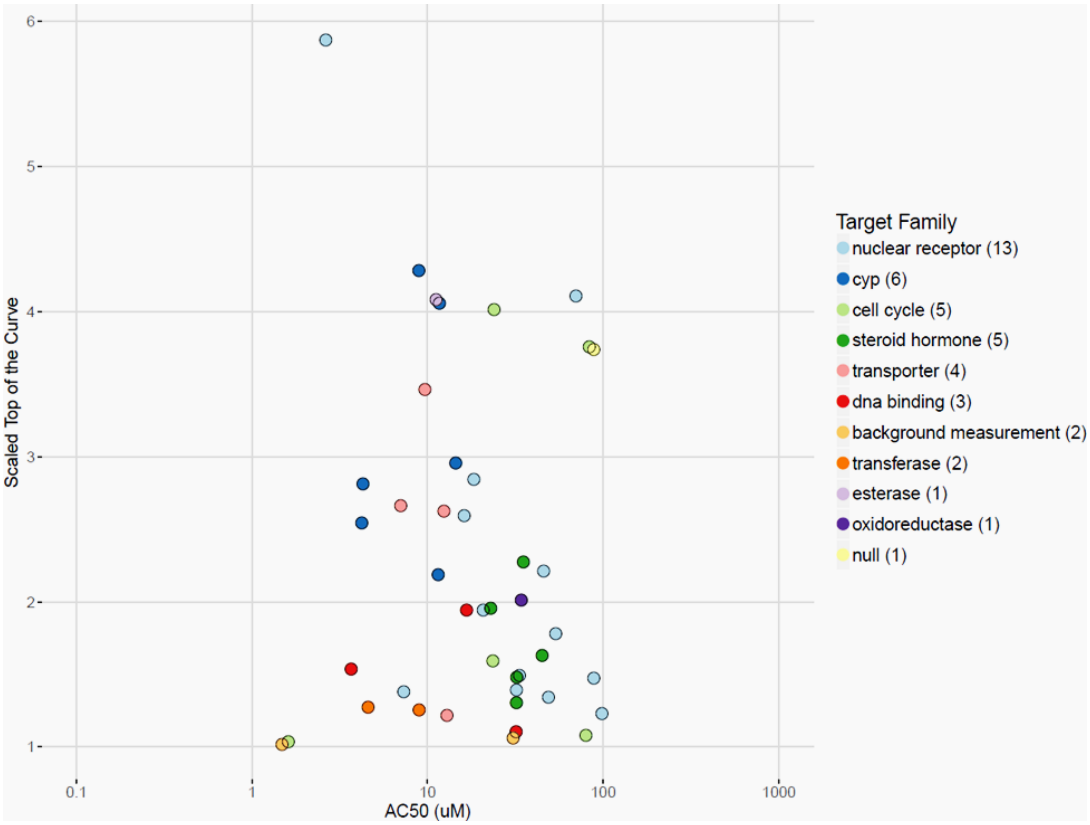


Figure 6.8-3. Summary of biological processes targeted by the assays in which cinmethylin was tested

Source: iCSS ToxCast Dashboard; *https://actor.epa.gov/dashboard/#chemical/87818-31-3*. The 43 assay endpoints in which Cinmethylin was active are all represented on this plot, colored according to the biological target ("intended_target_family" terms). The scaled top of curve on the y-axis was computed by dividing the maximum response by the activity threshold for the assay.

Cytotoxicity

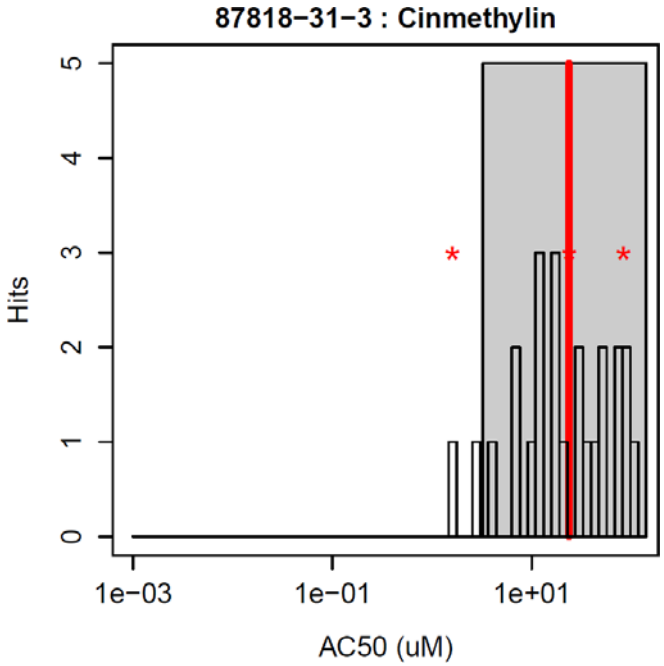
Cinmethylin was originally evaluated in 33 *in vitro* cytotoxicity assays at concentrations ranging from 0.001 to 200 µM (Judson *et al.*, 2016; 2016/1227708). Meanwhile the number of assays increased to at least 41. The cytotoxicity assays are all human cell based assays originating from a variety of tissues. Cinmethylin is active in several cytotoxicity assays, which are:

- ACEA_T47D_80hr_Negative (AC50 = 83.361 µM)
- BSK_3C_SRB_down (AC50= 23.537 µM)
- BSK_SAg_SRB_down (AC50 = 1.608 µM)
- NCCT_HEK293T_CellTiterGLO (AC50 = 24 µM)
- hNIS-HEK293T-EPA cells (AC50 = 30.2 µM)

Based on the originally 33 assays with the first 3 listed assays a cytotoxicity region has been defined (Judson *et al.*, 2016; 2016/1227708), in which any assay endpoint hit may be confounded by cytotoxicity. The cytotoxicity region is computed as a z-score greater than 3 from the median cytotoxicity AC50, as shown in Figure 6.8-4, the grey box centered around the median cytotoxicity AC50 (red line).

Source: Judson *et al.*, 2016; 2016/1227708. Summary of assay activities (bar plot) both within and outside the region of cytotoxicity (grey shaded area) for Cinmethylin. The vertical red line shows the median of the AC50

Figure 6.8-4. Cytotoxicity summary relative to active assay AC50 values for cinmethylin



across the cytotoxicity assays in which Cinmethryin was active (denoted by red asterisks (*)). The cytotoxicity region is calculated based on a z-score distribution of 3 around the median cytotoxicity AC50

For cinmethylin, the cytotoxicity region was defined as centered around the **median cytotoxicity AC50 of 23.54 µM**, and the **lower limit of the cytotoxicity region is 3.63 µM.** All active endpoints within the cytotoxicity region are potentially confounded by cytotoxicity and therefore not considered specific endpoints. This applies also for cell-free assays because some mechanisms leading to cytotoxicity are acting through disruption at the molecular/physical level. Hence, potent assays that are below the lower limit of the cytotoxicity region are considered to be specific for cinmethylin and of interest.

The BSK_SAg_SRB_down needs to be interpreted with care as the hit call is based on the highest concentration tested (40 µM) being only slightly outside the cutoff region, so that the hit call is potentially confounded by overfitting. In this case, the **median cytotoxicity AC50 would not change considerable from 23.54 µM to 24 µM and the cytotoxicity region would be slightly higher than calculated before.**

Therefore, within this assessment, the median cytotoxicity AC50 of 23.54 µM was used as first tier starting point to eliminate unspecific and potentially confounded responses.

All active endpoints are presented in the following with their AC50 value, the ACC concentration where the response cutoff is reached, and the concentration of measured maximum response. Those assays belonging to the endocrine endpoints (Estrogen, Androgen, Steroidogenesis, Aromatase, and Thyroid related endpoints) are discussed in B.6.8.3. Details on the assays can be found in the excel file (Anonymous, 2018 ; 2018/1090846) under the assay endpoint names and the plots of all assays are summarised in 2018/1090847 (Anonymous, 2018).

Hit calls of Cinmethylin

There are only three assays with AC50 values at or below the lower limit of the cytotoxicity region of 3.63 µM. Two of these endpoints (ATG_M_32_TRANS_up and BSK_SAg_SRB_down) are flagged to be borderline active and potentially confounded by overfitting and therefore less relevant. One of them is exactly the already mentioned cytotoxicity endpoint BSK_SAg_SRB_down, which is an unspecific protein content based assay. The response curve was so gently inclining that the response cutoff is reached not before 89 µM. The other unspecific active endpoint is the ATG_M_32_Trans_up, which measures mRNA induction in HEPG2 cells via a co-transfected reporter gene and the exogenous transcription factor GAL4_M32. This assay endpoint is used as internal marker for background mRNA induction.

The only specific endpoint for cinmethylin is the nuclear receptor assay named **ATG_PXRE_Cis_up**.

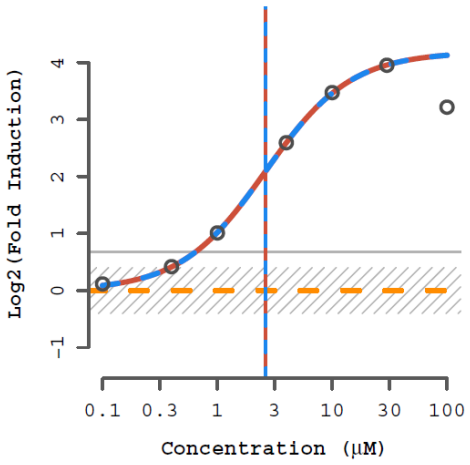
Table 6.8-12. Assay endpoints where cinmethylin elicited bioactivity below the cytotoxicity region

Assay Name	Biological target ("intended_target_family")	AC50 (µM)	ACC (µM)	Max_mean (Fold induction) (Concentration)
ATG_PXRE_CIS_up	Nuclear Receptor	2.631	0.64	3.95 (30 µM)
ATG_M_32_TRANS_up [7, 11, 16]	Background Measurement	1.48	5.44	0.3 (4 µM)
BSK_SAg_SRB_down [11, 16]	Cell Cycle	1.608	89.24	0.088 (40 µM)

[7] Only one conc. above baseline, [11] borderline active [16] Hit-call potentially confounded by overfitting, n.a. not applicable

ATG_PXRE_Cis_up measures the mRNA induction unique for the reporter gene response element PXRE, which is responsive to the endogenous human nuclear receptor PXR / NR1I2. The assay is performed in human liver cells (HEPG2). PXR as orphan nuclear receptor, primarily expressed in the liver, regulates the expression of phase I (mainly Cyp3A) and phase II metabolizing enzymes and transporters.

ATG_PXRE_CIS_up exceeded the response cutoff at 0.64 µM (ACC), reached half-maximal response (AC50) at 2.631 µM and maximal response of 3.95-fold induction at 30 µM. **Thereby, the ToxCast data indicate that cinmethylin specifically upregulates the response element of the gene PXR (PXRE) in the low µM range.** The corresponding plot is shown in Figure 6.8-5 below.




```
ASSAY:    AEID103 (ATG_PXRE_CIS_up)

NAME:     Cinmethylin
CHID:     34456    CASRN: 87818-31-3
SPID(S): TP0000075C10
M4ID:     8398254

HILL MODEL (in red):
          tp        ga        gw
val:   4.18      0.42      1.17
sd:    0.0241    0.00774   0.0162

GAIN-LOSS MODEL (in blue):
          tp        ga        gw        la        lw
val:   4.18      0.42      1.17      3.87      6.99
sd:    NA        NA        NA        NA        NA

          CNST         HILL         GNLS
AIC:   36.06        1            5
PROB:  0            0.88         0.12
RMSE:  2.56         0.34         0.34

MAX_MEAN: 3.95      MAX_MED: 3.95      BMAD: 0.135

COFF: 0.673   HIT-CALL: 1    FITC: 41    ACTP: 1

FLAGS:
```

Figure 6.8-5. Plot of the specific active endpoint ATG_PXRE_Cis_up

Phase I (Cytochrome P450 and Esterase) and Phase II (transferase) enzyme-related Assays

Cinmethylin was evaluated in 21 assays mapped to the "cyp", in 9 assays mapped to the "transferase", and in 2 assays related to "esterase" intended_target_family term. The "cyp" term reflects assays that target cytochrome P450 (CYP450) xenobiotic metabolizing enzymes, the "transferase" term tackles alkyl and aryl-, sulfo- and glucuronosyl-transferase assay endpoints, and the "esterase" assays are measuring butyryl cholinesterase activity. The assays originate from Tox21, CellzDirect (CLD) and NovaScreen. The "cyp" and "transferase" assays cover three timepoints (6h, 24h, and 48h) and the esterase assay is a single point assay. While the Tox21 and the NovaScreen assays measure enzyme activity or receptor binding, the CLD assays measure the gene expression of transcripts using a quantitative nuclease protection assays with the intention of these targets informing on transcription factor activity (i.e., AhR, CAR, PXR, FXR, and PPARα mediated transcription). General information is reported by Rotroff *et al.* (2010; 2010/1233112) and Sipes *et al.* (2013; 2013/1371960).

Cinmethylin was inactive in the three Tox21 assays mapped to CYP450 (TOX21_Aromatase_Inhibition (measuring CYP19A1 enzyme inhibition), TOX21_VDR_BLA_agonist_ratio (measuring CYP24A1 binding/activation), and TOX21_VDR_BLA_antagonist_ratio (measuring CYP24A1 antagonism)) and in the 3

CLD assays mapped to CYP1A1, CYP2C19, CYP2C9. Furthermore, cinmethylin was found to be inactive in the alkyl and aryl-, as well as the sulfo-transferase assays.

Cinmethylin was found to be active in six of the 21 CYP450-related assays in human hepatocytes, in 2 of the 9 transferase-related assays and in one esterase assay. The respective active assay endpoints as well as the enzyme transcript targeted, the AC50, the ACC (modeled concentration at response cutoff exceedance), and the measured concentration with maximal response (max-mean) are provided (Table 6.8-13). Activity was seen after 24h and 48h induction for cyp and transferase assays, while the corresponding 6h assay endpoints did not show activity.

Table 6.8-13. CYP450-related and Transferase-related assays in which cinmethylin was active

Assay Endpoint Name	Endpoint Targeted	AC50 (µM)	ACC (µM)	Max_mean (Fold induction or percent activity) (Concentration)
CLD_CYP1A2_24hr [6]	CYP1A2	14.435	7.07	2.96 (40µM)
CLD_CYP1A2_48hr [6]	CYP1A2	11.482	7.28	2.16 (40µM)
CLD_CYP2B6_24hr	CYP2B6	4.286	2.46	2.8 (40µM)
CLD_CYP2B6_48hr [6]	CYP2B6	4.208	3.99	2.47 (40µM)
CLD_CYP3A4_24hr [6]	CYP3A4	8.918	7.12	4.36 (40µM)
CLD_CYP3A4_48hr [6]	CYP3A4	11.691	2.95	4.04 (40µM)
CLD_UGT1A1_24hr [6, #]	UGT1A1	8.95	20.32	1.25 (40µM)
CLD_UGT1A1_48hr [6, #]	UGT1A1	4.59	5.6	1.25 (40µM)
NVS_ENZ_hES	BCHE	11.181	3.0	81.7% (50µM)

[#] Effect at AC50 is still below the efficacy cutoff; [6] only the highest conc. above baseline;

In conclusion, cinmethylin altered the gene expression of three CYP450 enzyme transcripts (CYP1A2, CYP2B6, and CYP3A4), each at the time points 24h and 48h. The UDP-Glucuronosyltransferase gene expression was rather marginal. These assays do not inform on the effect of cinmethylin on CYP450 enzyme activity and should not be overinterpreted beyond this context. In addition, the butyryl cholinesterase was activated.

Transporter-related Assays

Cinmethylin was evaluated in 17 assays mapped to the "transporter" intended_target_family. Four assays from NovaScreen (NVS) showed activity (Table 6.8-14). The CLD assays for ABC and organic anion transporter were inactive. The NVS assays are based on radioligand translocation from a transporter protein in a cell-free assay system measured 1 h after chemical dosing of up to 50 µM cinmethylin. The transporter proteins examined cell free were the Translocator protein TSPO, a transmembrane protein of the outer mitochondrial membrane that is involved in the cholesterol and coproporphyrinogen III transport, and the Solute Carrier Protein SLC6, an integral membrane protein responsible for translocation of small amino acids or amino acid-like substrates in the intestinal nutrient absorption, renal reabsorption, and synaptic transmission in the central nervous system.

Table 6.8-14. Transporter-related assays in which cinmethylin was active

Assay Endpoint Name	Endpoint Targeted	AC50 (µM)	ACC (µM)	Max_mean (% activity) (Concentration)
NVS_MP_hPBR[#, 17]	Cholesterol transporter (TSPO)	12.906	49.74	25.9 (50 µM)
NVS_TR_hDAT	Neurotransmitter transporter (SLC6A3)	12.379	9.88	52.5 (50µM)
NVS_TR_hSERT	Neurotransmitter transporter (SLC6A4)	7.019	2.73	84.6 (50µM)
NVS_TR_rSERT	Neurotransmitter transporter (Slc6a4)	9.663	3.03	88.1 (50µM)

[#] Effect at AC50 is still below the efficacy cutoff; [17] Biochemical assay with <50% efficacy;

Cinmethylin reduced the radioligand binding to the human transporters SLC6A3 and SLC6A4 at the top dose level of 50 µM by 52 – 8 %. The AC50 ranged at 7 - 12 µM. The correlating rat transporter protein Slc6a4 showed comparable interaction. The impact on the cholesterol transporter measured by NVS_MP_hPBR is considered to be unspecific as it was flagged for "biochemical assay with less than 50 % efficacy". The highest dose tested (50µM) just reached the response cutoff of 21.3 %, which means that the AC50 concentration was still below the efficacy cutoff for significant response.

In conclusion, cinmethylin can interact with specific carrier proteins at concentrations between 7 and 12 µM.

Nuclear-receptor Assays others than those related to endocrine pathways

Cinmethylin was evaluated to be active in 7 non-steroidal "nuclear receptors" (Table 6.8-15) beside the already discussed "specific" ATG_PXRE_Cis_up. The Attagene (ATG) assays measure induction of transcription factor activity by quantification of the level of mRNA reporter sequence unique to the transfected trans-acting reporter gene and exogenous transcription factor in human liver cells (HepG2) 24 hours after induction. The Odyssey Thera (OT) assays are based on protein-protein interaction using protein-fragment complementation technology in human kidney (HEK293T) based cell-assays.

Table 6.8-15. Nuclear Receptor assays in which cinmethylin was active

Assay Endpoint Name	Endpoint Targeted	AC50 (µM)	ACC (µM)	Max_mean (Fold induction or percent activity) (Concentration)
ATG_PPRE_CIS_up [6, 16, #]	PPARA, PPARD, PPARG	48.874	61.4	1.19 (100 µM)
ATG_VDRE_CIS_up	VDR	20.757	18.48	1.42 (100 µM)
ATG_LXRa_TRANS_up[#]	NR1H3	32.064	43.6	1.6 (100 µM)
ATG_PPARg_TRANS_up[#]	PPARG	33.316	39.68	1.78 (100 µM)
ATG_PXR_TRANS_up[#]	NR1I2	7.311	11	1.81 (30 µM)
OT_FXR_FXRSRC1_0480	NR1H4	70.021	33.72	90.2% (100 µM)
OT_NURR1_NURR1RXRa_0480 [6]	RXRA	88.478	88.17	29.1% (100 µM)

[#] Effect at AC50 is still below the efficacy cutoff; [6] only the highest conc. above baseline; [16] Hit-call potentially confounded by overfitting; [a] Plots are available in KCA 5.8.2/10 2018/1090847

The most sensitive nuclear receptor within this list was **ATG_PXR_Trans_up**, which belongs, as the specific **ATG_PXRE_Cis_up,** to the endogenous human nuclear receptor PXR / NR1I2 nuclear receptor subfamily 1 group I member 2, responsible for regulation of CYP 3A4. The induction remains below a value of 2-fold at 30 µM, and at the AC50 concentration of 7.3 µM, the effect is still below the efficacy cutoff, which is reached at 11 µM. Neither the max_mean value nor the ACC value indicate that induction of the PXR receptor is very prominent.

ATG_VDRE_CIS_up is the second most sensitive nuclear receptor. The assay is designed to make measurements of the mRNA induction unique to the cis-acting reporter gene response element VDRE, which is responsive to the endogenous human vitamin D receptor. At 100 µM the induction was 1.42-fold increased, and at the AC50 (20.757 µM) just calculated to be slightly above the efficacy cutoff of 0.734-fold induction.

All other listed nuclear receptors reached the efficacy cutoff not before the median cytotoxicity of cinmethylin (23.54 µM), and it is therefore reasonable to consider all these endpoints as unspecific high concentration endpoints, which are potentially confounded by cytotoxicity.

In conclusion, based on the low max_mean values or the high ACC concentrations, none of the non-steroidal nuclear receptor endpoints is considered to be a relevant and prominent actor *in vivo.*

DNA binding Assays

Cinmethylin was investigated in 84 assays related to transcription factors. Three were identified to be hits (Table 6.8-16), all belonging to the 52 assay components of the Attagene Cis assay (ATG_CIS assay). The ATG_CIS assay measures induction of transcription factor activity in human liver cells (HepG2) 24 hours after chemical

incubation. The quantification of the transcription factor activity is done via specific reporter DNA constructs and by measurements of specific reporter mRNA induction. The transcription factors formally identified to be induced by cinmethylin are the NRF2 (Nuclear factor (erythroid-derived 2)-like 2), the SREBP1 (Sterol Regulatory Element Binding Transcription Factor 1) and the UPS1 (Upstream transcription factor 1).

NRF2 (Nuclear factor (erythroid-derived 2)-like 2), also known as NFE2L2, regulates the expression of antioxidant proteins that contain the antioxidant response elements (ARE) in their promoters. The threshold of induction was reached at the ACC of 6.12 µM, however, the induction remains up to 100 µm at a very low level of 1.21-fold induction. SREBP1 is the Sterol Regulatory Element Binding Transcription Factor 1 which is involved in the sterol biosynthesis. The induction remained low up to the highest tested concentration of 100 µM. The ATG_E_BOX_CIS is most likely a false positive hit as only the last measured concentration was above the efficacy level.

Table 6.8-16. DNA-binding assays in which cinmethylin was active

Assay Endpoint Name	Endpoint Targeted	AC50 (µM)	ACC (µM)	Max_mean (Fold induction) (Concentration)
ATG_NRF2_ARE_CIS_up[#]	NFE2L2	3.67	6.12	1.21 (100 µM)
ATG_SREBP_CIS_up	SREBF1	16.642	11.8	0.7 (100 µM)
ATG_E_BOX_CIS_up [6, 11, 16]	USF1	31.927	n.a.	0.5 (100 µM)

[#] Effect at AC50 is still below the efficacy cutoff; [6] Only highest conc above baseline; [11] borderline active; [16] Hit-call potentially confounded by overfitting, n.a. not applicable;

In conclusion, based on the low max_mean values, ranging between 0.5 and 1.2 fold induction, none of the transcription factor endpoints is considered to be a relevant and prominent actor *in vivo.*

Further endpoints (excluding the endocrine related) found to be active in Tox Cast

The below listed endpoints were also found to be active, however, based on the dose response curve these effects are considered unspecific effects, likely to be confounded by overfitting or cytotoxicity and not relevant for risk assessment consideration.

Table 6.8-17. Further assays in which cinmethylin was active

Assay Endpoint Name	intended target family	AC50 (µM)	ACC (µM)	Maximal Fold induction or Percent Activity (Concentration)
APR_HEPG2_CellCycleArrest_24h_up [#, 11]	Cell cycle	79.845	171.1	0.76 (200 µM)
ATG_CMV_CIS_up [#, 6, 11, 16]	Background measurement	30.633	144.8	0.71 (100 µM)
NHEERL_ZF_144hpf_TERATOSCORE_up	Null	88.272	49.9	74.8 % (80 µM)

[#] Effect at AC50 is still below the efficacy cutoff; [6] Only highest conc above baseline; [11] borderline active; [16] Hit-call potentially confounded by overfitting;

Overall conclusion

Cinmethylin was tested in 689 assay endpoints and generated 43 hit calls in these assay endpoints (12 are discussed under the evaluation of endocrine disrupting properties). The cytotoxicity region, relevant to identify assays potentially confounded by cytotoxicity, is centered around the median cytotoxicity AC50 of **23.54 µM**, with the lower limit of the cytotoxicity region being 3.63 µM. Up to **23.54 µM**, only a few assays were active. The most sensitive endpoint has been shown to be the induction of the gene response element PXRE. PXRE induction reached half-maximal response (AC50) at 2.631 µM and maximal response of near 4-fold induction at 30 µM. Furthermore, ToxCast identified induction of gene expression of three human CYP450 enzyme transcripts, activity towards butyryl cholinesterase and towards the solute carrier protein SLC6 as endpoints reactive up to the median cytotoxicity level. All other endpoints were not sensitive up to the median cytotoxicity region. Overall, cinmethylin can be considered as a rather inactive substance. All ToxCast data are of limited value to the risk assessment and are considered to be supplemental information.

Overall, the information presented (the two relatively old liver mechanistic studies conducted in the 1980s and the ToxCast data) is not sufficient to either propose or establish a MoA for the liver effects seen in all three

species investigated (rat, mouse and dog). The only conclusion that can be drawn is that some liver CYP activities are marginally increased in rats and mice, possibly as a consequence of PXR activation.

B.6.8.3. Studies on endocrine disruption

This section includes an assessment of the adverse effects potentially related to an endocrine mode of action as observed in the regulatory toxicological studies conducted with cinmethylin and an assessment of the available *in vitro* endocrine activity assays, as reported under ToxCast. The assessment of endocrine disruption of cinmethylin is based on the criteria for endocrine disruption (Commission Regulation (EU) 2018/605 of 19 April 2018) and the definition of an endocrine disruptor is based on the WHO/IPCS (2002). The criteria as listed in Annex II point 3.6.5 are as follows:

'From 20 October 2018, an active substance, safener or synergist shall be considered as having endocrine disrupting properties that may cause adverse effect in humans if, based on points (1) to (4) of the sixth paragraph, it is a substance that meets all of the following criteria, unless there is evidence demonstrating that the adverse effects identified are not relevant to humans:

1) *it shows an adverse effect in an intact organism or its progeny, which is a change in the morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress or an increase in susceptibility to other influences;*
2) *it has an endocrine mode of action, i.e. it alters the function(s) of the endocrine system;*
3) *the adverse effect is a consequence of the endocrine mode of action.'*

For evaluation of the first criterion, it has to be determined whether adverse effects potentially related to an endocrine mode of action are observed. In this section the evaluation of effects on reproductive and endocrine related organs from all valid subchronic, chronic and reproductive toxicity studies are compiled and the adversity and specificity of the observed effects is assessed. For evaluation of the second criterion, the available *in vitro* endocrine activity assays are evaluated. To fulfil the third part of the criteria, an assessment is followed, whether the determined adverse endpoints are a consequence of an endocrine-mediated mechanism.

The assessment follows the ECHA/EFSA guidance for the identification of endocrine disruptors in the context of Regulations (EU) 528/2012 and (EC) No 1107/2009. The guidance proposes a workflow for assessing the endocrine disrupting properties of pesticides and biocides, which starts by collecting all available data in the format of an Excel File (Appendix E).

Gather all relevant information

The following regulatory apical studies (Table 6.8-18) conducted with cinmethylin, as well as the data published under ToxCast (https://comptox.epa.gov/dashboard/dsstoxdb/results?search=DTXSID3034456) are assessed in detail in Appendix E (2019/2034520). Relatively old apical studies considered unacceptable by HSE due to severe limitations and shortcomings (as described in the relevant sections of the B6 document) have not been included in this assessment.

Table 6.8-18. Outline of the dataset considered in the mammalian toxicology ED assessment - studies conducted with cinmethylin (summarised in Appendix E).

Type of toxicity	Study type	Species	OECD Test Guideline No.	Reference
Repeated dose toxicity studies in mammals	28-day oral toxicity study	Rat	407 (2008)	[redacted] 2015 (2015/1076329) Study ID Matrix: 10
	28-day oral toxicity study	Mouse	407 (2008)	[redacted] 2016 (2014/1162710) Study ID Matrix: 20
	90-day oral toxicity study	Rat	408 (1998)	[redacted] 2018a (2014/1228370) Study ID Matrix: 30
	90-day oral toxicity study	Mouse	408 (1998)	[redacted] 2018b (2015/1005983) Study ID Matrix: 40
	13-week oral toxicity study	Dog	EPA-guideline	[redacted], 1987

				(CI-425-003) Study ID Matrix: 50
	1-year oral toxicity study	Dog	EPA-guideline	Cagen, 1985 (CI-427-002) Study ID Matrix: 51
	1-year oral toxicity study	Dog	EPA-guideline	Larson, 1988a (CI-427-003) Study ID Matrix: 52
	1-year oral toxicity study (with 6 month reversibility)	Dog	EPA-guideline	Larson, 1988b (CI-427-003)
	28-day dermal toxicity study	Rat	410 (1981)	Flick *et al.,* 2018c (2017/1094162 and 2018/1091459) Study ID Matrix: 60
	Combined chronic toxicity/carcinogenicity studies	Rat	OECD 453 (2009)	Buesen *et al*., 2018 (2017/1093414) Study ID Matrix: 70a (chronic) and 70b (cancer)
	Carcinogenicity studies	Mice	OECD 451 (2009)	Flick *et al*., 2018d (2017/1094161) Study ID Matrix: 80
	Two-generation reproductive toxicity	Rat	OECD 416 (2001) including landmarks of sexual maturation	Schneider *et al.,* 2018 a (2017/1094504) and Schneider, 2018 (2018/1099151) Study ID Matrix: 90a (F0/F1 adults) and 90b (F1/F2 pups)
	Prenatal developmental toxicity study	Rat	Not recorded in study report.	Lochry *et al.,* 1984 (CI-432-001) Study ID Matrix: 100a (dams) and 100b (fetus)
	Prenatal developmental toxicity study	Rabbit	414 (2001)	Schneider *et al.,* 2018b (2015/1158053) Study ID Matrix: 110a (dams) and 110b (fetus)
In vitro mechanistic	Enzyme induction study (7 weeks feeding)	Rat	No guideline	CI-440-001 Study ID Matrix: 120
	Enzyme induction study (7 weeks feeding)	Mice	No guideline	CI-440-002 Study ID Matrix: 130
	ToxCast ER prediction model	*in vitro*	No guideline	Source: US_EPA Study ID Matrix: 140
	ToxCast AR prediction model	*in vitro*	No guideline	Source: US_EPA Study ID Matrix: 150
	ToxCast Aromatase inhibition	*in vitro*	No guideline	Source: US_EPA Study ID Matrix: 160
	ToxCast Steroidogenesis	*in vitro*	No guideline	Source: US_EPA Study ID Matrix: 170-181
	ToxCast Thyroid Assays	*in vitro*	No guideline	Source: US_EPA Study ID Matrix: 190-195, 200, 201

The mammalian *in vivo* parameters (listed in Table 14 of the ECHA/EFSA Guidance) considered '*in vivo* mechanistic' (highlighted in orange), 'EATS[7]-mediated' (highlighted in blue) or 'sensitive-to-but-not-diagnostic-of EATS' (highlighted in purple) were compared to the parameters investigated in each of the above *in vivo*

[7] EATS - Estrogen, Androgen, Thyroid, Steroidogenic.

guideline studies (see Appendix E, 'Summary Mammals' tab). The vast majority of parameters were assessed in the toxicological studies performed with cinmethylin; exceptions are discussed below.

The data assembled in Appendix E (2019/2034520) were assessed following '*Table 2: Example showing how to assemble, integrate and assess the lines of evidence for thyroid disruption in mammals*' of the ECHA/EFSA guidance as an example.

The assessment is divided into i) a review of the EAS (Estrogen, Androgen and Steroidogenic) modalities and ii) a review or the T (Thyroid) modality. Discussion mainly follows the order provided by the lines-of-evidence tables i.e. 'EAS-mediated' parameters followed by the 'sensitive to but not diagnostic of EATS' parameters. Finally, the '*in vitro* mechanistic' data are discussed. All parameters with changes were discussed in the Data tab of Appendix E (2019/2034520) to differentiate those considered primary effects from those considered secondary to other toxic effects or not considered to be treatment-related as these changes were not dose related or within the normal physiological range. In the corresponding lines of evidence, a subset of effects were further discussed.

EAS-modalities

Adversity
(see below)

Assessment of the integrated lines of evidence and weight of evidence for EAS-mediated adversity and endocrine activity

Lines of evidence for **adverse effects** and **endocrine activity** related to EAS-modality:

Table 6.8-19. Lines of evidence for EAS-modality (*in vivo* studies)

Study ID	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect[a]	Assessment of each line of evidence	Assessment on the integrated line of evidence
10	Epididymis histopathology	Rat	4	Weeks	Oral		mg/kg bw/day	No effect		No Effect on Epipidymis (histo-pathology). Size reduction secondary to moribundity	
20	Epididymis histopathology	Mice	4	Weeks	Oral		mg/kg bw/day	No effect			
30	Epididymis histopathology	Rat	13	Weeks	Oral		mg/kg bw/day	No effect			
40	Epididymis histopathology	Mice	13	Weeks	Oral		mg/kg bw/day	No effect			
50	Epididymis histopathology	Dog	13	Weeks	Oral		mg/kg bw/day	No effect			
51	Epididymis histopathology	Dog	52	Weeks	Oral		mg/kg bw/day	No effect			
52	Epididymis histopathology	Dog	52	Weeks	Oral		mg/kg bw/day	No effect			
70a	Epididymis histopathology	Rat	52	Weeks	Oral		mg/kg bw/day	No effect			

70b	Epididymis histopathology	Rat	104	Weeks	Oral		mg/kg bw/day	Change	Gross pathology: Size reduction Increased Incidence in high dose: Incidence in Control, low, mid, high dose: 0-0-1-4 Combined with aspermia/oligospermia ==> consequence of bad general status of these animals and not a direct effect of the testsubstance		
80	Epididymis histopathology	Mice	78	Weeks	Oral		mg/kg bw/day	No effect			
90a	Epididymis histopathology	Rat	10 (♂ and ♀ premating) +3 (♀ gestation) +3 (♀ lactation)	weeks	Oral		ppm	No effect			
10	Epididymis weight	Rat	4	Weeks	Oral	1522	mg/kg bw/day	Increase	rel. (% change to Ctrl) at highest dose: +16% considered secondary to body weight reduction (-10.6%).	Epididymis weight changes sec.to body weight	
20	Epididymis weight	Mice	4	Weeks	Oral		mg/kg bw/day	No effect			
30	Epididymis weight	Rat	13	Weeks	Oral	792	mg/kg bw/day	Increase	rel. (% change to Ctrl) at highest dose: +29% considered secondary to body weight reduction (-16%).		

40	Epididymis weight	Mice	13	Weeks	Oral		mg/kg bw/day	No effect			
70a	Epididymis weight	Rat	52	Weeks	Oral		mg/kg bw/day	No effect			
70b	Epididymis weight	Rat	104	Weeks	Oral	242	mg/kg bw/day	Increase	abs. & rel. (% change to Ctrl) at highest dose: (+8%) and (+18%) considered secondary to body weight reduction (-9%).		
80	Epididymis weight	Mice	78	Weeks	Oral	25	mg/kg bw/day	Change	rel. at low and mid dose and abs. & rel. (% change to Ctrl) at high dose: 10.4% , 6.6%, and -6.5% / 14.3%; considered secondary to body weight reduction in low,mid, high dose: -7.3%, -4.8%, -19.1%).		
90a	Epididymis weight	Rat	10 (♂ and ♀ premating) +3 (♀ gestation) +3 (♀ lactation)	weeks	Oral		ppm	No effect			
10	Ovary histopathology	Rat	4	Weeks	Oral		mg/kg bw/day	No effect		No adverse effect on ovary histo-pathology	
20	Ovary histopathology	Mice	4	Weeks	Oral		mg/kg bw/day	No effect			

30	Ovary histopathology	Rat	13	Weeks	Oral	240	mg/kg bw/day	Increase	Vacuolation, intersti. glands, Incidence: 0, 0, 7, 9; Mean severity grading: 0, 0, 1.9, 2.9; dose-related increase of incidence and severity ==> not seen in any other rat study despite higher dosing: 28d, 2-gen, Cancer ==> this finding is in contradiction to the decreased ovary weight (w. HCD). Without further reproductive tract findings vacuoltion is regarded as treatment related but not adverse.		
40	Ovary histopathology	Mice	13	Weeks	Oral		mg/kg bw/day	No effect			
50	Ovary histopathology	Dog	13	Weeks	Oral		mg/kg bw/day	No effect			
51	Ovary histopathology	Dog	52	Weeks	Oral		mg/kg bw/day	No effect			
52	Ovary histopathology	Dog	52	Weeks	Oral		mg/kg bw/day	No effect			
70a	Ovary	Rat	52	Weeks	Oral		mg/kg	No			

	histopathology						bw/day	effect			
70b	Ovary histopathology	Rat	104	Weeks	Oral		mg/kg bw/day	No effect			
70b	Ovary histopathology	Rat	104	Weeks	Oral		mg/kg bw/day	No effect			
80	Ovary histopathology	Mice	78	Weeks	Oral		mg/kg bw/day	No effect			
90a	Ovary histopathology	Rat	10 (♂ and ♀ premating) +3 (♀ gestation) +3 (♀ lactation)	weeks	Oral		ppm	No effect			
10	Ovary weight	Rat	4	Weeks	Oral		mg/kg bw/day	No effect		No ED related effect on ovary weight	
20	Ovary weight	Mice	4	Weeks	Oral		mg/kg bw/day	No effect			
30	Ovary weight	Rat	13	Weeks	Oral		mg/kg bw/day	No effect			
40	Ovary weight	Mice	13	Weeks	Oral		mg/kg bw/day	No effect			
50	Ovary weight	Dog	13	Weeks	Oral		mg/kg bw/day	No effect			
51	Ovary weight	Dog	52	Weeks	Oral		mg/kg bw/day	No effect			
52	Ovary weight	Dog	52	Weeks	Oral		mg/kg bw/day	No effect			
70a	Ovary weight	Rat	52	Weeks	Oral		mg/kg bw/day	No effect			
70b	Ovary weight	Rat	104	Weeks	Oral		mg/kg bw/day	No effect			
80	Ovary weight	Mice	78	Weeks	Oral	939	mg/kg bw/day	Increase	abs. & rel. (% change to Ctrl) at highest dose: +33.8% and +74.6%		

									Abs. weight - ↑ due to a cyst / isolated finding; Rel. weight - ↑ related to reduced body weight (-23%)		
90a	Ovary weight	Rat	10 (♂ and ♀ premating) +3 (♀ gestation) +3 (♀ lactation)	weeks	Oral		ppm	No effect			
10	Prostate histopathology (with seminal vesicles and coagulating glands)	Rat	4	Weeks	Oral		mg/kg bw/day	No effect		No effect on prostate histo-pathology in rat and mice. Size reduction secondary to moribundity	
30	Prostate histopathology (with seminal vesicles and coagulating glands)	Rat	13	Weeks	Oral		mg/kg bw/day	No effect			
70a	Prostate histopathology (with seminal vesicles and coagulating glands)	Rat	52	Weeks	Oral		mg/kg bw/day	No effect			
70b	Prostate histopathology (with seminal vesicles and coagulating glands)	Rat	104	Weeks	Oral		mg/kg bw/day	Change	Gross pathology: Size reduction Increased Incidence in high dose: Incidence in Control, low, mid, high dose: 0-0-1-6		

									==> consequence of bad general status of these animals and not a direct effect of the testsubstance		
90a	Prostate histopathology (with seminal vesicles and coagulating glands)	Rat	10 (♂ and ♀ premating) +3 (♀ gestation) +3 (♀ lactation)	weeks	Oral		ppm	No effect			
20	Prostate histopathology (with seminal vesicles and coagulating glands)	Mice	4	Weeks	Oral		mg/kg bw/day	No effect			
40	Prostate histopathology (with seminal vesicles and coagulating glands)	Mice	13	Weeks	Oral		mg/kg bw/day	No effect			
80	Prostate histopathology (with seminal vesicles and coagulating glands)	Mice	78	Weeks	Oral		mg/kg bw/day	No effect			

50	Prostate histopathology (with seminal vesicles and coagulating glands)	Dog	13	Weeks	Oral		mg/kg bw/day	No effect	Slightly reduced prostate glandular development: Incidence: 0, 0, 0, 0, 1, 2; Mean severity grading: 2.3, 2.5, 2.8, 3.0, 2.0, 1.8; No corresponding effect on testes or spermatogenesis; Considered body weight / Age related; no unusual findings for juvenile beagles - not adverse	Slightly reduced prostate glandular development in Beagle dogs mainly related to body weight and strain - not adverse	
51	Prostate histopathology (with seminal vesicles and coagulating glands)	Dog	52	Weeks	Oral	83.4	mg/kg bw/day	Decrease	Slightly reduced prostate glandular development: Incidence: 0, 1,4, 5; Mean grading 4.0, 3.8, 3.3, 2.6; No impairment of spermatogenesis, Body weight related at high dose (-79%); Not observed in Beagles from other supplier;		
52	Prostate histopathology (with seminal vesicles and coagulating glands)	Dog	52	Weeks	Oral		mg/kg bw/day	No effect			

10	Seminal vesicles histopathology	Rat	4	Weeks	Oral		mg/kg bw/day	No effect		No Effects on seminal vesicles (histo-pathology and weight). Size reduction secondary to moribundity	
20	Seminal vesicles histopathology	Mice	4	Weeks	Oral		mg/kg bw/day	No effect			
30	Seminal vesicles histopathology	Rat	13	Weeks	Oral		mg/kg bw/day	No effect			
40	Seminal vesicles histopathology	Mice	13	Weeks	Oral		mg/kg bw/day	No effect			
70a	Seminal vesicles histopathology	Rat	52	Weeks	Oral		mg/kg bw/day	No effect			
70b	Seminal vesicles histopathology	Rat	104	Weeks	Oral		mg/kg bw/day	Change	Gross pathology: Size reduction Increased Incidence in high dose: Incidence in Control, low, mid, high dose: 0-0-1-7 ==> consequence of bad general status of these animals and not a direct effect of the testsubstance		
80	Seminal vesicles histopathology	Mice	78	Weeks	Oral		mg/kg bw/day	No effect			

90a	Seminal vesicles histopathology	Rat	10 (♂ and ♀ premating) +3 (♀ gestation) +3 (♀ lactation)	weeks	Oral		ppm	No effect			
10	Seminal vesicles weight	Rat	4	Weeks	Oral		mg/kg bw/day	No effect			
20	Seminal vesicles weight	Mice	4	Weeks	Oral		mg/kg bw/day	No effect			
90a	Seminal vesicles weight	Rat	10 (♂ and ♀ premating) +3 (♀ gestation) +3 (♀ lactation)	weeks	Oral		ppm	No effect			
10	Testis weight	Rat	4	Weeks	Oral	1522	mg/kg bw/day	Increase	rel. (% change to Ctrl) at highest dose: +18% considered secondary to body weight reduction (-20.9%)-no histopath. correlate	Testis weight change related to body weight	
20	Testis weight	Mice	4	Weeks	Oral		mg/kg bw/day	No effect			
30	Testis weight	Rat	13	Weeks	Oral	211	mg/kg bw/day	Increase	rel. (% change to Ctrl) at mid and high dose: +12%, +29% considered secondary to body weight reduction (-4.9%, -16%) -no histopath. Correlate		
40	Testis weight	Mice	13	Weeks	Oral		mg/kg bw/day	No effect			
50	Testis weight	Dog	13	Weeks	Oral		mg/kg bw/day	No effect			
51	Testis weight	Dog	52	Weeks	Oral		mg/kg bw/day	No effect			

52	Testis weight	Dog	52	Weeks	Oral		mg/kg bw/day	No effect			
70a	Testis weight	Rat	52	Weeks	Oral		mg/kg bw/day	No effect			
70b	Testis weight	Rat	104	Weeks	Oral	242	mg/kg bw/day	Increase	rel. (% change to Ctrl) at high dose: +11%, considered secondary to body weight reduction (-9%) - no histopath. Correlate		
80	Testis weight	Mice	78	Weeks	Oral	25	mg/kg bw/day	Increase	rel. (% change to Ctrl) at low and high dose: +7.7%, +18.9%, considered secondary to body weight reduction (-7.3%, -19.1%) - no histopath. correlate		
90a	Testis weight	Rat	10 (♂ and ♀ premating) +3 (♀ gestation) +3 (♀ lactation)	weeks	Oral		ppm	No effect			
10	Uterus weight (with cervix)	Rat	4	Weeks	Oral		mg/kg bw/day	No effect		Uterus weight changes related to body weight	
20	Uterus weight (with cervix)	Mice	4	Weeks	Oral		mg/kg bw/day	No effect			
30	Uterus weight (with cervix)	Rat	13	Weeks	Oral		mg/kg bw/day	No effect			
40	Uterus weight (with cervix)	Mice	13	Weeks	Oral		mg/kg bw/day	No effect			
70a	Uterus weight (with cervix)	Rat	52	Weeks	Oral		mg/kg bw/day	No effect			
70b	Uterus	Rat	104	Weeks	Oral		mg/kg	No			

	weight (with cervix)						bw/day	effect			
80	Uterus weight (with cervix)	Mice	78	Weeks	Oral	939	mg/kg bw/day	Increase	rel. (% change to Ctrl) at highest dose: +23.2% ==> secondary to terminal body weight (-22.2%)		
90a	Uterus weight (with cervix)	Rat	10 (♂ and ♀ premating) +3 (♀ gestation) +3 (♀ lactation)	weeks	Oral		ppm	No effect			

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

a: For brevity and clarity's sake, only effect targets which showed one or more positive observed effects (rather than no effect in all studies) are listed in this table. This allows targeted discussion of EAS-mediated effects. The full list of positive and negative observed effects is available in Appendix E.

EAS-mediated effects

Sexual maturation: There were no treatment-related effects on the age at balanopreputial separation, age at vaginal opening, ano-genital distance and/or areola/nipple development in the 2-generation study (2018a; , 2018).

Cervix and coagulating gland: There were no treatment-related effects on cervix histopathology (cervix and coagulating gland), evaluated in the 2-generation study (., 2018a; 2018), 28-, 90-day and chronic rodent studies (2015; ., 2016; 2018a; 2018b; , 2018; 2018d), as well as in 1-year dog studies (, 1985; , 1988a; , 1988b) (histopathological evaluation of the cervix but not the coagulating gland). No histopathological changes were observed and no coagulating gland weight changes were detected.

Epididymis histopathology and weight: No histopathological changes in the epididymides were observed in any studies and/or species and application routes. In the 2-year study in rats (2018), in males of the top dose group there was a higher incidence of size reduction in the epididymides, prostate and seminal vesicle. All affected animals died on test or were sacrificed in a moribund state (mortality was 24 % in top dose males compared to 12 % in controls). Therefore, this finding was regarded to have been caused by the poor general status of these animals and not as a direct effect of cinmethylin. In general, regulatory toxicity studies with an almost life-time exposure (e.g. 24-month carcinogenicity or chronic study), are not relevant for assessment of reproductive findings (e.g. oligospermia/aspermia) except of pre-/neoplastic lesions. A high number of aged rats show degenerative lesions, e.g. tubular degeneration or tubular mineralisation/calcification, inflammation to varying degrees as a common spontaneous background lesion by 2-years of age (Boorman's Pathology of the Rat, Chap 28: Testis and Epididymis, 2nd editon, Academic Press, 2018). Often oligospermia and/or azoospermia is seen as direct secondary effect, due to decreased sperm production. Therefore, in long-term studies, effects in the epididymides (e.g. oligospermia) should not be assessed in isolation, but considered together with findings in the testes. The incidence of findings in the testes which might lead to decreased sperm production (tubular degeneration) was comparable between control and high dose test group (13 vs 10 in control), therefore the slightly increased incidence of oligospermia/aspermia in epididymides was not considered to be indicative of a treatment-related effect.

Statistically-significant increases in relative epididymides weights were noted in the 28-day, 90-day rat study (., 2015; 2018a) and of absolute and relative organ weights in the long-term rodent studies (2018 ; 2018d). These changes were secondary to altered terminal body weights. For example, relative epididymides weights in the high dose rats of the 28-day study (2015) were increased by 16 % (statistically-significant), secondary to 13 % decrease of terminal body weights, while absolute weights were comparable. In the combined chronic toxicity/carcinogenicity study in rats (2018) at the end of the 24-month exposure period, relative epididymides weights in the high dose rats were increased by 18 % (statistically-significant) and absolute weights were increased by 8 % (statistically-significant) secondary to 9 % (statistically-significant) decrease of terminal body weights. In the 2-generation study (., 2018a; , 2018), effects on epididymides weight was not considered treatment-related, as values were within the HCD and showed no dose-response. Overall, the parameter was measured 8 times; 4 measuments showed no treatment-relation and 4 showed weight effects secondary to terminal body weight reduction. Cauda epididymis weights, measured in F_0 and F_1 males in the 2-generation study, were not changed.

Overall, the significance of the epididymal sperm changes in aged sexually senescent rats is highly questionable, especially since in the 2-generation study (2018a; , 2018) no effects on fertility or sperm parameters were observed.

Oestrus cyclicity: as investigated in the 28-day rodent (2015; 2016) and the 2-generation study (., 2018a; , 2018), was not affected by cinmethylin.

Genital abnormalities: In the 2-generation study (., 2018a; , 2018) and the rat and rabbit developmental toxicity studies (., 1984; ., 2018b) no (potentially) EA(T)S mediated genital abnormalities/presence of anomalies were noted.

Mammary gland effect: Histopathology of female and male mammary gland was unremarkable in all studies in which these parameters were investigated (female gland - in 90-day and long-term studies in the rat, mice and dog, and male mammary gland - in 90-day dog study and in isolated animals in the rat carcinogenicity study).

Ovary histopathology and weight: Various ovary changes were noted in rats and mice but not in dogs.

A change in ovary histopathology was noted in the 90-day rat study ([redacted]., 2018a). In mid and high dose females increased incidence and severity of interstitial glands vacuolation was observed. This finding was not seen in the 28-day study [redacted] 2015) and was not reproduced in the chronic study ([redacted] 2018). Therefore, it is considered to be either a chance finding or of limited toxicological significance.

Reduced in ovary weights recorded in the combined chronic toxicity/carcinogenicity study in rats (which were within the HCD range) were not considered treatment-related due to much higher mean ovary weights in control animals (caused by either tumours or cysts in some control animals), far beyond the HCD values. There was a higher incidence of cystic/papillary hyperplasia observed in the high dose terminal animals (34 compared to 24 in controls). This is a common lesion in some rat strains, smaller than a normal corpus luteum and does not show signs of malignancy ([redacted] 2014). As in this study there were no increased numbers of ovarian tumors this finding was regarded to be incidental and not treatment-related. In the 90-day rat study ([redacted]., 2018a), statistically-significant changes in the ovary weights were not considered to be treatment-related. This was due to a lack of a a dose-response relationship and/or values falling within the range of the HCD.

In the mouse studies, ovary weight changes were seen in all studies (28-day, 90-day and chronic study) without any consistent pattern. In the 28-day study ([redacted]., 2016), absolute ovary weights were increased in the high dose (11 % with statistical-significance) and relative ovary weights were reduced at mid dose (10 % with statistical-significance). This is supported by the 90 day study ([redacted] 2018b), in which absolute ovary weights were decrease only in the mid dose (13 %, with statistical-significance). Due to lack of dose-response and with no histopathological correlate, these ovary weight change were not considered treatment-related.

In the long term study in mice ([redacted] 2018d), a statistically-significant increased in relative ovary weight was observed at the top dose; change compared to controls was 75 % for relative weight and 34 % for absolute weight. However, this was based on an extremely high individual ovarian weight (473 mg) in one female (# 372, due to a cyst) and was not regarded as treatment-related due to its isolated occurrence. There was no treatment-related increase in the number of ovary cysts. No effect on ovaries was seen in the 28-day dermal rat study [redacted]., 2018c). Overall, there is no indication of a primary adverse effect on the ovaries in a total of 11 subacute to chronic studies in rats, mice and dogs.

Histopathology of oviduct was unremarkable in all (8) studies in which this parameters was examined (90-day, 1-year, long-term and 2-generation studies).

Prostate histopathology and weight: No effects on prostate weights were recorded in the 28-day and 90-day rat studies ([redacted]., 2015; [redacted] 2018a). In the combined chronic toxicity/carcinogenicity study in Wistar rats ([redacted] 2018), 1 mid dose and 6 high dose males revealed a size reduction of the prostate, frequently combined with reduced size of epididymides and seminal vesicles. However, all affected animals died on test or were sacrificed in a moribund state (mortality was 24 % in top dose males compared to 12 % in controls). Therefore, this finding was regarded to have been caused by the poor general status of these animals and not as a direct effect of cinmethylin.

In the multigeneration study ([redacted] 2018a; [redacted], 2018), reductions in absolute and relative prostate weights were recorded in the F_0 generation in the low dose (by 11 % and 10 %, respectively, both with statistical-significance), and in the high dose (by 11 % and 9 %, respectively, both with statistical-significance). However, as it showed no dose dependency or histopathological correlate and was within the HCD range, the effect was not considered to be treatment-related.

No effect on prostate weights was seen in mice (in the 28-days, 90-days and/or 18-month studies).

Dog studies were performed in Beagles from two different suppliers; one 1-year study was conducted with [redacted], while the 90-day, second and third 1-year study was performed in Beagles from [redacted]. [redacted] are reported in the peer-reviewed literature to reach maturity rather late, this had an impact on the interpretation of the prostate findings. In the 90-day study ([redacted], 1987), prostatic glandular development (severity) was slightly reduced in males of the 3,000 and 6,000 ppm dose groups. The decrease of the prostatic glandular development in the 200 ppm dog that died intercurrently in week 7, at an age of 6 - 7 months, is clearly age-related rather than treatment-related as the animal was sacrificed at a younger age. An effect related to body weight development is likely for the male of the 3000 ppm dose group and one of the top dose males; both showed low body weight and high food consumption, typical of young animals. The other top dose male revealed a starting body weight that was comparable with the group means. In

this dog, however, reduced prostatic glandular development was supported by the reduced organ size. Therefore, taking into account variations in the age of reaching maturity and the individual body weights of the animals showing the finding, the delay in glandular development of the prostate at 3,000 and 6,000 ppm appears to be the consequence of impaired body weight development and not a direct effect of treatment. There was no functional impairment seen in any dog with regard to spermatogenesis, therefore, the changes in this study are not considered to be adverse. The prostate glandular development after 1-year treatment of [redacted] at doses of 300, 3000 and 10000 ppm was slightly reduced from the mid dose. The finding for the high dose animals and for the one low dose animals are related to a reduced or generally low body weight. As in the 90-day study, this finding was considered treatment-related and adverse but secondary to the impaired body weight development seen at these doses. For the mid dose animals no corresponding testicular atrophy or reduced spermatogenesis was seen that would indicate a hormone-related effect. There was no functional impairment seen in any dog with regard to spermatogenesis. This is supported by the second and third 1-year dog studies with beagles from [redacted] at dose levels of 0, 2, 30, 100, 200, and 3000 ppm. Prostate glands were small for 1 dog of the control, 1 of the 200 ppm group and 2 dogs of the 3000 ppm group in the second study. However, this finding had no correlating lesions at microscopic examination. Prostates from all males were examined by six veterinary pathologists, and prostates from treated and control dogs were considered to be histologically similar and normal for Beagle dogs of this age.

Overall, prostate effects seen in the dog are considered mainly secondary to reduced body weight and most likely strain specific. Without any functional impairment of spermatogenesis, the finding is not regarded to be adverse. Therefore, there is no indication of a primary adverse effect on the prostate in a total of 12 studies in rats, mice and dogs.

Seminal vesicle histopathology and weight: Seminal vesicle weight was investigated in the 28-day studies in rats and mice ([redacted] 2015; [redacted]., 2016), as well as in the 2-generation study ([redacted] 2018a; [redacted] 2018) in F_0 and F_1 parental males. In the multigeneration study, reductions in seminal vesicle (with coagulating gland) weights (absolute and relative) were seen in F_0 males. However, as it showed no dose-dependency or histopathological correlate, was within the HCD range and was not repeated in F_1 males the effect was not considered to be treatment-related. Seminal vesicles histopathology was unremarkable in the 28-day and 90-day rat studies [redacted] 2015; [redacted] 2018a). In the combined chronic toxicity/carcinogenicity study in Wistar rats ([redacted] 2018), 1/50 mid dose and 7/50 high dose males revealed a size reduction (macroscopically) of the seminal vesicles, frequently combined with reduced size of prostate and epididymides. However, all affected animals died on test or were sacrificed in a moribund state (mortality was 24 % in top dose males compared to 12 % in controls). Therefore, this finding was regarded to have been caused by the poor general status of these animals and not as a direct effect of cinmethylin. No effect on seminal vesicles histopathology was seen in mice (in the 28-days, 90-days and/or 18-month studies). Seminal vesicles were not weighed or investigated histopathologically in the dog studies. Overall, there was no indication of a primary adverse effect on the seminal vesicles in a total of 7 subacute to chronic studies in rats and mice. No information is available for the dog, but this is not considered a critical deficiency, in view of the lack of impairment in the rodents.

The investigation of the number of testicular and caput epididymis homogenisation resistant sperm heads as well as epididymal sperm motility and morphology of F_0 and F_1 parental males did not reveal any findings.

Testes histopathology and weight: Testes were investigated for weight and histopathological changes in all 28-day, 90-day and chronic studies in rats, mice and dogs, as well as in the 2-generation study in both parental generations. In the 28-day study in rats ([redacted] 2015), relative testes weights of high dose revealed an 18 % (statistically-significant) weight increase. However, this was regarded as secondary to a decrease of body weight (13 %). Similarly, the 90-day study in rats ([redacted] 2018a) revealed increased relative testes weights in the mid and high dose group (12 % and 29 % at 3,000 ppm and 10,000 pp,, respectively). However, as there were no histopathological changes that could explain the weight increases, this effect was not considered to be treatment-related. In the chronic study in rats ([redacted] 2018), in the satellite group (12-months), testes weights and body weight were not affected. At 24 months, relative testes weights were increased, however, as no concomitant histopathology was found, this increase was most likely the consequence of the decreased body weights and body weight gains observed. In the 2-generation study in rats ([redacted] 2018a; [redacted], 2018), testes were not affected up to the highest dose tested (5000 ppm). In the 28- and 90-day studies in mice ([redacted] 2016; [redacted] 2018b), testes were not affected. In the carcinogenicity study in mice, relative weight of testes was increased (by 19 %). However, due to a lack of dose-response and/or concomitant histopathological findings were considered to be a secondary effect of the reduced body weight in mice (19 %). Testis atrophy was seen at the top dose of 10,000 ppm in the first 1-year study in dogs ([redacted], 1985). This effect was however, the secondary consequence of the reduced body weight observed at this dose. Overall, the testes are not specific target organs in rodents and dogs. There was no indication of a primary adverse effect on testes in any species investigated.

Uterus histopathology and weight: In the 28-day and 90-day study in rats ([redacted] 2015; [redacted] 2018a), no treatment-related uterus weight or histopathological changes were observed. In the carcinogenicity study in rats ([redacted] 2018), after 1-year, 1 endometrial stromal polyp was recorded in the uterus of 1 low dose female; however, due to lack of dose-response this was considered to be of spontaneous origin. After 2-years, gross pathology revealed a slightly higher number of females of the low and high dose with masses in the uterus (11/50 and 9/50, respectively compared to 5 in the control). Masses were observed in a higher number of females in all treatment groups but with no dose-response relationship, therefore, this was not considered to be treatment-related. Neoplasia and neoplastic findings in the uterus after 2-years, including a higher incidence of endometrial adenocarcinoma and endometrial stromal polyps in the uterus of the high dose, were not considered treatment-related, but part of normal variation (as incidences were within the HCD range). Large changes in absolute and relative uterus weights were seen at 12- and 24-months, however, no dose-reponse and/or statistical-significance was observed. The values reflect the large variance of uterus weights within the oestrous cycle and are not considered treatment-related. In the 2-generation study ([redacted] 2018a; [redacted], 2018), in F_0 dams, statistically-significantly increased relative uterus weights were recorded in all treated groups. However, there was no dose-response and statistically-significant values were close to the HCD means. In addition, the mean uterus weight of the control group was low compared to HCD. There was also a lack of histopathological correlate. In mice, no effect on uterus was seen in the 28- and 90-day studies ([redacted] 2016; [redacted] 2018b). In the long-term study in mice ([redacted] 2018d), statistically-significant changes in uterus weights seen in the top dose were not considered treatment-related due to a lack of dose-response and/or concomitant histopathological findings. In the dog studies, uterus weights were not taken, however the uterus with cervix was investigated histopathologically and revealed no remarkable findings. Overall, there was no indication of any functional impairment of the uterus and consequently no specific adverse toxicity is seen in any of the tested species.

Gravid uterus weights were unchanged in the new/current rabbit developmental toxicity study ([redacted] 2018b) dosed up to 320 mg/kg bw and in the older study dosed up to 100 mg/kg bw. Lower (gravid) uterus weights were observed in the second rabbit developmental toxicity study at 500 and 750 mg/kg bw/d (29 % and 25 %, respectively), although without a dose-response and/or statistical-significance. This reduction was based on the lower litter size from 500 mg/kg bw/d. This finding was not considered treatment related, as it was based on events which occurred prior to the administration period. Overall, no substance-related effect was observed on gravid uterus weights.

No macro- or microscopic vaginal findings were observed in rodents in any study. In the dog studies, vagina was not part of the organs examined in histopathology, only in case of gross lesions it was examined. No indications for any critical effect was mentioned in the reports. The vaginal smears taken in the 2-generation study ([redacted] 2018a; [redacted], 2018) did not indicate any effect of treatment. Overall, the vagina is no target organ in rodents.

Overall, there is no indication of direct EAS-mediated adverse effects of cinmethylin. All observed changes were either incidental or secondary to effects on body weight development.

Sensitive to but not diagnostic of EAS

Table 6.8-20. Lines of evidence for EAS Modality 'Sensitive to but not diagnostic of EAS effects'

Study ID	Effect classification*	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect[a]	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
10	STBNDO	Adrenals weight	Rat	4	Weeks	Oral		mg/kg bw/day	No effect		Adrenal weight effects related to body weight reduction		N
20	STBNDO	Adrenals weight	Mice	4	Weeks	Oral		mg/kg bw/day	No effect				N
30	STBNDO	Adrenals weight	Rat	13	Weeks	Oral	814	mg/kg bw/day	Decrease	Abs.(% change to Ctrl.) at low, mid, high dose ♀ : -14%*, ±0%, -18%* & Rel.(% change to Ctrl.) at low, mid, high dose ♀: -12%*, -1%, -4%; no dose response, no histopathologic correlate; no changed rel. weights at top dose: incidental weight change at low dose, body weight related abs. weight change at high dose (-15%)			N
30	STBNDO	Adrenals weight	Rat	13	Weeks	Oral		mg/kg bw/day	No effect				N
40	STBNDO	Adrenals weight	Mice	13	Weeks	Oral		mg/kg bw/day	No effect				N
50	STBNDO	Adrenals weight	Dog	13	Weeks	Oral		mg/kg bw/day	No effect				N
51	STBNDO	Adrenals weight	Dog	52	Weeks	Oral		mg/kg bw/day	No effect				N
51	STBNDO	Adrenals weight	Dog	52	Weeks	Oral	285	mg/kg bw/day	Increase	Rel.(% change to Ctrl.) at high dose ♀: +41%; no histopathologic correlate; related to body weight (-29%)			N
52	STBNDO	Adrenals weight	Dog	52	Weeks	Oral		mg/kg bw/day	No effect				N
70a	STBNDO	Adrenals weight	Rat	52	Weeks	Oral		mg/kg bw/day	No effect				N

70a	STBNDO	Adrenals weight	Rat	52	Weeks	Oral	351	mg/kg bw/day	Decrease	Abs.(% change to Ctrl.) at mid and high dose ♀: -15%**%, -15%*; no histopathologic correlate; no dose response; no significantly changed rel.weights (-3%, -8%): incidental weight change at mid dose due to missing dose response, body weight related abs. weight change (-8%, -13%*)			N
70b	STBNDO	Adrenals weight	Rat	104	Weeks	Oral		mg/kg bw/day	No effect				N
70b	STBNDO	Adrenals weight	Rat	104	Weeks	Oral		mg/kg bw/day	No effect				N
80	STBNDO	Adrenals weight	Mice	78	Weeks	Oral	904	mg/kg bw/day	Increase	Rel.(% change to Ctrl.) at high dose ♂: +50%**; no histopathologic correlate; related to body weight (-19.1%)			N
80	STBNDO	Adrenals weight	Mice	78	Weeks	Oral	184	mg/kg bw/day	Increase	Rel.(% change to Ctrl.) at mid and high dose ♀: +17%*, +38%**; no histopathologic correlate; related to body weight (-9.2%**, -22.2%**)			N
90a	STBNDO	Adrenals weight	Rat	10 (♂ and ♀ prematin g) +3 (♀ gestation) +3 (♀ lactation)	weeks	Oral		ppm	No effect				N
10	STBNDO	Brain weight	Rat	4	Weeks	Oral		mg/kg bw/day	No effect		Brain weight changes sec. to body weight		N
20	STBNDO	Brain weight	Mice	4	Weeks	Oral		mg/kg bw/day	No effect				N
30	STBNDO	Brain weight	Rat	13	Weeks	Oral	792	mg/kg bw/day	Increase	abs. & rel. (% change to Ctrl) in ♂: 0, -3%, -4%*, -6%** (w HCD) / 0, -0%, +2%, +13%**; sec. to body weight (-17%)			N

30	STBNDO	Brain weight	Rat	13	Weeks	Oral	814	mg/kg bw/day	Increase	Rel. (% change to Ctrl) in ♀: +12%**; sec. to body weight reduction (-14%)			N
70a	STBNDO	Brain weight	Rat	52	Weeks	Oral		mg/kg bw/day	No effect				N
70b	STBNDO	Brain weight	Rat	104	Weeks	Oral	242	mg/kg bw/day	Increase	rel. (% change to Ctrl) in ♂ top dose: +8%** sec. to body weight reduction (-9%)			N
70b	STBNDO	Brain weight	Rat	104	Weeks	Oral	317	mg/kg bw/day	Increase	rel. (% change to Ctrl) in ♀ top dose: +14%**) sec. to body weight reduction (-12%)			N
90a	STBNDO	Brain weight	Rat	10 (♂ and ♀ prematin g) +3 (♀ gestation) +3 (♀ lactation)	weeks	Oral		ppm	No effect				N
90b	STBNDO	Brain weight	Rat	3 (gestation) +3 (lactation)	weeks	Oral		ppm	No effect				N
40	STBNDO	Brain weight	Mice	13	Weeks	Oral		mg/kg bw/day	No effect				N
80	STBNDO	Brain weight	Mice	78	Weeks	Oral	25	mg/kg bw/day	Change	abs. & rel. (% change to Ctrl) in ♂ : Low dose: -0.1% /+8.1%**; High dose: -1.9%** /+20%**, sec. to body weight (low dose -7%, high dose -19%)			N
80	STBNDO	Brain weight	Mice	78	Weeks	Oral	184	mg/kg bw/day	Change	abs. & rel. (% change to Ctrl) in ♀ : Mid dose: -1.1%/+ 9.1%**; High dose: -2.5%**/+24%**; sec. to body weight (mid dose -9 %; high dose: -22%)			N
50	STBNDO	Brain weight	Dog	13	Weeks	Oral		mg/kg bw/day	No effect				N
51	STBND	Brain weight	Dog	52	Weeks	Oral		mg/kg	No				N

	O							bw/day	effect				
51	STBNDO	Brain weight	Dog	52	Weeks	Oral	285	mg/kg bw/day	Increase	rel. (% change to Ctrl) in ♀ top dose: +32%**) sec. to body weight reduction (-30%)			N
52	STBNDO	Brain weight	Dog	52	Weeks	Oral		mg/kg bw/day	No effect				N
90a	STBNDO	Litter size	Rat	10 (♂ and ♀ prematin g) +3 (♀ gestation) +3 (♀ lactation)	weeks	Oral		ppm	No effect	F0: 12.5, 12.2, 12.1, 11.8; F1: 11.8, 11.1, 11.8, 11.5; w HCD (9.9 – 12.7) ;	No ED related effect on litter size		N
100a	STBNDO	Litter size	Rat	1.5 (GD6-15)	weeks	Oral		mg/kg bw/day	No effect	Litter size Ctrl. vs 1000, 2000 mg/kg bw): 13.6 ± 2.4, 13.4 ± 1.9, 12.8 ± 4.5; No significant change			N
110a	STBNDO	Litter size	Rabbit	3 (GD 6-28)	weeks	Oral		mg/kg bw/day	No effect	Top dose litter size higher than control			N
111a	STBNDO	Litter size	Rabbit	2 (GD 6-19)	weeks	Oral		mg/kg bw/day	No effect	Parameter in control, 3, 30, 100 mg/kg bw: 6.7 ± 3.1, 6.0 ± 3.0, 6.7 ± 2.5, 6.3 ± 4.2; No dose-response			N
112a	STBNDO	Litter size	Rabbit	2 (GD 7-19)	weeks	Oral		mg/kg bw/day	No effect	Parameter in control, 30, 200, 500 and 750 mg/kg bw 7.7 ± 2.4, 8.1 ± 2.1, 7.5 ± 2.4, 5.7 ± 2.6, 5.0 ± 2.8; No substance effect, Effect is related to low No.of corpora lutea & implantation sites.			N
90b	STBNDO	Litter/pup weight	Rat	3 (gestation) +3 (lactation)	weeks	Oral	5000/2500	ppm	Decrease	F1 BWG (PND 14-21): -6%; most likely as consequence of direct exposure	Effects on litter/pup weights with no obvious relation to ED		N
90b	STBNDO	Litter/pup weight	Rat	3 (gestation) +3	weeks	Oral		ppm	No effect				N

				(lactation)									
100b	STBNDO	Litter/pup weight	Rat	1.5 (GD6-15)	weeks	Oral	2000	mg/kg bw/day	Decrease	Mean fetal weight Ctrl vs 2000 mg/kg bw [g]: 3.51, 3.20**; as consequence of maternal systemic toxicity (BW -10%)			N
110a	STBNDO	Litter/pup weight	Rabbit	3 (GD 6-28)	weeks	Oral	250	mg/kg bw/day	Decrease	Mean litter/fetal weight [%] at 250, 320 mg/kg bw (%Change to Ctrl.): -3 %/ -14.4 %**, -4%/ -11.2 %**; ==> related to maternal toxicity (BWG GD0-29: -22.3, -22,4%) as well as influenced by a larger litter size (+1, +0.5 vs. Ctrl. Fetuses/Litter) as can be seen by the less reduced litter weights.			N
111b	STBNDO	Litter/pup weight	Rabbit	2 (GD 6-19)	weeks	Oral		mg/kg bw/day	No effect				N
112b	STBNDO	Litter/pup weight	Rabbit	2 (GD 7-19)	weeks	Oral		mg/kg bw/day	No effect				N
90a	STBNDO	Numbers of embryonic or foetal deaths and viable foetuses	Rat	10 (♂ and ♀ prematin g) +3 (♀ gestation) +3 (♀ lactation)	weeks	Oral		ppm	No effect		No ED related effect on embryonic or foetal deaths and viable foetuses		N
100a	STBNDO	Numbers of embryonic or foetal deaths and viable foetuses	Rat	1.5 (GD6-15)	weeks	Oral	2000	mg/kg bw/day	Change	2000 mg/kg bw vs Ctrl: Number of early resoptions: 51 vs 17; Number of late resoptions: 0, 0; Number of viable foetuses: 295 vs 339; Number of early resorptions increased and consequently number of viable foetuses reduced at 2000 mg/kg bw, accompanied by maternal toxicity BW GD16: -10%, Body weight loss			N

										during treatment,			
110a	STBNDO	Numbers of embryonic or foetal deaths and viable foetuses	Rabbit	3 (GD 6-28)	weeks	Oral		mg/kg bw/day	No effect				N
111a	STBNDO	Numbers of embryonic or foetal deaths and viable foetuses	Rabbit	2 (GD 6-19)	weeks	Oral		mg/kg bw/day	No effect				N
112a	STBNDO	Numbers of embryonic or foetal deaths and viable foetuses	Rabbit	2 (GD 7-19)	weeks	Oral		mg/kg bw/day	No effect				N
100a	STBNDO	Post implantation loss	Rat	1.5 (GD6-15)	weeks	Oral	2000	mg/kg bw/day	Increase	Parameter in control, 30, 300, 1000, and 2000 mg/kg bw: Post implantation loss: 4.7 ± 6.4, 5.7 ± 7.5, 10.2 ± 13.8, 6.9 ± 7.1, 17.1 ± 27.5; Number of Litters with total resorptions: 0, 0, 0, 0, 2; ==> The observed increase in post-implantation losses at 2000 mg/kg was entirely due to 2 animals with total resorptions, one of which had only one implantation; this animal therefore had a postimplantation loss of 100%, resulting in an increased group mean with large standard deviation for this parameter.	No ED related implantation loss		N

110a	STBNDO	Post implantation loss	Rabbit	3 (GD 6-28)	weeks	Oral		mg/kg bw/day	No effect				N
111a	STBNDO	Post implantation loss	Rabbit	2 (GD 6-19)	weeks	Oral		mg/kg bw/day	No effect				N
112a	STBNDO	Post implantation loss	Rabbit	2 (GD 7-19)	weeks	Oral		mg/kg bw/day	No effect				N
100a	STBNDO	Pre implantation loss	Rat	1.5 (GD6-15)	weeks	Oral		mg/kg bw/day	No effect				N
110a	STBNDO	Pre implantation loss	Rabbit	3 (GD 6-28)	weeks	Oral		mg/kg bw/day	No effect				N
111a	STBNDO	Pre implantation loss	Rabbit	2 (GD 6-19)	weeks	Oral		mg/kg bw/day	No effect				N
112a	STBNDO	Pre implantation loss	Rabbit	2 (GD 7-19)	weeks	Oral		mg/kg bw/day	No effect				N
90b	STBNDO	Presence of anomalies (external, visceral, skeletal	Rat	3 (gestation) +3 (lactation)	weeks	Oral		ppm	No effect	Dilated renal pelvis in 6 F2 pups at 5000 ppm slightly increased above HCD: Pup incidence 2.6%; Pup/litter incidence: 2.4%; (HCD Ø 0-2.3%, 0-2.1%;)Litter incidence: 16%; w HCR: 0-20%; mainly caused by one litter (#376) with 11 pups in total of which 3 were affected. Three further litters had single affected pups. single incident, not statistically significantly increased, mainly unilateral, within the historical control range on a litter base, no such effect on the F1 pups, => no association to the treatment assumed.	Developmental effects often within or close to HCD , at limit dose and above increased, no obvious relation to ED effects		N

100b	STBNDO	Presence of anomalies (external, visceral, skeletal	Rat	1.5 (GD6-15)	weeks	Oral	1000	mg/kg bw/day	Increase	Visceral and skeletal variations (affected fetuses/litter % from Ctrl to 2000 mg/kg bw): Visceral : 0, 0, 0, 2.61 ± 6.18, 13.66 ± 27.63; Skeletal : 6.2 ± 12.2, 5.7 ± 12.2, 5.8 ± 9.5, 9.4 ± 15.4, 9.5 ± 16.7; Total variations: 3.23 ± 6.32, 2.85 ± 6.09, 2.99 ± 4.84, 5.84 ± 7.79, 11.3 ± 19.54; Individual findings: (Ctrl to 2000 mg/kg bw) Lateral ventricles dilation in brain: 0, 0, 0, 0.80 ± 4.0, 13.07 ± 27.79%** Wavy ribs: 0.57 ± 2.86, 2.0 ± 10.0, 0, 1.71 ± 8.57, 2.57 ± 7.09; Not or incompletely ossified skeletal structures: 3.64 ± 9.20, 4.68 ± 11.5, 3.75 ± 8.09, 4.36 ± 9.92, 6.10 ± 12.1; Declined fetal ossification sites for caudal vertebrae and metacarpals: Mean at at 2000 mg/kg bw vs Ctrl: 4.35** vs. 5.23 and 3.34** vs 3.67;			N
110a	STBNDO	Presence of anomalies (external, visceral, skeletal	Rabbit	3 (GD 6-28)	weeks	Oral		mg/kg bw/day	No effect	Skeletal malformation and variations (affected fetuses/litter % from Ctrl 1 to 250 mg/kg bw and Ctrl 2 to 320 mg/kg bw): Total malformations: 1.8, 0.7, 0.5, 2.9; 0.4,0.4; no effect; Total variations: 95.8, 96.6, 97.0, 95.1; 89.4, 94.7; no effect; Individual findings: (Ctrl 1 to 250 mg/kg bw and Ctrl 2 to 320 mg/kg bw):) Misshapen thoracic vertebra: 0, 0, 0,			N

										1.7*; 0, 0; no dose response No association of 'misshapen thoracic vertebrae' to the treatment is given.			
111b	STBNDO	Presence of anomalies (external, visceral, skeletal	Rabbit	2 (GD 6-19)	weeks	Oral		mg/kg bw/day	No effect	No effect Skeletal malformation and variations (affected fetuses/litter % from Ctrl to 100 mg/kg bw): Total skeletal malformations: 8.9, 2.1, 0.0, 0.0; no effect; Total skeletal variations: 34.0, 18.3, 20.2, 12.5; no effect;			N
112b	STBNDO	Presence of anomalies (external, visceral, skeletal	Rabbit	2 (GD 7-19)	weeks	Oral		mg/kg bw/day	No effect	No effect Skeletal malformation and variations (affected fetuses/litter % from Ctrl to 750 mg/kg bw): Total skeletal malformations: 0.0, 0.0, 0.8, 5.0 ± 15.8, 0.0; no effect; Total variations: 68.5, 67.0, 69.6, 43.9, 57.1; no effect;			N

*STBNDO: Sensitiv to, but not diagnostic of, EAS; *: $p \leq 0.05$; **: $p \leq 0.01$;

a: For brevity and clarity's sake, only effect targets which showed one or more positive observed effects (rather than no effect in all studies) are listed in this table. This allows targeted discussion of effects. The full list of positive and negative observed effects is available in Appendix E.

'Sensitive to but not diagnostic of EAS'
The review of the 'sensitive to, but not diagnostic of EAS' (STBNDO) parameters, revealed no indication for adverse effects (Table 6.8-20). There were some changes in parameters which were either considered secondary to other toxic effects or not considered to be treatment-related (as changes were not dose-related or within the normal physiological range). These changes are discussed below.

Adrenal weight: In the 90-day study in rats, in females statistically-significant changes in absolute (at the low and top dose) and relative (at the low dose only) adrenal weights were seen. In the combined chronic toxicity and carcinogenicity study in rats, at 12-months statistically-significant decreases in absolute adrenal gland weights (15 % change compared to control) were seen in females from the mid dose. There was no accompanying statistically-significant change in relative adrenal weight. In the 2-gen study in the rat, in females a statistically-significant decrease was recorded at the low dose (7 %). In the carcinogenicity study in mice, statistically-significant changes in adrenal weights were observed in males and females after 18-months. However, all the above changes were not considered treatment-related due to a lack of dose-response and/or concomitant histopathological findings. Increased relative adrenal weights were detected in the first 1-year dog study, in high dose females (41 % and statistically-significant) and also in males (22 % but not statistically significant). However, this was considered a consequence of significantly lower body weights and was not considered treatment-related. In the second 1-year dog study at lower doses (up to 80 mg/kg bw) no effect on adrenal weights were observed. In all dog studies, no pathological findings were observed. Overall, there is no evidence for any functional impairment of the adrenals and consequently no specific adverse toxicity observed in any of the tested species.

Fertility and gestation length: Neither a general delay of parturition or any parturition complications (dystocia) nor effects on male or female fertility were noted in the 2-generation study. The mean gestation length was similar in all test groups (22.1 - 22.3 days) in the 2-generation study.

Litter size: Litter size was not affected by treatment in the 2-generation study (within the historical control), as well as in the developmental studies up to limit dose (1000 mg/kg bw/d). In the rat developmental study, at 2000 mg/kg bw/d (above limit dose and a dose which induced severe maternal toxicity) litter size was slightly redueced (12.8 vs 13.6 in the control). This effect however was considered the unspecific secondary consequence of maternal toxicity. In one of the rabbit developmental studies, the litter size was reduced at 500 and 750 mg/kg bw/d; a consequence of the lower number of corpora lutea and implantation sites. Litter size reduction was not considered to be treatment-related as inseminated animals were used in these studies. Overall, there were no specific adverse effects on litter size.

Litter viability: as determined in the 2-generation studies by means of viability (Survival PND 0-4) and lactation index (Survival PND 4-21), litter viability was not affected by treatment in any dose group.

Litter/pup weight: Foetal development, as indicated by foetal weights, in the rat developmental toxicity study was not affected up to 1000 mg/kg bw/d. At doses inducing severe maternal toxicity including mortality in rats (2000 mg/kg bw), mean foetal weights were reduced by 8.8 % (with statistical-significance), similar to the weight reductions in dams (9 % at GD 20). This effect was only seen at a dose much higher than the limit dose and was considered the unspecific secondary consequence of maternal toxicity. In rabbits, the mean foetal weight of all viable foetuses at 250 and 320 mg/kg bw/d were statistically-significantly reduced (14 % and 11 %, respectively). However, the reduced foetal weight was the unpecific secondary consequence of the maternal toxicity (observed from 250 mg/kg bw/d) elicited by cinmethylin.

In the 2 generation study in rats, statistically-significant decreases in body weight gain were observed in high dose F_1 pups, during PND 14 – 21, in both males and females. However, percent change compared to controls was < 10 %, overall (PND 1 – 21) body weight gain was not statistically-significant, and there were no statisticall-significant effects on body weight gain in the F_2 generation.

Number of implantations, corpora lutea: There were no treatment-related effects on copora lutea counts or implantations.

Number of live births: The number of live births was not affected in the 2-generation study, neither in the F_0 nor in the F_1 generation.

Numbers of embryonic or foetal deaths and viable foetuses and post-implantation loss: There were no indications for any treatment-related intrauterine embryo-/foeto lethality in F_0 and F_1 generations of the 2-

generation study. In the rat developmental study, no treatment-related differences between control and treate groups were seen up to the limit dose (1000 mg/kg bw/d). At the top dose (2000 mg/kg bw/d – a dose which induced severe maternal toxicity and mortality), two dams suffered total resorptions, consequently post-implantation loss and resorptions were increased. This effect was considered the unspecific, secondary consequence of the maternal toxicity recorded from 300 mg/kg bw/d. As this effect is only seen at a dose much higher than the limit dose, it is not further considered for the ED assessment. Overall, there is no indication for a specific adverse effect on post- and pre-implantation loss.

Developmental toxicity: In the 2 generation study in rats, pup development was not affected by treatment. The higher incidence of dilated renal pelvis in F_2 pups (6 pups out of 233; pup incidence of 2.6 % and pup/litter incidence of 2.4 %) was above the HCD range (pup incidence: 0 - 2.3 %; pup/litter incidence: 0 - 2.1 %), but within the HCD range on a litter basis (4 of 24 litters; litter incidence of 16 %; HCD: 0 - 20%). The increased pup incidence was mainly caused by one litter (#376) with 11 pups in total, of which 3 were affected. Three further litters had single affected pups. As this was a single incident, not statistically significantly, within the HCD range for litters and there was no similar effect on the F_1 pups, it was not considered treatment related.

In the developmental toxicity study in the rat, the developmental effects observed (mild dilation of brain ventricles, skeletal variations, incomplete ossification) were considered the unspecific, secondary consequence of the maternal toxicity recorded from 300 mg/kg bw/d (limited number of clinical signs of toxicity and decreases in body weight gain). It is noted that at the top dose of 2000 mg/kg bw/d maternal toxicity was particularly severe, with deaths, significant reductions in body weights, numerous clinical signs of toxicity and liver effects; effects at this dose were therefore not considered for the ED assessment.

Similarly, when cinmethylin was administered daily to rabbits in the new/modern developmental toxicity study, no developmental toxicity was observed at doses up to and including the highest dose of 320 mg/kg bw/d. Furthermore, no developmental toxicity was observed in two older studies which are considered as supportive information.

Pituitary histopathology and weight: The histopathological examination of the pituitary in 10 short- and long-term studies in rats, mice and dogs did not reveal any treatment related effects. There is no indication for any functional impairment of the pituitary gland or any adverse effect on pituitary in any species investigated.

Sex ratio: This was not affected in the 2-generation study as well as in the developmental toxicity studies.

Time to mating: The mean time to mating did not show any test-substance related effect.

Differential ovarian follicle count: In the 2 generation study in rats, there was no effects of treatment on differential ovarian follicle counts up to the top dose of 5000/2500 ppm (394 mg/kg bw/d).

Overall, the ‘sensitive to, but not diagnostic of, EAS’ parameters evaluated above did not reveal any specific adversity. All observed changes were either not treatment-related or secondary to other toxicities including effects on body weight development.

Endocrine activity

***In vitro* mechanistic (level 2 studies) for EAS**

ToxCast data are available; no specific level 2 or 3 studies have been conducted by the applicant.

The ToxCast/Tox21 data for cinmethylin, relating to EAS endocrine activity, include:

- 18 assays for estrogenic and anti-estrogenic activity (E),
- 11 assays for androgenic and anti-androgenic activity (A),
- 20 assays for steroidogenesis alteration and 1 for aromatase-inhibition (S),
- Several specificity assays and viability assays.

Estrogen Receptor Agonist/Antagonist

In vitro assays in Tox21 and ToxCast Phase II specifically related to estrogen receptor (ER) pathway signaling include: estrogen receptor transcriptional activation assays; ER cofactor recruitment and dimerization assays; ER binding assays and an estrogen-dependent cell proliferation assay. A systems biology model has been generated by the US EPA, which integrates the 18 ToxCast/Tox21 assay endpoints for ER-based pathway activity to produce an area under the curve (AUC) scoring metric which can be used to prioritise a chemical's estrogen agonist or antagonist potential.

Cinmethylin was tested in all 18 assay endpoints for ER-based pathway activity. Results for cinmethylin were compared to the AC50 values of a strong and weak ER agonist (17ß-estradiol and genistein) and to an ER antagonist (tamoxifen).

Table 6.8-21. ToxCast ER assays for cinmethylin (tested up to 100 µM) and reference chemicals

Biological Process Indicator [Estrogen (E) or Anti-Estrogen (Anti-E) Signal Detection]	**Assay ID**	**Assay Endpoint Name**	**AC50 [µM]**			
			17β-estradiol	**Genistein**	**Tamoxifen**	**Cin-methylin**
Binding to estrogen receptor [E/Anti-E]	A1	**NVS_NR_bER**	0.0002	0.1061	0.0867	inactive
	A2	**NVS_NR_hER**	0.00003	0.0145	0.0518	inactive
	A3	**NVS_NR_mERa**	0.0005	0.0948	0.1005	inactive
Receptor Dimerization [E/Anti-E]	A4	**OT_ER_ERaERa_0480**	0.1083	3.0788	0.4745	inactive
	A5	**OT_ER_ERaERa_1440**	0.0467	2.6000	0.9033	inactive
	A6	**OT_ER_ERaERb_0480**	0.0182	0.7914	0.3395	**53.635**
	A7	**OT_ER_ERaERb_1440**	0.021	1.3521	0.3354	inactive
	A8	**OT_ER_ERbERb_0480**	0.0087	0.1317	0.4977	**45.821**
	A9	**OT_ER_ERbERb_1440**	0.0091	0.113	0.1136	inactive
Co-factor recruitment [E/Anti-E]	A10	**OT_ERa_EREGFP_0120**	0.0001	1.2010	0.2292	inactive
	A11	**OT_ERa_EREGFP_0480**	0.00003	0.9253	0.2018	inactive
RNA transcription [E]	A12	**ATG_ERa_TRANS_up**	1.0669	0.1433	inactive	**18.365**
	A13	**ATG_ERE_CIS_up**	0.0009	0.0975	inactive	**16.140**
Protein production [E]	A14	**Tox21_ERa_BLA_Agonist_ratio**	0.0007	8.9789	71.8512	inactive
	A15	**Tox21_ERa_LUC_BG1_Agonist**	0.0001	2.7502	inactive	inactive
Cell prolifera-tion [E]	A16	**ACEA_T47D_80hr_Positive**	0.0062	0.0786	inactive	inactive
Transcription suppression [Anti-E]	A17	**Tox21_ERa_BLA_Antagonist_ratio**	70.5706	34.9238	1.4754	inactive
	A18	**Tox21_ERa_LUC_BG1_Antagonist**	inactive	inactive	13.8652	inactive
ER Agonist AUC Score (significant if ≥0.1)			0.935	0.538	0.0199	**0.011**
ER Antagonist AUC Score (significant if ≥0.1)			0.0153	0.000	0.447	0.000

Cinmethylin was active in 4 of the 18 estrogen-relevant assays. AC50 values were all above 16 µM, they were all above or near the median cytotoxicity for cinmethylin of 23.54 µM and the lower bound cytotoxicity of 6.6µM. Therefore, cinmethylin is considered to be inactive in ER assays at relevant concentrations. An ER Agonist AUC score of 0.01 implies a concentration of greater than 1 mM is required to elicit activity, this is several orders of magnitude higher than the cytotoxicity level of 6.6 µM.

In addition, 12 new ER assays are listed in the EDSP21 Dashboard Version 3.0.8, these new assays are special versions of previously included assays, measuring the specificity of the assay and/or the viability of the cells in the respective assays previously included in the Dashboard. In light of the cytotoxicity value of 6.6 µM, none of these new assays were active for cinmethylin.

Overall, the output AUC scores from the ER models do not predict any potential of cinmethylin to act as an ER agonist / antagonist at relevant concentrations. It should be noted that the ToxCast ER bioactivity model is considered sufficiently predictive of the OECD Level 2 and 3 tests for the E modality.

Androgen Receptor Agonist/Antagonist

In vitro assays in ToxCast/Tox 21 specifically related to androgen receptor (AR) mediated effects (of which there are 11) include: chemical AR binding assays, AR dimerization assays, AR transcriptional activation reporter assays and AR co-factor recruitment assays. Again, a computational systems biology modeling approach has been used to integrate the results of these 11 assay endpoints and produce a scoring metric (AUC), used to prioritise a chemical's androgen agonist or antagonist potential.

Table 6.8-22. ToxCast AR assays for cinmethylin and reference chemicals

Biological Process Indicator [Androgen (A) or Anti-Androgen (Anti-A) Signal Detection]	Assay ID	Assay Endpoint Name	AC50 [µM]		
			Testosterone Propionate	Hydroxy-flutamide	Cin-methylin
Binding to androgen receptor [A/Anti-A]	A1	**NVS_NR_hAR**	0.0438	0.1728	inactive
	A2	**NVS_NR_cAR**	0.7980	0.1497	inactive
	A3	**NVS_NR_rAR**	0.7017	6.0037	inactive
Cofactor recruitment [A/Anti-A]	A4	**OT_AR_ARSRC1_0480**	0.0135	7.1768	inactive
	A5	**OT_AR_ARSRC1_0960**	0.0070	8.8837	**98.367**
RNA transcription [A]	A6	**ATG_AR_TRANS_up**	0.5726	inactive	inactive
Transcription and Reporter Protein production [A/Anti-A]	A7	**OT_AR_ARELUC_AG_1440**	0.0031	2.9455	inactive
	A8	**TOX21_AR_BLA_Agonist_ratio**	0.0043	inactive	inactive
	A9	**TOX21_AR_LUC_MDAKB2_Agonist**	0.0014	4.2943	inactive
	A10	**TOX21_AR_BLA_Antagonist_ratio**	inactive	1.8396	inactive
	A11	**TOX21_AR_LUC_MDAKB2_Antagonist2**	inactive	0.0790	inactive
AR Agonist AUC Score (significant if ≥0.1)			1.53	0.0011	**0.000**
AR Antagonist AUC Score (significant if ≥0.1)			0.000	0.999	**0.000**

Cinmethylin was active in 1 of the 11 androgen-relevant assays with an AC50 value of 98 µM. However, this AC50 is above the median cytotoxicity for cinmethylin of 23.54 µM and the lower bound cytotoxicity of 6.6µM. Therefore, cinmethylin is considered to be inactive in AR assays at relevant concentrations.

In addition, 8 new AR assays are listed in the EDSP21 Dashboard Version 3.0.8, these are special versions of previously included assays measuring the specificity of the assay and/or the viability of the cells in the respective assays previously included in the Dashboard. In light of the cytotoxicity value of 6.6 µM, none of these assays were active for cinmethylin.

Overall, the output AUC (area under curve) scores from the AR models do not predict any potential of cinmethylin to act as an AR agonist / antagonist at relevant concentrations.

Steroidogenesis Pathway
ToxCast contains a high-throughput modification of the OECD 456-validated *in vitro* steroidogenesis assay in H295R human adrenocortical carcinoma cells. While the guideline study measures 2 hormones (Testosterone and Estradiol), the ToxCast system measures 13 hormones in a 96-well format. The validity of this modified test system was demonstrated by the correct identification of the 2 endpoints included in the OECD guideline assay for several core chemicals used to validate the OECD 456 study. The high-throughput steroidogenesis assays quantified the following 10 steroid hormones (including: progestogens, glucocorticoids androgens and estrogens via HPLC/MS/MS analysis):

- 17α-Hydroxypregnenolone (OHPREG)
- Progesterone (PROG)
- 17α-Hydroxyprogesterone (OHPROG)
- 11-Deoxycortisol (11DCORT)
- Cortisol
- 11-Deoxycorticosterone (DOC)
- Androstenedione (ANDR)
- Testosterone (TESTO)
- Estrone
- Estradiol

Levels of 3 further hormones: pregnenolone (PREG), dehydroepiandrosterone (DHEA) and corticosterone (CORT), were consistently below the limit of quantification (LOQ) and therefore omitted for most of the analyses.

The high-throughput assay was conducted in two steps:
1) a maximum tolerated testing concentration was identified (cell viability by MTT assay ≥70%) for chemicals at a single testing concentration to ensure cell viability was not confounding results
2) 6-point concentration-response analysis was conducted by titrating down from the maximum tolerated concentration in half-log increments.

Cinmethylin had a maximum tolerated testing concentration of 100 µM, at which 90 % cell viability was observed, and the final concentration-response evaluation ranged from 0.4 to 100µM. The MTT cytotoxicity assay is conducted on cells remaining in the well after media is removed for hormone quantification, ensuring cytotoxicity data are matched with hormone results. The cell viability obtained from the same wells as the hormone measurements for the concentration-response testing confirm that cinmethylin did not affect cell viability in H295R cells.

While the AC50 values from these hormone endpoints inform on potency, the efficacy of hormone level changes and the ACC (concentration at which the response exceeds the efficacy cut-off) are also important to evaluate this assay. The ACC value (concentration at which the change in hormone levels exceeded the response cut-off), the measured maximum achieved fold change (max_med), the modeled max.fold induction (modl_tp) and the AC50 values, per hormone analysed, is given for cinmethylin and compared to the reference substances Forskolin and Prochloraz (modeled max fold induction (modl_tp) and the AC50 values are given for comparison) (Tabble 6.8-22).

Table 6.8-23. ToxCast Steroidogenesis assays for cinmethylin and reference chemicals

Hormone quantified	Assay ID CEETOX_H295R_...	Forskolin		Prochloraz		Cinmethylin			
		AC50 (µM)	Max. Fold Change [modl_tp]	AC50 (µM)	Max. Fold Change [modl_tp]	AC50 (µM)	ACC (µM)	Measured Max. Fold Change (Concentration) [max_med]	Max. Fold Change [modl_tp] (CR Cutoff§)
17α-OH pregnenolone	**OHPREG**	0.666	**2.439***	inactive		**Inactive** (measured up to 30 µM)			
						46.77	42.6	-1.4 (100 µM)	
Progesterone	**PROG**	**inactive**		0.175	**5.596***	**inactive**			

17α-OH progesterone	**OHPROG**	0.722	**1.597***	inactive		**inactive**			
Deoxycortico-sterone	**DOC**	0.408	1.001	0.051	**4.207***	22.848	18.04	-1.802 (100 µM)	**-2.132* (1.9)**
Cortisol	**CORTISOL**	0.631	**2.421***	0.302	**-5.017***	44.970	43.57	-1.604 (100 µM)	**-2.021*(1.98)**
11-Deoxycortisol	**11DCORT**	0.457	**1.417***	0.346	**-1.845***	35.058	25.7	-1.322 (100 µM)	**-1.553* (1.5)**
Androstenedione[#]	**ANDR**	0.657	**1.900***	0.175	**-5.512***	32.147	35.25	-1.130 (100 µM)	-1.130 (1.7)
Testosterone[1] [#]	**TESTO**	0.941	**1.891***	0.175	**-4.816***	32.016	37.18	-1.348 (100 µM)	-1.348 (2.05)
Estrone	**ESTRONE**	0.622	**2.466***	0.279	**-6.047***	**inactive**			
Estradiol	**ESTRADIOL**	0.659	**2.572***	0.121	**-3.014***	**inactive**			

[#] Effect at AC50 is still below the efficacy cut-off for cinmethylin;
[1] Only the highest conc. above baseline for cinmethylin;
***** *Indicates statistically significant fold change relative to a factor of the baseline median absolute deviation;*
[§]*CR cutoff is 6xBMAD*

Cinmethylin was active for the reduction of the mineralocorticoids and the androgens. The maximal reduction in mineralocorticoids and androgens at the highest tested concentration of 100 µM does not exceed a level of 1.8- or 1.3-fold, respectively. The effects of cinmethylin at the AC50 are all very close to the efficacy cut-off value (ACC), near/above the median cytotoxicity for cinmethylin of 23.54 µM and above the lower bound cytotoxicity of 6.6µM.

Karmaus *et al.* (2016) identified a sample as having a significant effect on a hormone if it had an active hit and the efficacy exceeded a specified threshold (CR cut-off) defined by a cut-off of ≥ 6 times the BMAD of all response values at the lowest 2 tested concentrations. According to this, the threshold of efficacy for active hit call affecting cortisol levels is 1.98-fold (CR Cut-off CORTISOL); cinmethylin achieved a maximum modeled efficacy of 2.021-fold decrease in cortisol levels. Similarly, the cut-offs for deoxycorticosterone (CR Cut-off DOC: 1.90- fold) and for 11-deoxycortisol (CR Cut-off 11DCORT: 1.50-fold) were also very close to the maximum achieved efficacy elicited by cinmethylin. The effects on androgens did not reach the CR cut-off (1.7-fold) and are therefore not considered to be significantly changed. A comparison with the reference substances supports the marginal response of cinmethylin.

Using a statistical modeling approach, the 20 assays were integrated to produce a statistical metric, used to prioritise a chemical's steroidogenesis disrupting potential. According to this approach, as 5 of 10 hormones generated a hit, independent of the effect efficacy, cinmethylin would be considered a candidate for further investigations. However, based on *in vivo* data, no adverse effect on adrenals or reproductive organs were identified.

While some effects achieved statistical-significance, the low efficacy and high AC50 at which these effects were observed do not suggest that cinmethylin has a robust or targeted effect on steroidogenesis. This is in line with the fact that adrenal gland and reproductive organs were not affected by cinmethylin. In light of the cytotoxicity value of 6.6 µM, these assays would be classified as false actives, as there is no activity up to 6.6 µM.

Aromatase Inhibition
The aromatase (CYP19A1) enzyme is responsible for the conversion of androgens to estrogens, namely testosterone to estradiol and androstenedione to estrone. This last step in the steroidogenesis pathway is critical for the biosynthesis of estrogens (female sex hormones).

Though there are 3 aromatase assays in ToxCast/Tox21, cinmethylin was only evaluated in 2 assays:
1) Tox21_Aromatase_Inhibition - this assay is based in MCF-7 human breast cells, and relies upon a reduction in luciferase-coupled ATP quantitation after 24 hours of chemical treatment.
2) Viability of MCF-7 cells.

Cinmethylin was evaluated in the Tox21_Aromtatase_Inhibition assay across a 15-point concentration-response, in triplicate, ranging from 0.001 to 90 µM. No aromatase inhibition was detected in response to cinmethylin over the concentration range tested, thus no interaction with aromatase is expected. The 2 assays for aromatase inhibition were flagged as boarderline inactive, however, in light of the cytotoxicity value of 6.6 µM, there is no concern for a borderline aromatase inhibition given.

Overall
Cinmethylin was tested at multiple concentrations up to around 100 µM. EAS effects were observed (Table 6.8-24) at relative high concentrations which were confounded by significant cytotoxicity i.e. exceeded

the median (23.45 µM) and lower bound (6.6 µM) cytotoxicity levels for cinmethylin. Overall, the ToxCast/EDSP21 '*in vitro* mechanistic' dataset indicate that cinmethylin does not specifically perturb the pathways related to endocrine activity for the E, A and S-modalities.

Table 6.8-24. Lines of evidence for EAS-modality (*in vitro* studies) from ToxCast

Study ID	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
140	*In vitro* mechanistic	Estrogen receptor				Uptake from the medium*	0.0107 (Agonist) 0 (Antagonist)		Change	No effect on the Estrogen Receptor pathway up to Cytotoxicity-cutoff.	No effect on ER up to the cytotox-cutoff	Overall negative evidence for specific endocrine activity *in vitro*	EAS
150	*In vitro* mechanistic	Androgen receptor				Uptake from the medium*	0 (Agonist) 0 (Antagonist)		No effect	No effect on Androgen Receptor pathway	No effect on AR		EAS
176	*In vitro* mechanistic	11-Deoxycorticosterone (*in vitro*)	Human adrenal gland cell line	48	hours	Uptake from the medium*	22.8	µM	Change	False positive; No effect seen up to 6.6µM	No effect on Steroidogenesis up to the cytotox-cutoff		EAS
170	*In vitro* mechanistic	11-Deoxycortisol (*in vitro*)	Human adrenal gland cell line	48	hours	Uptake from the medium*	35.1	µM	Change	False positive; No effect seen up to 6.6µM			EAS
171	*In vitro* mechanistic	17-alpha-hydroxypregnelone (*in vitro*)	Human adrenal gland cell line	48	hours	Uptake from the medium*	46.77	µM	Change	False positive; No effect seen up to 6.6µM			EAS
172	*In vitro* mechanistic	17-alpha-hydroxypregnelone (*in vitro*)	Human adrenal gland cell line	48	hours	Uptake from the medium*	-	µM	No effect				EAS
173	*In vitro* mechanistic	17-alpha-hydroxyprogestero	Human adrenal gland cell line	48	hours	Uptake from the medium*	33.1	µM	No effect	No exceedance of response			EAS

		ne (*in vitro*)								cutoff			
174	*In vitro* mechanistic	Androstenedione (*in vitro*)	Human adrenal gland cell line	48	hours	Uptake from the medium*	32.15	µM	Change	False positive; No effect seen up to 6.6µM			EAS
175	*In vitro* mechanistic	Cortisol (*in vitro*)	Human adrenal gland cell line	48	hours	Uptake from the medium*	45	µM	Change	False positive; No effect seen up to 6.6µM			EAS
160	*In vitro* mechanistic	CYP19	human breast cell line	24	hours	Uptake from the medium*	49.07	µM	No effect	No exceedance of response cutoff			EAS
177	*In vitro* mechanistic	Estradiol level (*in vitro*)	Human adrenal gland cell line	48	hours	Uptake from the medium*	-	µM	No effect				EAS
178	*In vitro* mechanistic	Estrone (*in vitro*)	Human adrenal gland cell line	48	hours	Uptake from the medium*		µM	No effect				EAS
179	*In vitro* mechanistic	Progesterone (*in vitro*)	Human adrenal gland cell line	48	hours	Uptake from the medium*	15.1	µM	No effect	No exceedance of response cutoff			EAS
180	*In vitro* mechanistic	Progesterone (*in vitro*)	Human adrenal gland cell line	48	hours	Uptake from the medium*		µM	No effect				EAS
181	*In vitro* mechanistic	Testosterone level (*in vitro*)	Human adrenal gland cell line	48	hours	Uptake from the medium*	32	µM	Change	False positive; No effect seen up to 6.6µM			EAS

WoE for EAS-mediated adversity

In all species investigated (rat, mouse and dog) there were no specific adverse effects on reproductive organs and related endocrine glands (e.g. adrenal, pituitary, mammary). In addition, there were no specific adverse effects on reproduction in the rat and on development in rats and rabbits. The effects on the prostate in the dog and on post-implanation loss in rats were the unspecific secondary consequence of generalised/maternal toxicity.

WoE for EAS-mediated endocrine activity

Overall, the ToxCast/EDSP21 '*in vitro* mechanistic' dataset indicates that cinmethylin does not specifically perturb the pathways related to endocrine activity for the E, A and S-modalities.

Have EAS-mediated parameters been sufficiently investigated?

EAS-mediated parameters have been sufficiently investigated, based on a modern two-generation reproductive toxicity study by [redacted] 2018a) (OECD TG No. 416; test protocol according to latest version of January 2001).

Analysis of the evidence and identification of relevant scenario for the ED assessment of EATS-modality
Seclection of relevant scenario

Adversity based on EAS-mediated parameters	**Positive mechanistic OECD CF level 2/3 Test**	**Scenario**	**Next step of the assessment**	**Scenario selected**
No (sufficiently investigated)	Yes/**No**	**1a**	Conclude: ED criteria not met because there is no **'EAS-mediated'** adversity	X
Yes (sufficiently investigated)	Yes/No	1b	Perform MoA analysis	
No (not sufficiently investigated)	Yes	2a (i)	Perform MoA analysis (additional information may be needed for the analysis)	
No (not sufficiently investigated)	No (sufficiently investigated)	2a (ii)	Conclude: ED criteria not met because no EAS-mediated endocrine activity observed	
No (not sufficiently investigated)	No (not sufficiently investigated)	2a (iii)	Generate missing level 2 and 3 information. Alternatively, generate missing "EATS-mediated" parameters. Depending on the outcome move to corresponding scenario	
Yes (not sufficiently investigated)	Yes/No	2b	Perform MoA analysis	

Conclusion of the assessment of EAS-modalities

Based on scenario 1a of the ECHA/EFSA guidance for the identification of endocrine disruptors in the context of Regulations (EU) 528/2012 and (EC) No 1107/2009, there is no indication of adversity for the EAS modalities. In addition, EAS adversity has been sufficiently investigated. There is also robust evidence from the ToxCast ER bioactivity model of a lack of endocrine activity for the E modality. The first condition of the ED criteria is not met; therefore, it is possible to conclude that cinmethylin does not meet the ED criteria for the EAS modalities and that these modalities have been sufficiently investigated.

T-modality

The review of the T parameters determined in the existing toxicological database indicate that the thyroid is a target organ in the rat, but not in mice and dogs. The effects on the thyroid consisted of increased thyroid weights, increased incidences of follicular cell hypertrophy/hyperplasia and/or altered colloid. The data are compiled in Appendix E and relevant parameters for the T-modality are extracted in the lines of evidence below (Table 6.8-25). The data are discussed below.

Assessment of the integrated lines of evidence and weight of evidence for T-mediated adversity and endocrine activity

Lines of evidence for **adverse effects** and **endocrine activity** related to T-modality:

Table 6.8-25. Lines of evidence for T-modality (*in vivo* & *in vitro* mechanistic)

Study ID	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
10	EATS-mediated	Thyroid weight	Rat	4	Weeks	Oral		mg/kg bw/day	No effect		Increased thyroid weight in rats. In dogs the effect is seen only at doses exceeding MTD.	Overall positive evidence for Thyroid effects in rats.	T
30	EATS-mediated	Thyroid weight	Rat	13	Weeks	Oral	814	mg/kg bw/day	Increase	♀ high dose: abs. +8%, rel. +25%*. both outside HCD ==> partly sec. to lower body weight, corresponding liver changes at same or lower doses			T
50	EATS-mediated	Thyroid weight	Dog	13	Weeks	Oral		mg/kg bw/day	No effect				T
51	EATS-mediated	Thyroid weight	Dog	52	Weeks	Oral	253.9	mg/kg bw/day	Increase	♂ high dose: abs. 44.5%, rel. +71%*. No clear dose dependency or microscopic correlate. Considered sec. to ↓ body weight (-21%), MTD exceeded at high dose			T
51	EATS-mediated	Thyroid weight	Dog	52	Weeks	Oral	81.4	mg/kg bw/day	Increase	♀ mid. & high dose: abs. 39% and 8%, rel. 55% and 54%. No dose dependency, no microscopic correlate. Considered sec. to ↓ body weight (-13% and -30%), MTD exceeded at high dose.			T
52	EATS-	Thyroid	Dog	52	Weeks	Oral		mg/kg	No effect				T

	mediated	weight						bw/day					
70a	EATS-mediated	Thyroid weight	Rat	52	Weeks	Oral		mg/kg bw/day	No effect				T
90a	EATS-mediated	Thyroid weight	Rat	10 (♂ and ♀ prematin g) +3 (♀ gestation) +3 (♀ lactation)	weeks	Oral	394 - 481	mg/kg bw/day	Increase	High dose F0 & F1: ♂(w.HCD)/♀(slightly outside HCD) abs. (15%-22%) and rel. (+17%-24%), dose-dependently increased, correlating histopathological finding,			T
10	EATS-mediated	Thyroid histopathology	Rat	4	Weeks	Oral	477	mg/kg bw/day	Increase	Follicular hypertrophy/hyperplasia Hypertropy mean grading: ♂ : 0-0-1.3-1.6; ♀ : 0-0-1.0-1.8 ==>likely a consequence of liver enzyme induction	Increased follicular hypertrophy/hyperplasia in rat, accompanied by liver effects.		T
30	EATS-mediated	Thyroid histopathology	Rat	13	Weeks	Oral	211/240	mg/kg bw/day	Increase	Hypertrophy/Hyperplasia: Incidence in ♂: 0, 0, 4, 9; ♀: 0, 0, 1, 1 Mean severity grading in ♂: 0, 0, 1.5, 2; ♀: 0, 0, 1, 2; ==>likely a consequence of liver enzyme induction			T
70a	EATS-mediated	Thyroid histopathology	Rat	52	Weeks	Oral		mg/kg bw/day	Change	Hypertrophy/Hyperplasia Incidence in ♂: 0,0,0,3 ==>likely a consequence of liver enzyme induction			T
70a	EATS-mediated	Thyroid histopathology	Rat	52	Weeks	Oral		mg/kg bw/day	No effect				T
70b	EATS-mediated	Thyroid histopathology	Rat	104	Weeks	Oral	242	mg/kg bw/day	Increase	Hyperplasia, follicular cells: Incidence in ♂: 2, 2, 4, 10 no increase in tumors			T
70b	EATS-mediated	Thyroid histopathology	Rat	104	Weeks	Oral		mg/kg bw/day	No effect				T

90a	EATS-mediated	Thyroid histopathology	Rat	10 (♂ and ♀ prematin g) +3 (♀ gestation) +3 (♀ lactation)	weeks	Oral	394 - 481	mg/kg bw/day	Change	Follicular hypertrophy/hyperplasia Incidences: F0: ♂ :0, 2, 3, 10; ♀ : 0, 0, 0, 16; F1: ♂ : 0, 1, 1, 15; ♀ : 0, 0, 0, 8; ==> treatment-related in high dose.			T
20	EATS-mediated	Thyroid histopathology	Mice	4	Weeks	Oral		mg/kg bw/day	No effect		No thyroid histo-pathological changes in mice or dogs.		T
40	EATS-mediated	Thyroid histopathology	Mice	13	Weeks	Oral		mg/kg bw/day	No effect				T
80	EATS-mediated	Thyroid histopathology	Mice	78	Weeks	Oral		mg/kg bw/day	No effect				T
50	EATS-mediated	Thyroid histopathology	Dog	13	Weeks	Oral		mg/kg bw/day	No effect				T
51	EATS-mediated	Thyroid histopathology	Dog	52	Weeks	Oral		mg/kg bw/day	No effect				T
52	EATS-mediated	Thyroid histopathology	Dog	52	Weeks	Oral		mg/kg bw/day	No effect				T
10	EATS-mediated	Colloid area (thyroid histopathology)	Rat	4	Weeks	Oral	1522/1331	mg/kg bw/day	Increase	Altered colloid: ↑minimal to slight flaky appearance of colloid at top dose Incidence in ♂: 1,2,2,3 ♀:0,0,0,2; Mean severity grading ♂ : 1-1-1-1.3; ♀ :0-0-0-1.0	Increased incidence of altered colloid (flaky appearance) in rats.		T
30	EATS-mediated	Colloid area (thyroid histopathology)	Rat	13	Weeks	Oral	792/814	mg/kg bw/day	Increase	Altered colloid: Incidence ♂: 1, 1, 0, 8; ♀: 0, 1, 0, 2; Mean severity grading ♂: 1, 1, 0, 1.4; ♀: 0, 1, 0, 1;			T
70a	EATS-mediated	Colloid area	Rat	52	Weeks	Oral	51	mg/kg bw/day	Change	Altered colloid in mid and high dose ♂ : 1,2,4,8;			T

		(thyroid histopathology)											
70a	EATS-mediated	Colloid area (thyroid histopathology)	Rat	52	Weeks	Oral	351	mg/kg bw/day	Change	Altered colloid in high dose ♀:0,1,1,6;			T
70b	EATS-mediated	Colloid area (thyroid histopathology)	Rat	104	Weeks	Oral	242	mg/kg bw/day	Increase	Altered colloid in high dose: Incidence in ♂: 7, 4, 12, 24** Mean severity grading in ♂: 1.6, 1.8, 1.3, 1.8;			T
70b	EATS-mediated	Colloid area (thyroid histopathology)	Rat	104	Weeks	Oral	317	mg/kg bw/day	Increase	Altered colloid: Incidence in ♀: 5, 6, 7, 33* Mean severity grading in ♀: 1.0, 1.3, 1.3, 1.3;			T
90a	EATS-mediated	Colloid area (thyroid histopathology)	Rat	10 (♂ and ♀ prematin g) +3 (♀ gestation) +3 (♀ lactation)	weeks	Oral		mg/kg bw/day	No effect	Altered colloid ==> no dose-dependency, individual findings in high dose animals= not treatment related F0: ♂ :0, 0, 3, 1; ♀ : 0, 0, 1, 1; F1: ♂ : 0, 2, 3, 1; ♀ : 0, 0, 0, 0;			T
20	EATS-mediated	Colloid area (thyroid histopathology)	Mice	4	Weeks	Oral		mg/kg bw/day	No effect		No change of colloid in mice.		T
40	EATS-mediated	Colloid area (thyroid histopathology)	Mice	13	Weeks	Oral		mg/kg bw/day	No effect				T
80	EATS-mediated	Colloid area (thyroid	Mice	78	Weeks	Oral		mg/kg bw/day	No effect				T

		histopathology)											
10	Target organ toxicity	Liver weight	Rat	4	Weeks	Oral	477	mg/kg bw/day	Increase	abs. & rel. (% change to Ctrl) in ♂ and ♀: >10%, dose dep., with hypertrophy at higher dose	Liver weight increased in rat, mice, dog, and rabbits.		T
20	Target organ toxicity	Liver weight	Mice	4	Weeks	Oral	1016	mg/kg bw/day	Increase	abs. & rel. (% change to Ctrl) in ♀: >20%, highest dose, no histopath.correlate			T
20	Target organ toxicity	Liver weight	Mice	4	Weeks	Oral	296	mg/kg bw/day	Increase	abs.(not signif.) & rel. (% change to Ctrl) in ♂: >15% / >10%, dose dependent, no histopath. correlate			T
30	Target organ toxicity	Liver weight	Rat	13	Weeks	Oral	211/240	mg/kg bw/day	Increase	abs. & rel. (% change to Ctrl) in ♂ and ♀: ≥12% / ≥11% , with hypertrophy at higher dose			T
40	Target organ toxicity	Liver weight	Mice	13	Weeks	Oral	201	mg/kg bw/day	Increase	abs. & rel. (% change to Ctrl) in ♂: >9% / >10%, dose dependent,			T
40	Target organ toxicity	Liver weight	Mice	13	Weeks	Oral	1304	mg/kg bw/day	Increase	abs. & rel. (% change to Ctrl) in ♀: 20% / 24%			T
50	Target organ toxicity	Liver weight	Dog	13	Weeks	Oral	96.5	mg/kg bw/day	Increase	abs. & rel. (% change to Ctrl) in ♂ and ♀: ≥16%/ ≥16%, no histopath. correlate;			T
50	Target organ toxicity	Liver weight	Dog	13	Weeks	Oral	91.9	mg/kg bw/day	Increase				T
51	Target organ toxicity	Liver weight	Dog	52	Weeks	Oral	83.4	mg/kg bw/day	Increase	abs. & rel. (% change to Ctrl) in ♂ and ♀: ≥11%/ ≥26%, no histopath. Correlate; limited evidence for adversity from clin. Chem.			T
51	Target organ toxicity	Liver weight	Dog	52	Weeks	Oral	81.4	mg/kg bw/day	Increase				T
52	Target organ toxicity	Liver weight	Dog	52	Weeks	Oral	81	mg/kg bw/day	Increase	abs. & rel. (% change to Ctrl) in ♂ and ♀: ≥5.6%/ ≥6.3% slight changes, no histopath correlate, limited evidence for adversity from clin. Chem.			T
52	Target organ toxicity	Liver weight	Dog	52	Weeks	Oral	71	mg/kg bw/day	Increase				T
70a	Target organ toxicity	Liver weight	Rat	52	Weeks	Oral	265	mg/kg bw/day	Increase	abs. & rel. (% change to Ctrl) in ♂ 16% (w. HCD)/ 14%, with histopath correlate, treatment-related;			T

70a	Target organ toxicity	Liver weight	Rat	52	Weeks	Oral	351	mg/kg bw/day	Increase	rel. (% change to Ctrl) in ♀: +17% considered secondary to body weight reduction (-13%). However, hypertrophy indicates liver as being induced			T
70b	Target organ toxicity	Liver weight	Rat	104	Weeks	Oral	45	mg/kg bw/day	Increase	rel. (% change to Ctrl) in ♂ at mid dose +5% secondary to body weight reduction, at high dose +13%, treatment related, at high dose with histopath Correlate, no neoplastic findings			T
70b	Target organ toxicity	Liver weight	Rat	104	Weeks	Oral	59	mg/kg bw/day	Increase	rel. In mid dose (+11%), and abs. & rel. (% change to Ctrl) in high dose ♂ 6%/ 20%, with histopath correlate, treatment-related;			T
80	Target organ toxicity	Liver weight	Mice	78	Weeks	Oral	162	mg/kg bw/day	Increase	rel. (% change to Ctrl) in ♂ at mid dose 12%secondary to body weight reduction, at high dose abs.&rel. +12%/38% treatment realted			T
80	Target organ toxicity	Liver weight	Mice	78	Weeks	Oral	27	mg/kg bw/day	Change	abs. (% change to Ctrl) in ♀ at low (-5%) and rel. at mid dose (+7%), secondary to body weight, at high dose rel. +27%, treatment related;			T
90a	Target organ toxicity	Liver weight	Rat	10 (♂ and ♀ prematin g) +3 (♀ gestation) +3 (♀ lactation)	weeks	Oral	81 - 97	mg/kg bw/day	Increase	abs. &rel. (% change to Ctrl) in Adult F0 ♂ and ♀ from mid dose onwards:≥ 8 %/≥ 4%), being adaptive first, adverse at higher dose levels			T
90a	Target organ toxicity	Liver weight	Rat	10 (♂ and ♀ prematin g) +3 (♀ gestation) +3 (♀ lactation)	weeks	Oral	384 - 481	mg/kg bw/day	Increase	abs. &rel. (% change to Ctrl) in Adult F1 ♂ and ♀ at high dose:≥ 19 %/≥ 19%), treatment related			T

100a	Target organ toxicity	Liver weight	Rat	1.5 (GD6-15)	weeks	Oral	1000	mg/kg bw/day	Increase	abs. &rel. (% change to Ctrl) in ♀ from 1000 mg/kg bw onwards:≥ 12 %/≥ 16%), treatment related			T
110a	Target organ toxicity	Liver weight	Rabbit	3 (GD 6-28)	weeks	Oral	80	mg/kg bw/day	Increase	abs. &rel. (% change to Ctrl) in ♀ from 80 mg/kg bw onwards:≥ 13 %/≥ 12%), dose dependent, treatment related,adaptive at 80 mg/kg bw			T
111a	Target organ toxicity	Liver weight	Rabbit	2 (GD 6-19)	weeks	Oral		mg/kg bw/day	No effect				T
112a	Target organ toxicity	Liver weight	Rabbit	2 (GD 7-19)	weeks	Oral		mg/kg bw/day	No effect				T
120	Target organ toxicity	Liver weight	Rat	7	weeks	Oral	87/99	mg/kg bw/day	Increase	abs. &rel. (% change to Ctrl) in ♂ & ♀ at top dose:≥ 9 %/≥ 8.5%), treatment related,			T
130	Target organ toxicity	Liver weight	Mice	7	weeks	Oral		mg/kg bw/day	No effect				T
10	Target organ toxicity	Liver histopathology	Rat	4	Weeks	Oral	1522/1331	mg/kg bw/day	Increase		Increased hepatocellular hypertrophy, cytoplasmatic alterations, fatty change and pigment storage.		T
30	Target organ toxicity	Liver histopathology	Rat	13	Weeks	Oral	211/240	mg/kg bw/day	Increase				T
70a	Target organ toxicity	Liver histopathology	Rat	52	Weeks	Oral	265/351	mg/kg bw/day	Change				T
70b	Target organ toxicity	Liver histopathology	Rat	104	Weeks	Oral		mg/kg bw/day	No effect				T
70b	Target organ toxicity	Liver histopathology	Rat	104	Weeks	Oral	242	mg/kg bw/day	Change	Cytoplasmatic alterations, periportal pigment storage in ♂			T
70b	Target organ toxicity	Liver histopathology	Rat	104	Weeks	Oral	59	mg/kg bw/day	Change	Hypertrophy in all high dose ♂ & ♀.			T
90a	Target organ toxicity	Liver histopathology	Rat	10 (♂ and ♀ prematin	weeks	Oral		mg/kg bw/day	No effect	Hypertrophy in mid dose, accompanied by peripheral fatty change and pigment storage in top			T

				g) +3 (♀ gestation) +3 (♀ lactation)						dose animals			
20	Target organ toxicity	Liver histopathology	Mice	4	Weeks	Oral		mg/kg bw/day	No effect	Cytoplasmatic alterations in males and Hypertrophy in both sexes			T
40	Target organ toxicity	Liver histopathology	Mice	13	Weeks	Oral	1200/1304	mg/kg bw/day	Change				T
80	Target organ toxicity	Liver histopathology	Mice	78	Weeks	Oral	904	mg/kg bw/day	Change	Eosinophilic alterations and centrilobular hypertrophy, reduced macrovesicular fatty change in ♂, no tumors			T
80	Target organ toxicity	Liver histopathology	Mice	78	Weeks	Oral	939	mg/kg bw/day	Change	Periportal hypertrophy, Hyperplasia, reduced macrovesicular fatty change in ♀, no tumors			T
50	Target organ toxicity	Liver histopathology	Dog	13	Weeks	Oral	1200/1304	mg/kg bw/day	No effect		Dog's Liver histopathology unchanged.		T
51	Target organ toxicity	Liver histopathology	Dog	52	Weeks	Oral	1200/1304	mg/kg bw/day	No effect				T
52	Target organ toxicity	Liver histopathology	Dog	52	Weeks	Oral		mg/kg bw/day	No effect				T
120	*In vivo* mechanistic	Phase I enzyme induction (*in vivo*)	Rat	7	weeks	Oral	26.4 / 29.2	mg/kg bw/day	Induction	slight CYP induction	The ability for cinmethylin to induce hepatic CYP activities in rats cannot be excluded.	Evidence for liver enzym induction.	T
130	*In vivo* mechanistic	Phase I enzyme induction (*in vivo*)	Mice	7	weeks	Oral	38.9 / 40.7	mg/kg bw/day	Induction	slight CYP induction			T
120	*In vivo* mechanistic	Phase II enzyme induction (*in vivo*)	Rat	7	weeks	Oral		mg/kg bw/day	No effect				T

130	*In vivo* mechanistic	Phase II enzyme induction (*in vivo*)	Mice	7	weeks	Oral		mg/kg bw/day	No effect				T
195	*In vitro* mechanistic	Deiodination enzyme activity (*in vitro*)	human deiodinase type 1	3	hours	Uptake from the medium (*in vitro*)	-	µM	No effect		No changes on mechanistic events up to the Cytotox-cutoff.	Overall negative evidence for endocrine activity *in vitro*.	T
194	*In vitro* mechanistic	Sodium-iodide symporter (NIS) (*in vitro*)	human kidney cell line	2	hours	Uptake from the medium (*in vitro*)	59	µM	Change	False positive; No effect seen up to the Cytotox-cutoff (6.6µM)			T
190	*In vitro* mechanistic	Thyroid receptor	Human liver cell line	24	hours	Uptake from the medium (*in vitro*)	-	µM	No effect				T
191	*In vitro* mechanistic	Thyroid receptor	rat pituitary gland cell line	28	hours	Uptake from the medium (*in vitro*)	-	µM	No effect				T
192	*In vitro* mechanistic	Thyroid receptor	rat pituitary gland cell line	28	hours	Uptake from the medium (*in vitro*)	38.4	µM	Change	False positive; No effect seen up to the Cytotox-cutoff (6.6µM)			T
193	*In vitro* mechanistic	Thyroperoxidase activity (TPO) (*in*	rat thyroid gland	0.5	hours	Uptake from the medium	34.18	µM	Change	False positive; No effect seen up to the Cytotox-cutoff (6.6µM)			T

		vitro)				(*in vitro*)							
200	*In vitro* mechanistic	TSH receptor (*in vitro*)	human kidney cell line	0.5	hours	Uptake from the medium (*in vitro*)	64.44	µM	Change	False positive; No effect seen up to the Cytotox-cutoff (6.6µM)			T
201	*In vitro* mechanistic	TSH receptor (*in vitro*)	human kidney cell line	0.5	hours	Uptake from the medium (*in vitro*))	-	µM	No effect				T
10	Systemic toxicity	Clinical chemistry and haematology	Rat	4	Weeks	Oral	1522/1331	mg/kg bw/day	Decrease	Prothrombin time: high dose ♂ : -15.7%** ♀: -21.4% **;	Changes in clinical chemistry/ haematology secondary to reduced food intake at higher doses.		Sys
10	Systemic toxicity	Clinical chemistry and haematology	Rat	4	Weeks	Oral	477	mg/kg bw/day	Increase	GGT: mid dose ♂ : 36** ↑; ♀ : -65 ** ↑; low dose ♀: 6* (within HCD-incidental);			Sys
10	Systemic toxicity	Clinical chemistry and haematology	Rat	4	Weeks	Oral	477	mg/kg bw/day	Change	♂ Cholesterol, Calcium, Glucose from mid dose onwards; Protein, Alb, and Trig. from high dose onwards; ♀ Prot, Glob, Glucose (w HCD) from mid dose onwards; Cholesterol from high dose onwards; indicative of higher liver metabolism			Sys
20	Systemic toxicity	Clinical chemistry and haematology	Mice	4	Weeks	Oral	791/1016	mg/kg bw/day	Change	High dose:↓ albumin (♂+♀),↓ protein, globulin, cholesterol and triglycerides and bilirubin (♂);			Sys

30	Systemic toxicity	Clinical chemistry and haematology	Rat	13	Weeks	Oral	814	mg/kg bw/day	Decrease	HGB slightly reduced <10%, with HCT and RBC being within the normal range is considered treatment related but not adverse			Sys
30	Systemic toxicity	Clinical chemistry and haematology	Rat	13	Weeks	Oral		mg/kg bw/day	No effect				Sys
30	Systemic toxicity	Clinical chemistry and haematology	Rat	13	Weeks	Oral	814	mg/kg bw/day	Decrease	Prothrombin time: high dose ♂ : -6.9%**;			Sys
30	Systemic toxicity	Clinical chemistry and haematology	Rat	13	Weeks	Oral	240	mg/kg bw/day	Decrease	Prothrombin time: high dose ♀: -13.8%**; mid dose ♀: -5.9%**			Sys
30	Systemic toxicity	Clinical chemistry and haematology	Rat	13	Weeks	Oral	211	mg/kg bw/day	Decrease	EOS abs. in high dose ♀-44%** EOS rel. in high dose ♂ -26.3%* and mid dose ♂ -26.3%* no clear dose response, most probably due to some stress reaction.			Sys
30	Systemic toxicity	Clinical chemistry and haematology	Rat	13	Weeks	Oral	814	mg/kg bw/day	Decrease	EOS abs. in high dose ♀-44%** EOS rel. in high dose ♀ -35%** most probably as in males due to some stress reaction.			Sys
30	Systemic toxicity	Clinical chemistry and haematology	Rat	13	Weeks	Oral	211/240	mg/kg bw/day	Increase	GGT: high dose ♂ : 292**↑; ♀: 268** ↑; mid dose ♂ : 27* ↑; females: 46**↑;			Sys

30	Systemic toxicity	Clinical chemistry and haematology	Rat	13	Weeks	Oral	211/240	mg/kg bw/day	Change	♂: mid dose onwards: ↑ Calcium ; High dose: ↓Glucose (Stress indicator), ↑ Prot, Alb, Cholesterol, Na, K, Cl, P; ♀: mid dose onwards: ↑ Calcium, K, Glob, Chol, Alb, Prot; ↓ Bilirubin High dose: ↓ Creatinine, Glucose; ↑ Trig., indication of transporter proteins and liver enzyme induction, stress response and acidose at high dose			Sys
40	Systemic toxicity	Clinical chemistry and haematology	Mice	13	Weeks	Oral	1304	mg/kg bw/day	Increase	HGB, corpuscular volume (MCV) and corpuscular hemoglobin content (MCH), and mean corpuscular hemoglobin concentration (MCHC) were increased in top dose females: +10%**, +2.9%**, +4.4%**, +1.8%**			Sys
40	Systemic toxicity	Clinical chemistry and haematology	Mice	13	Weeks	Oral	43	mg/kg bw/day	Increase	MCV in Control, low, mid, high dose: 0, 0.4%, 1.3%**, 3.8%** MCH in Control, low, mid, high dose:: 0, 1.1%*, 2.3%**, 5.7%**			Sys
40	Systemic toxicity	Clinical chemistry and haematology	Mice	13	Weeks	Oral		mg/kg bw/day	No effect				Sys
40	Systemic toxicity	Clinical chemistry and haematology	Mice	13	Weeks	Oral	1200/1304	mg/kg bw/day	Decrease	White blood cell parameters: top dose males: WBC -31%**; NEUT -22%*; LYMPH -31%**; EOS -60%*; top dose females: EOS -50%**			Sys
40	Systemic toxicity	Clinical chemistry and haematology	Mice	13	Weeks	Oral	201	mg/kg bw/day	Decrease	♂: mid dose onwards: ↓ Prot, Alb, Cholesterol; Bilirubin high dose: additionally ↓TRIG			Sys

40	Systemic toxicity	Clinical chemistry and haematology	Mice	13	Weeks	Oral	1304	mg/kg bw/day	Decrease	♀: High dose: ↓TRIG;			Sys
50	Systemic toxicity	Clinical chemistry and haematology	Dog	13	Weeks	Oral		mg/kg bw/day	No effect				Sys
50	Systemic toxicity	Clinical chemistry and haematology	Dog	13	Weeks	Oral		mg/kg bw/day	No effect				Sys
51	Systemic toxicity	Clinical chemistry and haematology	Dog	52	Weeks	Oral	285	mg/kg bw/day	Change	Haematology: High dose ♂: ↓RBC , HCT, and HGB, ↑ Platelet count, WBC, lymphocyte, neutrophil count, monocyte			Sys
51	Systemic toxicity	Clinical chemistry and haematology	Dog	52	Weeks	Oral	83	mg/kg bw/day	Change	Haematology: High dose ♀: ↓RBC , HCT, and HGB, ↑ Platelet count, lymphocyte mid dose onwards: ♀: ↑ WBC, neutrophil count			Sys
51	Systemic toxicity	Clinical chemistry and haematology	Dog	52	Weeks	Oral	83/81	mg/kg bw/day	Change	Mid dose: ↑ ALP (♂ & ♀), ↓ Albumin (♂ only) High Dose ♀ additionally: ↓Total protein, Calcium, Inorganic phosphorus; (A relation to treatment is most likely secondary to the lower food intake in the high dose group females). ♂ only: Reduced ALT;			Sys
52	Systemic toxicity	Clinical chemistry and haematology	Dog	52	Weeks	Oral	81	mg/kg bw/day	Change	Haematology: High dose ♂: ↑ WBC, abs. neutrophil count week 13, 26 & 52, rel. neutrophils count week 26 & 52 week, ↓ lymphocytes (week 26 &			Sys

										52)			
52	Systemic toxicity	Clinical chemistry and haematology	Dog	52	Weeks	Oral	71	mg/kg bw/day	Change	Haematology: High dose: ↑ WBC, abs. neutrophil count week 13			Sys
52	Systemic toxicity	Clinical chemistry and haematology	Dog	52	Weeks	Oral		mg/kg bw/day	No effect				Sys
52	Systemic toxicity	Clinical chemistry and haematology	Dog	52	Weeks	Oral	71	mg/kg bw/day	Change	High dose: ↑ ALP (week 13, 26 & 52)			Sys
70a	Systemic toxicity	Clinical chemistry and haematology	Rat	52	Weeks	Oral	265	mg/kg bw/day	Change	Haematology: High dose ♂ (12 weeks): ↑ RET [%], MCV; These changes reflected an increased red blood cell synthesis of the bone marrow. This alteration was transient, since it did not occur in later study periods. Therefore, the mentioned changes were regarded as an adaptive effect.			Sys
70a	Systemic toxicity	Clinical chemistry and haematology	Rat	52	Weeks	Oral		mg/kg bw/day	No effect				Sys

70a	Systemic toxicity	Clinical chemistry and haematology	Rat	52	Weeks	Oral	51	mg/kg bw/day	Change	High Dose: ↑ GGT (♂, 3-12M); Cholesterol (3M, ♂) & Albumin (6M, ♂), ↓ Glucose (♂ 3M & 6M), Mid Dose: ↓ Glucose (♂, 3M) Treatment related but not adverse as this was the only relevantly changed clinical pathology parameter at mid dose.			Sys
70a	Systemic toxicity	Clinical chemistry and haematology	Rat	52	Weeks	Oral	351	mg/kg bw/day	Change	High Dose: ↑ GGT (♀, 3-12M); ↓ Glucose (♀ 3M); ↑ K (♀, 6M)			Sys
80	Systemic toxicity	Clinical chemistry and haematology	Mice	78	Weeks	Oral		mg/kg bw/day	No effect				Sys
110a	Systemic toxicity	Clinical chemistry and haematology	Rabbit	3 (GD 6-28)	weeks	Oral	250	mg/kg bw/day	Increase	GGT (compared to control at 25, 80,250, 320 mg/kg bw): -4%, -8%,+51%*,+87%**;			Sys
10	STBNDO*	Brain weight	Rat	4	Weeks	Oral		mg/kg bw/day	No effect		Brain weight changes secondary to body weight.		N
20	STBNDO*	Brain weight	Mice	4	Weeks	Oral		mg/kg bw/day	No effect				N
30	STBNDO*	Brain weight	Rat	13	Weeks	Oral	792	mg/kg bw/day	Increase	abs. & rel. (% change to Ctrl) in ♂: 0, -3%, -4%*, -6%** (w HCD) / 0, -0%, +2%, +13%**. Values within the HCD range, relative change <10 %, lack of correlated histopathology. Not treatment-related.			N
30	STBNDO*	Brain weight	Rat	13	Weeks	Oral	814	mg/kg bw/day	Increase	Rel. (% change to Ctrl) in ♀: +12%**; secondary to body weight reduction (-14%). Values within the HCD			N

										range, relative change <10 %, lack of correlated histopathology. Not treatment-related.			
70a	STBNDO*	Brain weight	Rat	52	Weeks	Oral		mg/kg bw/day	No effect				N
70b	STBNDO*	Brain weight	Rat	104	Weeks	Oral	242	mg/kg bw/day	Increase	rel. (% change to Ctrl) in ♂ top dose: +8%** secondary to body weight reduction (-9%). Not treatment-related.			N
70b	STBNDO*	Brain weight	Rat	104	Weeks	Oral	317	mg/kg bw/day	Increase	rel. (% change to Ctrl) in ♀ top dose: +14%**) secondary to body weight reduction (-12%). Not treatment-related.			N
90a	STBNDO*	Brain weight	Rat	10 (♂ and ♀ prematin g) +3 (♀ gestation) +3 (♀ lactation)	weeks	Oral		mg/kg bw/day	No effect				N
90b	STBNDO*	Brain weight	Rat	3 (gestation) +3 (lactation)	weeks	Oral		mg/kg bw/day	No effect				N
40	STBNDO*	Brain weight	Mice	13	Weeks	Oral		mg/kg bw/day	No effect				N
80	STBNDO*	Brain weight	Mice	78	Weeks	Oral	25	mg/kg bw/day	Change	abs. & rel. (% change to Ctrl) in ♂ : Low dose: -0.1% /+8.1%**; High dose: -1.9%** /+20%**, sec. to body weight (low dose -7%, high dose -19%)			N
80	STBNDO*	Brain weight	Mice	78	Weeks	Oral	184	mg/kg bw/day	Change	abs. & rel. (% change to Ctrl) in ♀ : Mid dose: -1.1%/+ 9.1%**; High dose: -2.5%**/+24%**; sec. to body weight (mid dose -9 %; high dose: -22%)			N

50	STBNDO*	Brain weight	Dog	13	Weeks	Oral		mg/kg bw/day	No effect				N
51	STBNDO*	Brain weight	Dog	52	Weeks	Oral		mg/kg bw/day	No effect				N
51	STBNDO*	Brain weight	Dog	52	Weeks	Oral	285	mg/kg bw/day	Increase	rel. (% change to Ctrl) in ♀ top dose: +32%**) sec. to body weight reduction (-30%)			N
52	STBNDO*	Brain weight	Dog	52	Weeks	Oral		mg/kg bw/day	No effect				N

STBNDO*: Sensitive to, but not diagnostic of, EATS; *: p≤0.05; **: p≤0.01;

Adversity

Thyroid

Thyroid findings (weight and histopathology): Thyroid findings (histopathological changes accompanied with organ weight increases at higher dose levels) were observed in all rat studies. The 28-day study in the rat revealed thyroid histopathological changes with no change in thyroid weight. An increase in the incidence of thyroid follicular hypertrophy/hyperplasia was observed from 477 mg/kg bw/d; incidence and severity were dose-dependently increased. In affected animals, the number of small follicles was increased or the follicular epithelium was higher, varying in size from cuboidal cells to columnar cells. The incidence of altered colloid (which showed a flaky appearance) was increased at the top dose.

The 90-day rat study showed treatment-related effects on thyroid weight and histopathology. Relative thyroid weight was statistically-significant increased in females of the top dose (relative change 25 % compared to controls). Both the absolute and relative values were outside the HCD range, therefore, this effect was considered to be treatment-related and adverse. In males from the mid dose (211 mg/kg bw/d), hypertrophy/hyperplasia of the follicular cells was observed with a dose-related increase of incidence and severity. The same was seen in females but to a lower extent. Most top dose males (8/10) and some top dose females (2/10) revealed altered (flaky) colloid of increased incidence and grading.

The chronic study in rats, showed no change in thyroid weight but revealed thyroid histopathological changes. No thyroid weight increase was observed up to the highest dose (5000 ppm, equivalent to 265 and 351 mg/kg bw/d in males and females, respectively) after 12-months. At 12-months, males revealed minimal to slight hypertropy/hyperplasia (3/10) and an increase in altered colloid was observed from the mid dose males and at top dose females. These findings were considered treatment-related and adverse at the top dose (265/351 mg/kg bw/d for males and females, respectively). At 24-months, findings in the thyroid at the top dose (242/317 mg/kg bw/d for males and females, respectively) - an increase in follicular cell hyperplasia in males and an increase in altered colloid in males and females - were considered treatment-related.

The 2 generation study in rats showed treatment-related effects on thyroid weight and histopathology. Thyroid weights (absolute and relative) were consistently statistically significantly increased at the top dose 5000 ppm (394 mg/kg bw/d), in parental males and females of both generations. Change compared to controls was between 15 – 24 %. In parental males statistically significant values were within the HCD but close to the HCD maximum; in parental females values were at the HCD maximum or outside the HCD range. Concomitant histopathology (an increase in the incidence and severity of hypertrophy/hyperplasia of follicular epithelial cells) was recorded at the top dose of both generations, in both males and females. Overall, treatment-related and adverse increases in thyroid weights with associated histopathology were seen at the top dose in parental males and females of both generations.

In mice and dogs, no histopathological changes were seen in the thyroid in any study. Dog thyroid weights showed large variability in one of three 1-year studies, however, the lack of dose dependency and microscopic correlate leads to the conclusion that the thyroid is not a target organ in dogs.

LOAELs and NOAELs of treatment-related thyroid findings are summarised below (Table 6.8-26). The NOAEL for thyroid findings across all studies was 1,000 ppm (51-81 mg/kg bw/d). The LOAEL for thyroid weight effects across all studies was 5,000 ppm (412/394 mg/kg bw/d in males and females, respectively), from the 2 generation study in rats. The LOAELs for histopathological effects across all studies were:

- 3,000 ppm (211/240 mg/kg bw/d in males and females, respectively), for hypertrophy/hyperplasia of follicular cells, from the 90-day study in rats.
- 5,000 ppm (242/317 mg/kg bw/d in males and females, respectively), for altered colloid (of flaky appearance) from the combined chronic toxicity and carcinogenicity study in rats.

Table 6.8-26. Lowest effect level (+) and no effect level (-) of treatment-related thyroid findings

Dose [ppm]	Males ♂ Study type, Dose level [mg/kg bw/day]				Females ♀ Study type, Dose level [mg/kg bw/day]			
	Thyroid w.	**Thyroid Hypertrophy/ Hyperplasia**	**Hyperplasia of follicular cells**	**Altered colloid**	**Thyroid w.**	**Thyroid Hypertrophy/ Hyperplasia**	**Hyperplasia of follicular cells**	**Altered colloid**
200								
1000	2gen NOAEL = 79.	90d NOAEL = 67. 2gen NOAEL = 79. chr/carc, 12m NOAEL = 51.	90d NOAEL = 67. 2gen NOAEL = 79. chr/carc, 24m NOAEL = 45.	chr/carc, 12m NOAEL = 51. chr/carc, 24m NOAEL = 45.	2gen NOAEL = 81	90d NOAEL = 79. 2gen NOAEL = 81.	90d NOAEL = 79. 2gen NOAEL = 81.	chr/carc, 12m NOAEL = 69. chr/carc, 24m NOAEL = 59.
3000	90d NOAEL = 211	90d LOAEL = 211.	90d LOAEL = 211.	90d NOAEL = 211.	90d NOAEL = 240.	90d LOAEL = 240	90d LOAEL = 240	90d NOAEL = 240.
5000	chr/car, 12m NOAEL = 265. chr/car, 24m NOAEL = 242. 2gen LOAEL = 412	chr/carc, 12m LOAEL = 265. 2gen LOAEL = 412.	chr/carc, 24m LOAEL = 242. 2gen LOAEL = 412. 28d LOAEL = 477.	chr/car, 12m LOAEL = 265. chr/car, 24m LOAEL = 242.	chr/car, 12m NOAEL = 351. chr/car, 24m NOAEL = 317. 2gen LOAEL = 394	chr/carc, 12m NOAEL = 351. 2gen LOAEL = 394.	chr/carc, 24m NOAEL = 317. 2gen LOAEL = 394. 28d LOAEL = 477.	chr/car, 12m LOAEL = 351. chr/car, 24m LOAEL = 317.
10000	90d LOAEL = 792			90d LOAEL = 792.	90d LOAEL = 814	90d LOAEL = 814.		90d LOAEL = 814.

Study type: d = days; chr/car = combined chronic toxicity and carcinogenicity, m = month; gen = generation; w.: weight;

Liver

The applicant proposes that the thyroid effects seen in the rat are secondary to liver enzyme induction. Therefore, an analysis of the liver findings produced by cinmethylin is also presented below. Liver findings such as enlargement and weight increases (absolute and relative) were seen in all repeated dose studies, consistently in rat, mice and dogs. Liver weight increases were generally observed in combination with:

- Liver histopathology (e.g. hepaocellular hypertrophy and cytoplasmic alterations)
- Changes in clinical chemistry parameters, indicative of liver toxicity. The applicant suggested that the clinical pathology parameters observed were indicative of liver enzyme induction as follows:
  - shortened prothrombin time due to increased biosynthesis of coagulation factors,
  - increased total protein (albumin, globulin) as well as cholesterol because of an increased biosynthesis,
  - increased calcium levels because of higher levels of transporter proteins,
  - lower plasma bilirubin levels in absence of anaemia is likely due to an increased conjugation rate and accelerated excretion of bilirubin via the bile.

HSE notes that this is plausible but has not been demonstrated

In rats, the liver was affected from a dose of 211 mg/kg bw/d in the 90-day study, at 394 mg/kg bw/d in the 2-generation study and at 45 mg/kg bw/d in the chronic/carcinogenicity studies. These are the same dose levels at which the thyroid was affected.

Biochemical investigations conducted with cinmethylin in the rat ([redacted] 1983a) and mouse ([redacted] 1983b) demonstrated slight CYP induction, without liver weight increases or changes in hepatic glutathione levels (GSH). However, specific hepatic UDP-glucuronosyltransferases (UGTs as phase II enzymes) were not identified in these mechanistic studies, therefore a MoA for the thyroid effects involving increased clearance of thyroid hormones has not been fully demonstrated.

Overall, in studies in rats, mice and dogs, the liver (all species) and thyroid (rats only) were shown to be target organs. The applicant suggested that thyroid effects are a consequence of liver enzyme induction, however, this is not sufficiently substantiated. A detailed comparative MoA analysis is necessary to assess whether thyroid adversity is secondary to liver effects. The weight of evidence analysis should include a time- and dose-concordance analysis of liver and thyroid effects.

HSE notes that, as per Appendix A of the EFSA/ECHA guidance, as cinmethylin is seen to induce histopathological changes in the thyroid, it poses a potential hazard for human thyroid insufficiency in adults as well as pre- and post-natal neurological development of offspring. This issue has not been addressed by the applicant.

Pituitary

The lack of pituitary effects is already discussed for EAS modality. Overall, there is no indication for any functional impairment of the pituitary gland or any adverse effect on pituitary in any species investigated.

Endocrine activity

ToxCast Data

There are 13 assay endpoints (for which cinmethylin was evaluated) in the ToxCast/Tox21 *in vitro* high-throughput screening program that inform on thyroid perturbation. Three of these assays focus on the thyroid receptor (TR), one investigates thyroid peroxidase (TPO), one looks at deiodinase (DIO) and one is the sodium-iodide-symporter (NIS) inhibition assay. The last endpoint, deiodinase inhibition, is not yet included as a ToxCast endpoint but is available as a publication (Hornung *et al*., 2017). Furthermore, two assays on the Thyroid stimulating hormone receptor (TSHR) are now included in the ToxCast data. Several assays were supplemented by the respective cytotoxicity assays. There are also publications for TPO (Friedman *et al*., 2016), NIS (Wang *et al.,* 2018) and DIO (Hornung *et al.,* 2017) assays. Results from both ToxCast data and relevant publications are summarised below (Table 6.8-27).

Table 6.8-27. ToxCast Thyroid-related assays for cinmethylin

Process Indicator		**Assay Endpoint Name**	**Cinmethylin**		
[Agonist (A) or Antagonist (Anti-A) Signal Detection]			**AC50 (µM)**	**ACC (µM)**	**Measured Max. percent inhibition (Concentration) [max_med]**
Binding to Thyroid receptor	[A]	ATG_THRa1_TRANS_up / _dn	inactive		
	[A]	TOX21_TR_LUC_GH3_Agonist	inactive		
	[Anti-A]	TOX21_TR_LUC_GH3_Antagonist [2, 3.]			
			38.0	44.7	34.5% (90 µM)
	Tox	Tox21_TR_LUC_GH3_Antagonist viability	47.86		23.7% (90 µM)
Activation of TSH Receptor	[A]	TOX21_TSHR_Agonist_ratio	inactive		
	[Anti-A]	TOX21_TSHR_Antagonist_ratio[2]	64.57	63.1	25.7% (90µM)
TPO inhibition		NCCT_TPO_AUR_dn [2]	33.88	22.35	40.2% (90 µM)
	Tox	NCCT_HEK293T_CellTiterGLO	24	16.6	80.2% (100 µM)
DIO1 inhibition		Not yet defined	inactive		
NIS inhibition		NIS_RAIU_inhibition assay [1,2]	58.88	7.7	41.5% (100 µM)

	Tox	NIS_HEK293T_CTG_Cytotoxicity[1,2]	30	25	22.6% (100 µM)

TSH: Thyroid-stimulating hormone;
TPO: Thyroidperoxidase;
NIS: Sodium-Iodide-Symporter;
DIO: Deiodinase;
Tox: corresponding Cytotoxicity assay established within the set of studies
1 : Only highest conc above baseline, active;
2 : Less than 50% efficacy
3 : Noisy data

The ATG_THRa1_Trans_up (ATG_THRa1_Trans_dn) assay (study ID matrix 190), which investigated the regulation of the transcription factor activity of the thyroid hormone receptor α in human liver HepG2 cells was inactive (i.e. no effect of cinmethylin).

No agonistic activity of cinmethylin was identified in thyroid receptor ligand-binding assay, measuring the increase of luminescence resulting from thyroid receptor response element-driven expression of luciferase in rat pituitary GH3 cells (Tox21_TR_LUC_GH3_Agonist - study ID matrix 191).

By contrast, in the analogue assay in presence of 1 µM T3 (Tox21_TR_LUC_GH3_Antagonist - study ID matrix 192), an antagonistic activity of cinmethylin was observed, indicated by a decrease of T3 induced luciferase synthesis. This activity occurred with an AC50 of 38 µM. However, the response cut-off was only exceeded at the ACC value of 44.7 µM, which is close to the range of cytotoxicity (with an AC50 of 47.86 µM). Generally, the activity was quite weak, considering that maximum measured inhibition was 34.5 % at 90 µM; compared to the 23.7 % cytotoxicity reached at 90 µM. The antagonistic activity plot is flagged as *'less than 50% efficacy'* and *'noisy data'*. These data indicate that antagonistic acitivty is mainly detected at a concentration range inducing cytotoxicity. Any effect in this loss of signal assay is therefore considered likely to be the result of cytotoxicity. In light of the lower bound cytotoxicity level of 6.6 µM these would all be considered inactive. Any activity seen is above the median cytotoxicity level of 23.54 µM. Overall, the Tox21_TR_LUC_GH3_Antagonist assay is considered a false positive.

The TSH receptor assays demonstrate no agonistic activity (TOX21_TSHR_Agonist_ratio - study ID matrix 201) but measured antagonistic activity towards the thyroid stimulating hormone receptor in the human kidney cell line HEK293T (TOX21_TSHR_Antagonist_ratio - study ID matrix 200). Antagonisic activity is seen only at the highest concentration, with less than 50 % efficacy. The measured activity is 25.7 % inhibition at 90 µM. The AC50 is measured at 64.57 µM. This study is conducted in HEK293T cells; cytotoxicity of cinmethylin is found to be in the range of 24 – 30 µM, as seen in the NCCT_HEK293T_CellTiterGLO and the NIS_HEK293T_CTG_Cytotoxicity assays. Therefore, any effect in this loss of signal assay is considered likely to be the result of cytotoxicity. In light of the lower bound cytotoxicity of 6.6 µM and the median cytotoxicity level of 23.54 µM, no specific TSHR interaction is observed. Overall, the TOX21_TSHR_Antagonist_ratio assay is considered a false positive and cinmethylin is inactive in the TSH receptor assay.

Friedman *et al*. (2016) published the results of ToxCast high-throughput screening assay for thyroidperoxidase inhibition (study summarised below, BASF DocID 2016/1351637). The plots are now also available in ToxCast database (with AC50 values). The TPO activity plot is flagged for '*less than 50 % efficacy'*. The concentration activity curves for TPO inhibition demonstrate that loss of signal was only seen at the highest dose, with less than 50 % efficacy. The activity reached 40 % at 90µM, with a measured AC50 value at 33.88 µM and a ACC level at which the response cut off is exceeded of 22.35 µM. The corresponding cytotoxicity study (NCCT_HEK293T_CellTiterGLO - study ID matrix 193) revealed an AC50 of 24 µM. Therefore, any effect in the TPO loss of signal assay is considered likely to be the result of cytotoxicity. In light of the lower bound cytotoxicity of 6.6 µM and the median cytotoxicity level of 23.54 µM, no specific TPO interaction is observed. Overall, the NCCT_TPO_AUR_dn assay (study ID matrix 193) is considered a false positive and cinmethylin is inactive in the TPO assay.

The NIS inhibition assay (NIS_RAIU_inhibition - study ID matrix 194) and specific cytotoxicity in the assay system (NIS_HEK293T_CTG_Cytotoxicity - study ID matrix 194) are comparable. Both are flagged for *'only highest concentration above baseline active'* and *'less than 50 % efficacy'*, therefore, they are borderline actives. The AC 50 for NIS inhibition (58.88 µM) is above the AC50 for the corresponding cytotoxicity assay (30 µM). The modeled ACC for the NIS inhibition (7.7 µM) is below the cytotoxicity ACC (25 µM). However, low ACC value is only an artefact of modelling; no response cut-off was exceeded at the concentration of 10 µM. Only

the highest dose showed activity. Therefore, any effect in this loss of signal assay is considered likely to be the result of cytotoxicity. In light of the lower bound cytotoxicity of 6.6 µM and the median cytotoxicity level of 23.54 µM, no specific NIS inhibition is observed. Overall, the NIS_RAIU_inhibition assay is considered a false positive and cinmethylin is inactive in the NIS inhibition assay.

Overall
Cinmethylin had no effect on: the *in vitro* regulation of the transcription factor activity of the thyroid hormone receptor α in human liver HepG2, thyroid receptor activity (no agonist effect) and/or deiodinase inhibition.

Antagonistic activity on thyroid receptor binding (AC50 38 µM), activation of TSH receptor (AC50 64.57 µM), thyroidperoxidase (TPO) enzyme inhibition (AC50 33.88 µM) and sodium iodide symporter (NIS) inhibition (AC50 58.88 µM) was noted. However, those effects are observed at relatively high concentrations, above the lower bound cytotoxicity level of 6.6 µM, and the median cytotoxicity limit of 23.54 µM. All are therefore non-specific effects, mirroring cytotoxicity. This is supported by the corresponding cytotoxicity assays which show significant cytotoxicity in the respective cell systems at similar concentrations. Furthermore, these assays are flagged because the data are active only at the highest concentration (90 – 100 µM) and/or reach less than 50 % efficacy.

Overall, the ToxCast/Tox21 data suggest that thyroid-related assay endpoints are not primary target for cinmethylin. These data indicate that there is no specific interaction of cinmethylin with the thyroid.

Summay of publications
1)

Report reference	Friedman *et al.* (2016) BASF Study ID: 2016/1351687
Report title	Tiered high-throughput screening approach to identify Thyroperoxidase inhibitors within the ToxCast Phase I and II Chemical Libraries
Guidelines	None.
GLP	No.
Reliability	Reliable, noting more up-to-date ToxCast data are available.
Relevance	Supplemental information to be used alongside ToxCast data.
Acceptable	Yes, regarded as supplemental information only.
Conclusion	Cinmethylin is a non-selective TPO inhibitor; TPO is not a target for cinmethylin Generally consistent (with minor differences in specific numbers) with new/modern ToxCast data.

Executive Summary
High-throughput screening for potential thyroid-disrupting chemicals requires a system of assays to capture multiple molecular-initiating events (MIEs) that converge on perturbed thyroid hormone (TH) homeostasis. Screening for MIEs specific to TH-disrupting pathways is limited in the U.S. Environmental Protection Agency ToxCast screening assay portfolio. To fill 1 critical screening gap, the Amplex UltraRed-thyroperoxidase (AUR-TPO) assay was developed to identify chemicals that inhibit TPO, as decreased TPO activity reduces TH synthesis. The ToxCast phase I and II chemical libraries, comprised of 1074 unique chemicals, were initially screened using a single, high concentration to identify potential TPO inhibitors. Chemicals positive in the single-concentration screen were retested in concentration-response. Due to high false-positive rates typically observed with loss-of-signal assays such as AUR-TPO, we also employed 2 additional assays in parallel to identify possible sources of nonspecific assay signal loss, enabling stratification of roughly 300 putative TPO inhibitors based upon selective AUR-TPO activity. A cell-free luciferase inhibition assay was used to identify nonspecific enzyme inhibition among the putative TPO inhibitors, and a cytotoxicity assay using a human cell line was used to estimate the cellular tolerance limit. Additionally, the TPO inhibition activities of 150 chemicals were compared between the AUR-TPO and an orthogonal peroxidase oxidation assay using guaiacol as a substrate to confirm the activity profiles of putative TPO inhibitors. This effort represents the most extensive TPO inhibition screening campaign to date and illustrates a tiered screening approach that focuses resources, maximizes assay throughput, and reduces animal use.

Materials and Methods

The NCCT_TPO_AUR_dn inhibition assay is a loss-of-signal assay based on the conversion of the fluorogenic substrate Amplex ® UltraRed (AUR) to Amplex UltroxRed in the presence of excess H_2O_2 with an efficacy cut-off of 20 % enzyme inhibition. Thyroidperoxidase (TPO) was extracted from rat thyroid microsomes. Cinmethylin is part of the ToxCast program and as such defined via its CAS Nr., procured from an undisclosed commercial source. Stock solutions were prepared in DMSO at a target concentration of 20 mM. Cinmethylin was run in 3 separate trials at 8 concentrations with Methimazole (MMI) as positive control. Due to the high false-positive rate typically observed with loss-of-signal assays, Friedman *et al.* (2016) employed 2 additional assays in parallel to identify possible sources of non-specific assay signal loss, a cytotoxicity assay and a luciferase inhibition assay which are presented for cinmethylin (Figure 6.8-6).

Results

TPO inhibition exceeds the baseline of 20 % at the highest tested concentration of 90 µM and reached a level of 40 % inhibition. The effect of cinmethylin at the AC50 (34.176 µM) is close to the efficacy cut-off value. Cinmethylin did not influence luciferase activity but the cytotoxicity in HEK295R cells (AC50 = 23.98 µM) as well as the median cytotoxicity concentration of cinmethylin (23.54 µM) are below the AC50 of TPO inhibition.

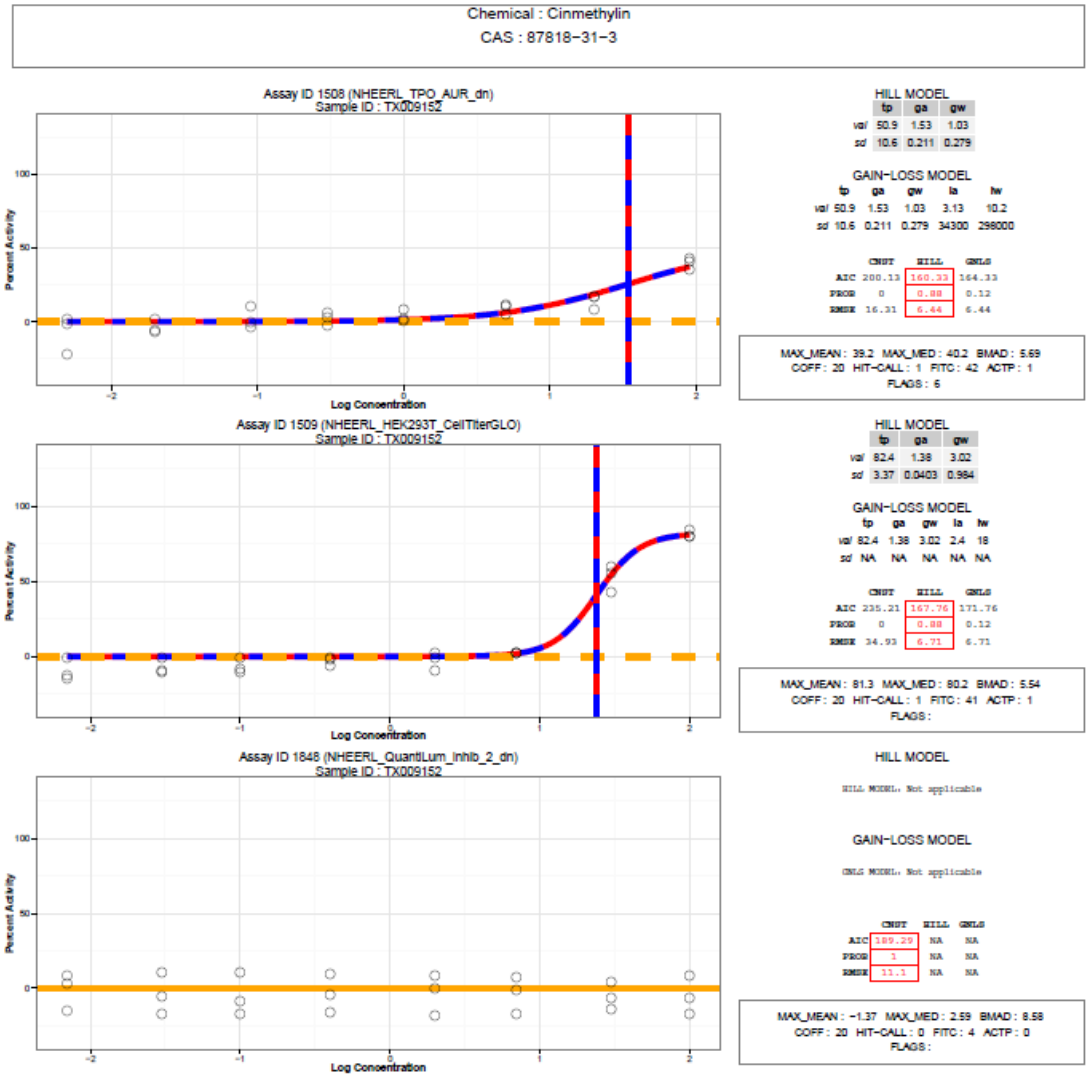


Figure 6.8-6. Dose response curve of TPO-inhibition (top), cytotoxicity in HEK293T cells (middle) and luciferase-inhibition (bottom)

Conclusion
Cinmethylin is ranked in the group of non-selective TPO inhibitors, characterized by TPO effects at relatively high concentration relative to the lower observed cytotoxicity limit, suggesting that TPO is not a target for cinmethylin.

HSE notes that the results of this study are generally consistent with the data from the new/modern ToxCast database, small differences in specific numbers were seen.

2)

Report reference	Wang *et al.* (2018) BASF Study ID: 2018/1086611
Report title	High-throughput screening and quantitative chemical ranking for sodium iodide symporter (NIS) inhibitors in ToxCast Phase I chemical library
Guidelines	None.
GLP	No.
Reliability	Reliable, noting more up-to-date ToxCast data are available
Relevance	Supplemental information to be used alongside ToxCast data.
Acceptable	Yes, regarded as supplemental information only.
Conclusion	No specific NIS inhibition was seen with cinmethylin. Generally consistent (with minor differences in specific numbers) with new/modern ToxCast data.

Executive Summary
Thyroid uptake of iodide via the sodium-iodide symporter (NIS) is the first step in the biosynthesis of thyroid hormones that are critical for health and development in humans and wildlife. Despite having long been a known target of endocrine disrupting chemicals such as perchlorate, information regarding NIS inhibition activity is still unavailable for the vast majority of environmental chemicals. This study applied a previously validated high-throughput approach to screen for NIS inhibitors in the ToxCast phase I library, representing 293 important environmental chemicals. Here 310 blinded samples were screened in a tiered-approach by an initial single-concentration (100 μM) radioactive-iodide uptake (RAIU) assay, followed with 169 samples further evaluated in multi-concentration (0.001 μM – 100 μM) testing in parallel RAIU and cell viability assays. A novel chemical ranking system that incorporates multi-concentration RAIU and cytotoxicity responses was also developed as a standardised method for chemical prioritisation in current and future screenings. Representative chemical responses and thyroid effects of high-ranking chemicals are further discussed. This study significantly expands current knowledge of NIS inhibition potentials in environmental chemicals, and provides critical support to U.S.EPA's Endocrine Disruptor Screening Program (EDSP) initiative to expand coverage of thyroid molecular targets as well as the development of thyroid adverse outcome pathways (AOPs).

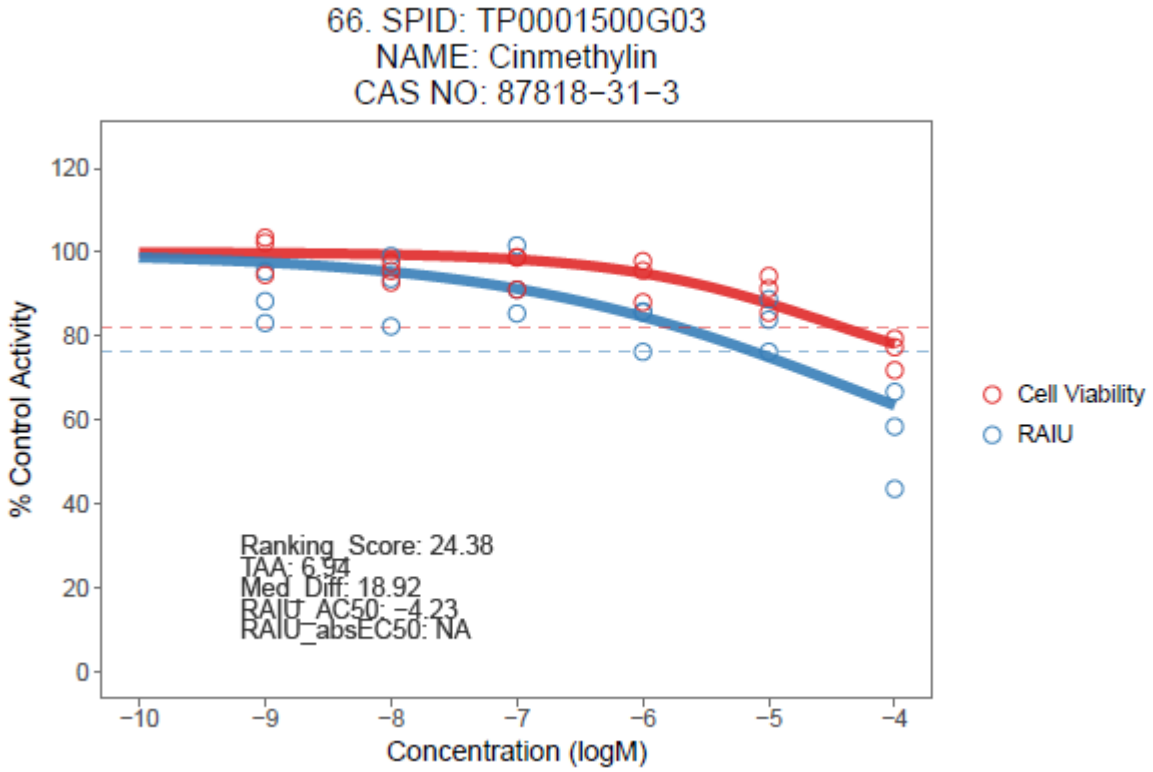
Materials and methods
Cinmethylin was screened for its sodium-iodide symporter inhibition properties in a tiered approach by an initial single concentration (100 μM) radioactive-iodide uptake (RAIU) assay, followed by a multi-concentration assay (0.001 – 100 μM) testing in parallel RAIU and cell viability in hNIS-HEK293T-EPA cells. Finally, a chemical ranking system that incorporates multi-concentration RAIU and cytotoxicity responses was used to characterise the specificity of the effect. Stock solutions were prepared in DMSO at a target concentration of 20 mM. Cinmethylin was run in 3 independent runs at 6 concentrations with several control substances. RAIU and cell viability assays were conducted with low passage hNIS-HEK293T-EPA cells (< 25 passes). The test principle is based on the exposure of cells to the test chemical at room temperature for 2 hours in addition to radioactive iodide 125I, termination of the assay by washing of the cells and quantification of the intracellular radioactive counts per minute with a scintillation counter. Cell viability is measured via CellTiter-Glo (Promega, Fitchburg, WI) and BMG FLUOstar Omega where luminescent signal was quantified as relative light units (RLU) indicative of ATP concentration.

Result and discussion

The activity threshold for RAIU was set on 23.8 % (as 3x bmad RAIU with DMSO). The threshold for significant cytotoxicity was set to 17.7 % (3x bmad of cell viability with DMSO). Sodium iodide symporter inhibition exceeds the baseline of 23.8 % at the highest tested concentration of 100 µM (max_mean not given in the publication, however according personal information from the author max_mean = 23.75 % inhibition which is below the baseline). The effect of cinmethylin at the AC50 (58.884 µM) is close to the efficacy cut-off value. The cytotoxicity in hNIS-HEK293T-EPA cells is defined via the AC50 = 30.2 µM, reaching significance (exceeding the abs EC 82.3 %) at the concentration of 37.15 µM. Therefore, the specific cytotoxicity as well as the median cytotoxicity concentration of cinmethylin (23.54 µM) are below the AC50 of NIS inhibition. There is no concentration showing RAIU inhibition without cytotoxicity.

In conclusion, cinmethylin is not a specific NIS inhibitor. Effects were detected at relatively high concentrations, above the cinmethylin median cytotoxicity limit.

HSE notes that the results of this study are generally consistent with the data from the new/modern ToxCast database, small differences in specific numbers were seen.



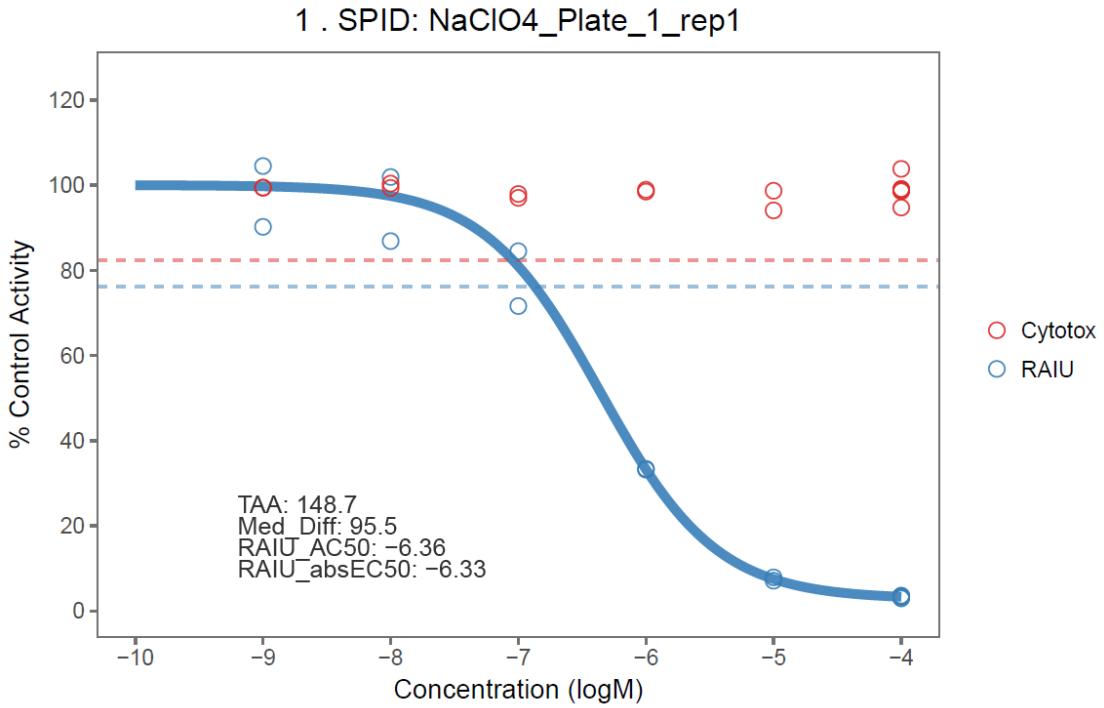


Figure 6.8-7. Dose response curve of Radioactive Iodide Uptake (RAIU) and cytotoxicity in HEK293T cells treated with cinmethylin (top) compared to the positive control $NaClO_4$ (bottom)

3)

Report reference	Hornung *et al.* (2017) BASF Study ID: 2017/1225381
Report title	Screening the ToxCast Phase 1 Chemical Library for inhibition of Deiodinase type 1 activity
Guidelines	None.
GLP	No.
Reliability	Reliable.
Relevance	Supplemental information.
Acceptable	Yes, regarded as supplemental information only.
Conclusion	Cinmethylin displayed no DIO1 activity at a concentration of 200 µM.

This study is part of the EPA ToxCast program.

Executive Summary

Thyroid hormone (TH) homeostasis is dependent upon co-ordination of multiple key events including iodide uptake, hormone synthesis, metabolism and elimination, to maintain proper TH signaling. Deiodinase enzymes catalyze iodide release from THs to interconvert THs between active and inactive forms and are integral to hormone metabolism. The activity of deiodinases has been identified as an important endpoint to include in the context of screening chemicals or TH disruption. To begin to address the potential for chemicals to inhibit these enzymes an adenovirus expression system was used to produce human deiodinase type 1 (DIO1) enzyme, established robust assay parameters for non-radioactive determination of iodide release by the Sandell-Kolthoff method, and employed a 96-well plate format for screening chemical libraries. An initial set of 18 chemicals was used to establish the assay, along with the known DIO1 inhibitor 6-propylthiouracil as a positive control. An additional 292 unique chemicals from the EPA's ToxCast phase 1_v2 chemical library were screened. Chemicals were initially screened at a single high concentration of 200 mM to identify potential DIO1 inhibitors. There were 50 chemicals, or 17 % of the TCp1_v2 chemicals tested, that produced >20 % inhibition of DIO1 activity. Eighteen of these inhibited DIO1 activity >50 % and were further tested in concentration-response mode to determine IC50s. This work presents an initial effort toward identifying chemicals with potential for affecting THs via inhibition of deiodinases and sets the foundation for further testing of large chemical libraries against DIO1 and the other deiodinase enzymes involved in TH function.

Cinmethylin was tested as part of the ToxCast Phase 1_v2 chemical library. Cinmethylin did not inhibit DIO1 activity.

Material and methods
Cinmethylin was prepared as 20 mM stock solution in DMSO. Adenoviruses expressing deiodinase were constructed by cotransfecting HEK293 cells with the subcloned DIO1 gene. The sensitivity of the assay was guaranteed by adding multiple samples of the positive control PTU to each 96 well plate. In addition, a set of benzothiazoles for which *in vitro* TPO inhibition data and *in vivo* responses in a Xenopus laevis metamorphosis assay were available were included (validation set).

Results
Cinmethylin (tested at 200 µM) displayed no DIO1 inhibition activity (% inhibition = 0.8; range: 0.6 – 1.1).

Discussion
Cinmethylin displayed no DIO1 activity at a concentration of 200 µM. DIO1 activity is generally considered to be one of the mechanisms by which a chemical can affect thyroid hormone homeostasis. Therefore, a lack of DIO1 activity provides evidence, that cinmethylin does not influence thyroid hormone homeostasis.

HSE agrees that cinmethyin did not inhibit DIO1 activity in this *in vitro* assay.

4)
Applicant remark:
This study with propylthiouracil (PTU) as positive control substance of thyroid toxicity is submitted to demonstrate that a correlation between morphological changes in the thyroid (follicular hypertrophy/hyperplasia) and hormone effects is seen. Therefore, in the absence of hormone level data, morphological changes are a sufficient indicator for identifying the LOEL of thyroid effects in rats.

Report reference	[redacted] (2011) BASF Study ID: 2011/1276730
Report title	BAS 455 H (Pendimethalin) - Developmental thyroid study in the Sprague-Dawley rat - Oral administration (diet)
Guidelines	EPA Guidance for thyroid assays in pregnant animals foetuses, postnatal and adult animals (2005)
GLP	Yes
Reliability	Reliable.
Relevance	Supplemental information.
Acceptable	Yes, regarded as supplemental information only.
Conclusion	PTU-treated rats of Wistar and Sprague-Dawley strains, demonstrated an effect on both thyroid hormones and hypertrophy/hyperplasia of the thyroid gland follicular cells. A dose-response was evident and a correlation between the effects was seen.

Methods
For the purpose of this dossier only the data on the positive control substance are summarised. In this extensive study a positive control comparative developmental thyroid study with 6-propyl-2-thiouracil (PTU) was performed. In this study summary the focus lies on the morphological and hormonal thyroid effects seen with (PTU) in dams and pups.

In two separate developmental thyroid studies, the relative sensitivity and potential differences in response to treatment of the Sprague-Dawley and Wistar rat strains were investigated using 6-propyl-2-thiouracil (PTU), a known thyroid modulator. For both studies, 35 time-mated females were dosed by gavage, once daily, from day 6 post coitum (GD 6) to day 21 post partum (PND 21), at dosages of 0.0, 0.1 or 2.5 mg/kg/day. Thyroid hormones (T3, T4 and TSH) were measured in dams on GD 20 and PND 21 and in foetuses/pups on GD 20, PND 4 and PND 21. At termination, thyroid weights were determined for the dams and histopathology of the thyroids was evaluated in both dams and foetuses/pups.

The study did not include any analytical determinations (see Volume 3 CA B5, section B.5.1.2).

Results:
There were no treatment-related deaths or clinical signs of toxicity (noted during gestation and lactation, for either rat strain. At the dose of 2.5 mg/kg/day, for both rat strains, maternal food consumption was reduced during the last week of the gestation period and throughout the lactation period. Also at this dose maternal body weight gain was retarded during the last week of the gestation period and overall body weight gain was increased during the lactation period.

Histopathology of the thyroid glands: For the GD 20 fetuses, PND 4 and PND 21 pups, the thyroid glands were removed together with adjacent tissue at necropsy in order to maintain optimal glandular architecture for histopathological evaluation. This precluded measurement of organ weights in these animals.

For the GD 20 and PND 21 dams at 2.5 mg/kg/day, both absolute and relative thyroid weights were increased and significantly different from controls. Enlarged thyroid glands were noted at 2.5 mg/kg/day (macroscopic examination). For both rat strains, there was a clear, dose-related increase in the incidence and severity of hypertrophy/hyperplasia of the thyroid follicular cells in the GD 20 and PND 21 dams, in the GD 20 foetuses and in the PND 4 pups (Tables 6.8-28, 6.8-29 and 6.8-30).

Table 6.8-28. Necropsy findings in dams administered PTU from GD 6 to PND 21

	GD 20 dams						**PD 21 dams**					
	Wistar rats			**Sprague Dawley**			**Wistar rats**			**Sprague Dawley**		
Dose Level [mg/kg]	**0**	**0.1**	**2.5**	**0**	**0.1**	**2.5**	**0**	**0.1**	**2.5**	**0**	**0.1**	**2.5**
Females examined	10	10	10	10	10	10	20	23	19	22	23	25
Hypertrophy/hyperplasia, follicular cells												
No finding	10	4		10	6		20	15		16	7	
Grade 1		5			4			8		5	10	
Grade 2		1								1	6	
Grade 3			2						7			
Grade 4			8			10			12			18
Grade 5												7

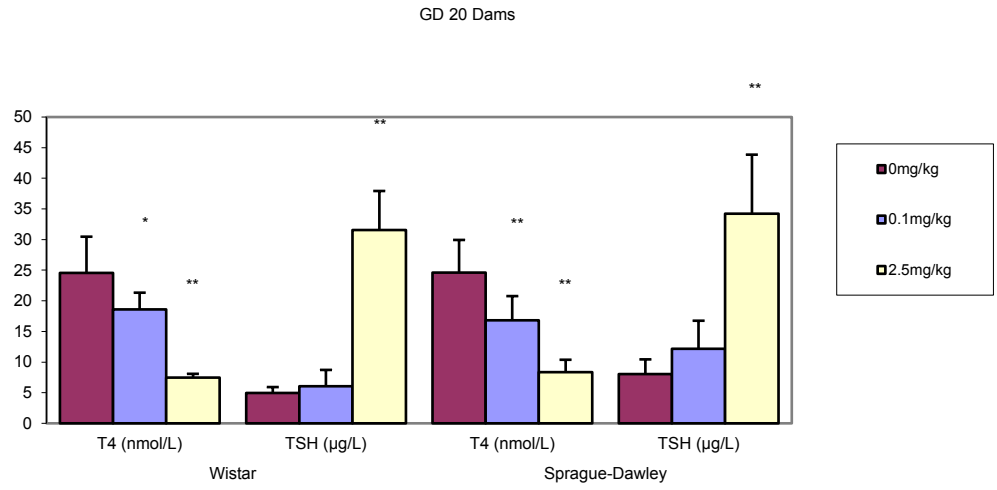
Table 6.8-29. Necropsy findings in foetuses administered PTU from GD 6 to PND 21

	GD 20 male fetuses						**GD 20 female fetuses**					
	Wistar rats			**Sprague Dawley**			**Wistar rats**			**Sprague Dawley**		
Dose Level [mg/kg]	**0**	**0.1**	**2.5**	**0**	**0.1**	**2.5**	**0**	**0.1**	**2.5**	**0**	**0.1**	**2.5**
Females examined	10	10	10	10	9	8	10	10	10	10	7	8
Hypertrophy/hyperplasia, follicular cells												
No finding	10			10	9	4	10	2		10	1	
Grade 1		10				1		8	1		2	1
Grade 2			4			2			9		4	5
Grade 3			6			1						2
Grade 4												

Table 6.8-30. Necropsy findings in pups administered PTU from GD 6 to PND 21

Sex	**PD 4 male pups**						**PD 4 female pups**					
Strain	**Wistar rats**			**Sprague Dawley**			**Wistar rats**			**Sprague Dawley**		
Dose Level [mg/kg]	**0**	**0.1**	**2.5**	**0**	**0.1**	**2.5**	**0**	**0.1**	**2.5**	**0**	**0.1**	**2.5**
Females examined	10	10	10	10	10	10	10	10	10	10	10	10
Hypertrophy/hyperplasia, follicular cells												
No finding	10	2		10	2		10	2		10		
Grade 1		8			6	4		7			3	1
Grade 2			8		2	2		1	5		6	8
Grade 3			2			4			5		1	1
Grade 4												

Thyroid hormone measurements: For both rat strains, TSH values were increased (statistically-significantly in some instances) at 2.5 mg/kg/day in dams at GD 20 and PND 21, in foetuses at GD 20 and in pups at PND 4 and 21 (Figures 6.8-8 and 6.8-9). At the low dose of 0.1 mg/kg/day, TSH values were also statistically-significantly increased for the GD 20 foetuses and pups on PND 4 and 21; values for dams on GD 20 and PND 21 were only minimally increased. For both rat strains, there was a dose-related and (in most instances) statistically-significant reduction at 2.5 mg/kg/day in the T4 values for dams on GD 20 and PND 21, foetuses on GD 20, pups on PND 4 and 21. T4 values were statistically-significantly reduced at 2.5 mg/kg/day (both rat strains) and at 0.1 mg/kg/day (Wistar strain only), in pups on PND 4 and 21. For both rat strains, T3 values were reduced in dams on GD 20 and the pups on PND 4 and 21 at 2.5 mg/kg/day, but not in dams on PND 21. Sample volumes were insufficient to measure T3 values in the GD 20 fetuses.



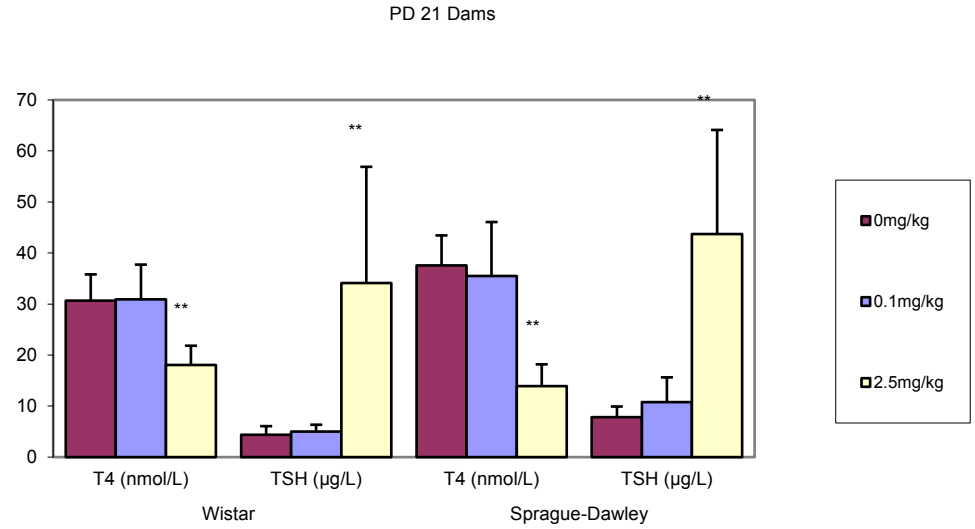
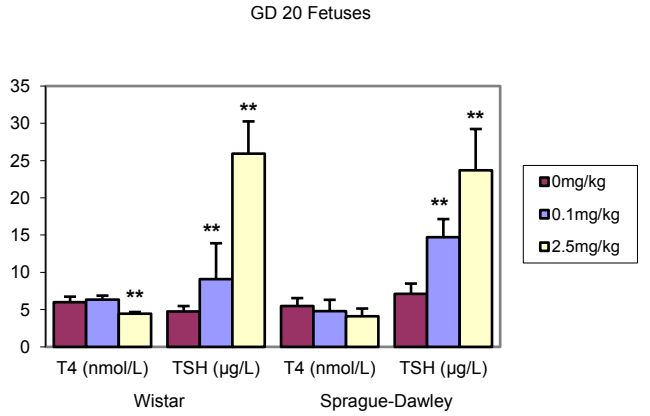
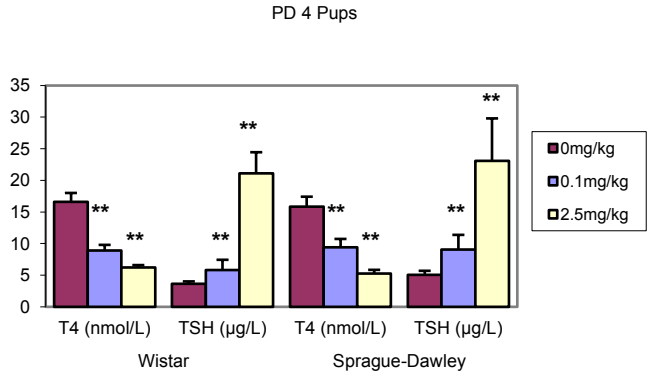


Figure 6.8-8. Thyroid hormone levels in dams administered PTU from GD 6 to PND 21





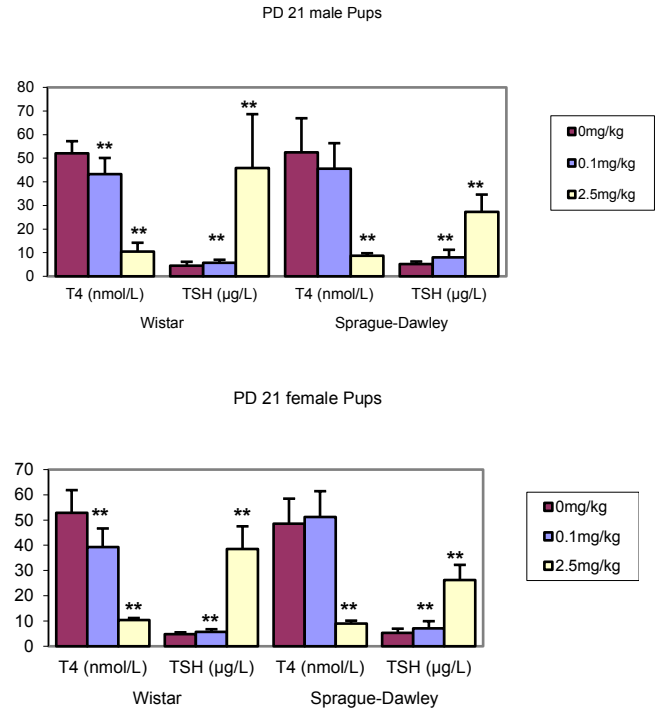


Figure 6.8-9. Thyroid hormone levels in foetuses and pups from dams administered PTU from GD 6 to PND 21

Conclusions

Results indicate that PTU-treated rats of Wistar and Sprague-Dawley strains, demonstrate clear dose-response relationships for thyroid hormones. Evident effects were decreased T4 and increased TSH levels for dams (GD 20, PD 21), foetuses (GD 20) and pups (PD 4 & 21). T3 responded with less sensitivity. Average variability of the data was acceptable. At these time points, dams, foetuses and pups of both rat strains showed comparable signs of diffuse hypertrophy and hyperplasia of the thyroid gland follicular cells, with a dose-dependent severity. A treatment-free period of 10 days (PD 11 – 21) did not result in any recovery. A correlation between the effects on morphology and hormone was seen. The results showed no obvious differences between the Wistar and Sprague-Dawley rat strains.

HSE agrees that there is a correlation between effects on thyroid hormone levels and morphological changes in the thyroid. However, as all doses tested showed morphological changes (no lower doses were tested which did not show morphological effects), it is not possible to conclude that in the absence of morphological changes in the thyroid any changes in hormone levels can be excluded.

Postulated MOA

The applicant notes that the liver is a target organ of cinmethylin and suggests that the pattern of thyroid effects is the consequence of liver enzyme induction.

Justification from the applicant:

Overall, the liver changes are consistent with adaptational responses to cinmethylin exposure rather than representing a pathological effect.

In a dose-response analysis of liver and thyroid findings, concordance is seen (Table 6.8-31). As liver enzyme induction is an early event, the liver and thyroid results from the 28-day, the 90-day and the 2-generation toxicity studies were compared in the table below. In the absence of thyroid hormone data, the thyroid morphological data were used as a marker for thyroid toxicity. It is assumed that the onset of treatment-related thyroid histopathological findings is - in the absence of hormone level data – a sufficient indicator for identifying the LOEL for thyroid effects in rats. This is supported by experimental studies with a positive control substance (pendimethalin), investigating thyroid hormones and thyroid histopathology occurring at the same doses ([redacted] 2011). In order to evaluate the potential MoA of thyroid toxicity in rats, the timing and onset

of hypertrophy/hyperplasia of follicular cells in the thyroid is compared with the onset of observed changes in the liver (Table 6.8-31).

The dose concordance of thyroid and liver effects in the subacute and subchronic rat studies supports the hypothesis that thyroid effects are secondary to liver enzyme induction. In all cases liver effects were seen at lower or comparable dose levels than hypertrophy/hyperplasia of follicular cells in the thyroid.

Table 6.8-31. Dose concordance of liver and thyroid findings

Dose ppm	Males ♂ (Study type, Dose level [mg/kg bw/day]				Females ♀ (Study type, Dose level [mg/kg bw/day]			
	Liver weight increases	**Liver Hypertrophy & cytoplasm. Alteration & clinical chemistry**	**Thyroid Hypertrophy/ Hyperplasia**	**Altered colloid**	**Liver w.**	**Liver Hypertrophy& cytoplasm. Alteration & clinical chemistry**	**Thyroid Hypertrophy/ Hyperplasia**	**Altered colloid**
1000	2 gen, 79							
3000	90 d, 211	90 d, 211	90 d, 211		90 d, 240	90 d, 240		
5000	28 d, 477	28 d, 477*	28 d, 477 2 gen, 412		28 d, 477 2 gen. 395	28 d, 477*	28 d, 477 2 gen, 395	2 gen, 395
10000				90 d, 792			90 d, 814	90 d, 814
15000			28 d, 1522	28 d, 1522		28 d, 1331		28 d, 1331

Study type: d = days; m = month; gen = generation; w.: weight
*At these doses, statistically significantly increased GGT levels (indicative for liver enzyme induction) were seen only

HSE notes that the assessment of the proposed thyroid MoA is very concise; in addition, there is a lack of thyroid hormone (e.g. T3, T4, TSH) measurements, CAR/PXR activation and UGT data (MIE - molecular initiating event). It is not possible to conclude (in the limited study by [redacted] 2011) that in the absence of morphological changes in the thyroid any changes in hormone levels can be excluded. Mechanistic data to support the postulated indirect MoA is required. In addition, a detailed comparative MoA analysis is necessary to assess whether thyroid adversity is secondary to liver effects. The weight of evidence analysis should include a more in-depth time- and dose-concordance analysis of liver and thyroid effects, including additional information on some key events. The applicant should follow Appendix A of the EFSA/ECHA guidance on the identification of endocrine disruptors, which states:

'To investigate whether liver enzyme induction is responsible for the effects seen on TH levels and/or thyroid histopathology and weight, as well as whether the effect is or not likely to be human-relevant, the following three pieces of information are needed:

1) *Results of analysis of serum/plasma samples (if available) for TSH, T3 and T4 in the existing repeated dose toxicity studies. If unavailable, a specifically designed in vivo toxicity study should be considered. In this study, TSH, T3 and T4 should be measured and, where possible, additional data on liver enzyme induction (e.g. measurement of UDPGT) should be included.*
2) *Comparative studies of enzyme activity induced by the test substance in liver in vitro systems should be measured in both the relevant test species (e.g. rat, mouse and dog) and humans. The metabolism of the specific substance (ADME properties) in both test species and humans, and the activity of possible metabolites must be considered when this comparison is conducted.*
3) *The presence of other possible thyroid-disrupting modes of action such as interference with TH synthesis should also be excluded, e.g. by evaluating in vitro the potential for inhibition of the sodium–iodide symporter (NIS) and thyroid peroxidase (TPO). It must, however, be acknowledged that substances may interfere with the thyroid hormone system through many different mechanisms of action, and that currently validated/standardised in vitro assays do not exist to investigate all these different pathways and a reasonable effort is anticipated, based on available tools and current understanding of thyroid physiology.'*

Excluding other modes of action
The applicant suggests that, based on available ToxCast data, a selective effect of cinmethylin can be excluded on the following thyroid (hormone) receptors/enzymes:

- Thyroid hormone receptor
- Thyroid stimulating hormone receptor
- Deiodinase inhibition
- Thyroidperoxidase (TPO) enzyme inhibition
- Sodium-iodide symporter (NIS) inhibition

In conclusion, the ToxCast/Tox21 data show that thyroid is not a direct target of cinmethylin.

HSE accepts that based on ToxCast and literature data, direct thyroid MoAs have been excluded. HSE considers that, as there are no guideline studies for these mechanistic investigations, the ToxCast data are sufficient..

Human relevance of hypothesised MoA
The applicant provided the following human relevance assessment:
The data support the postulated MoA of an indirect liver enzyme mediated rat-specific thyroid toxicity. In this case, cinemthylin activates the degradation of T4 as a consequence of increased phase II metabolising liver activities. The human relevance of that MoA, leading to thyroid hypertrophy/hyperplasia, caused by increased liver enzyme induction, increased excretion of T3/T4, increased TSH (via a feedback mechanism), which leads to thyroid hypertrophy (Dellarco *et al.*, Thiazopyr and thyroid disruption: Case study within the context of the 2006 IPCS Human Relevance Framework for Analysis of a cancer mode of action, Critical Reviews in Toxicology, 36, 793 – 801, 2006) is low, as the T4 half-life of rats is considerably lower compared to humans (5 – 9 days in humans compared to 0.5 – 1 day in rats) (Jahnke *et al.,* 2004).

There are strong quantitative differences in the thyroid function/homeostasis between rat and human. Rats have smaller reserve for thyroid hormones, shorter half-life for T4, and higher constitutive TSH levels reflecting higher activity of the thyroid, than human. Rats also have a different histological appearance of follicular epithelium cells which produce T4 at higher rate, compared to human, whose follicular cells appear quiescent and less responsive to elevated TSH (Kaptein *et al.* (1994), Lewandowski *et al.* (2004), Jahnke *et al*. (2004), , Dohler *et al*. (1979) and Hill *et al*., (1989)). Therefore, thyroid hypertrophy observed in rats treated with cinmethylin is considered not to be relevant in human based on quantitative differences.

Furthermore, dietary exposure to cinmethylin and its metabolites is estimated to stay below 2 % of the TTC level of general toxicity, thereby range at a level 0.03 µg/kg bw, which is 1.7×10^6 fold below the NOAEL of around 50 mg/kg bw/d for hypertrophy/hyperplasia of follicular cells. The relevance of this effect for human is therefore negligible.

HSE agrees with the applicant that given the large quantitative differences in thyroid homeostasis between rats and humans, these indirect thyroid effects are unlikely to be relevant to humans. This is further supported by the lack of thyroid effects in mice and dogs.

WoE for T-mediated adversity

Thyroid weight was measured in several oral repeated dose toxicity studies in rats, mice and dogs. Effects on thyroid weight (increases) were recorded in studies in rats (in the 90-day and 2-generation study) but no treatment-related changes were seen in studies in mice and dogs.

Thyroid histopahology was evaluated in several oral repeated dose toxicity studies in rats, mice and dogs. Effects on thyroid histopathology (hypertrophy/hyperplasia and altered colloid) were consistently recorded in study in rats (in the 28-day, 90-day, chronic toxicity/carcinogenicity and 2-generation study) but no treatment-related changes were seen in studies in mice and dogs.

WoE for T-mediated endocrine activity

Overall, the ToxCast/EDSP21 '*in vitro* mechanistic' dataset indicates that cinmethylin does not specifically perturb the pathways related to direct thyroid activity.

The applicant suggested that thyroid effects are a consequence of liver enzyme induction (postulated indirect MoA), however, this is not sufficiently substantiated. A detailed comparative MoA analysis is necessary to assess whether thyroid adversity is secondary to liver toxicity. The weight of evidence analysis should include a time- and dose-concordance analysis of liver and thyroid effects. The applicant should follow Appendix A of the EFSA/ECHA guidance on the identification of endocrine disruptors.

Have T-mediated parameters been sufficiently investigated?

T-mediated adversity has been sufficiently investigated, based on the following studies in which thyroid effects were identified:

- 28-day oral toxicity studies in the rat (OECD TG No. 407)
- 90-day oral toxicity studies in the rat (OECD TG No. 408)
- Chronic toxicity / carcinogenicity studies in the rat (OECD TG No. 453)
- 2-generation reproductive toxicity study in the rat (OECD TG No. 416).

However, T-mediated activity (in particular UGT and thyroid hormones) has not been sufficiently addressed.

Analysis of the evidence and identification of relevant scenario for the ED assessment of T-modality
Seclection of relevant scenario

Adversity based on T-mediated parameters	Positive mechanistic OECD CF level 2/3 Test	Scenario	Next step of the assessment	Scenario selected
No (sufficiently investigated)	Yes/No	1a	Conclude: ED criteria not met because there is no **'T-mediated'** adversity	
Yes (sufficiently investigated)	Yes/**No**	**1b**	**Perform MoA analysis**	**X**
No (not sufficiently investigated)	Yes	2a (i)	Perform MoA analysis (additional information may be needed for the analysis)	
No (not sufficiently investigated)	No (sufficiently investigated)	2a (ii)	Conclude: ED criteria not met because no EAS-mediated endocrine activity observed	
No (not sufficiently investigated)	No (not sufficiently investigated)	2a (iii)	Generate missing level 2 and 3 information. Alternatively, generate missing "EATS-mediated" parameters. Depending on the outcome move to corresponding scenario	
Yes (not sufficiently investigated)	Yes/No	2b	Perform MoA analysis	

Conclusion of the assessment of T-modality

Based on scenario 1b of the ECHA/EFSA guidance for the identification of endocrine disruptors in the context of Regulations (EU) 528/2012 and (EC) No 1107/2009, there is an overall indication of adversity. Some information from ToxCast on thyroid activity (direct thyroid MoAs) has been presented and direct thyroid MoAs have been excluded; however, more detailed information is required to substantiate the postulated indirect MoA.

HSE notes that the assessment of the proposed thyroid MoA is very concise; in addition, there is a lack of thyroid hormone (e.g. T3, T4, TSH) measurements, CAR/PXR activation and UGT data (MIE - molecular initiating event). It is not possible to conclude (in the limited study by [redacted] 2011) that in the absence of morphological changes in the thyroid any changes in hormone levels can be excluded. Mechanistic data to support the postulated indirect MoA is required. In addition, a detailed comparative MoA analysis is necessary to assess whether thyroid adversity is secondary to liver effects. The weight of evidence analysis should include a

more in-depth time- and dose-concordance analysis of liver and thyroid effects, including additional information on some key events.

Overall conclusion on the ED assessment for humans

HSE concludes that for the EAS modalities cinmethylin is not an ED and its ED potential has been sufficiently investigated. However, in relation to the T modality a conclusion cannot be reached as further information is required. The following further data and information is being generated by the applicant:

- *In vivo* thyroid hormone and enzyme induction study in rats
- *In vitro* comparative enzyme activity study in rat and human hepatocytes
- Description of the postulated MoA
- Empirical support of the postulated MoA
- Conclusion on MoA analysis
- A case to address the potential for effects on post-natal neurological development in offspring
- A case to address the potential relevance to humans (or lack thereof) of the proposed MoA

B.6.8.4. Immunotoxicity

No specific immunotoxicity study with cinmethylin is available. However, an assessment of the immunotoxicity potential of cinmethylin can be performed by considering the available repeat dose toxicity, carcinogencicity and reproductive toxicity studies. The standard regulatory studies conducted with cinmethylin have assessed its potential impact on a number of immune-related endpoints (Table 8.6-32) including haematological parameters such as white blood cell count, spleen and thymus weights, histopathology of the spleen, thymus, lymph nodes and bone marrow. There were no consistent treatment-related changes in white blood cell (WBC) count, select differential blood cell counts (lymphocytes, neutrophils, basophils, monocytes), or histology of the spleen, thymus, lymph node or bone marrow in any study. There was no evidence of a specific immunotoxic effect on any immune-related parameter. Sporadic effects on single parameters were observed in some studies but none supported a specific and consistent immunotoxic effect.

Total white and differential blood cell parameters were investigated in subacute to chronic studies in rats, mice and dogs and in the developmental toxicity study in rabbits. Only sporadic effects were seen (see below).

In the 90-day rat study ([redacted] 2018a), statistically-significantly decreased relative eosinophil counts (EOS) in top dose animals of both sexes and in mid dose males, as well as a reduced absolute eosinophil count in top dose females were observed. Relative change compared to controls was > 10 % for all mid and top dose animals. However, in isolation, without related effects on other WBC parameters, these changes were considered chance findings. Statistically-significantly increases in absolute and relative counts of large unstained cells (LUC) were observed in top dose males and females, respectively. Relative change compared to controls was > 10 % for top dose animals. Whilst values obtained for top dose males (absolute) and females (relative) were within the HCD range they were greater than their respective HCD means. However, a dose-response relationship was lacking, therefore HSE did not consider these LUC changes to be related to treatment.

In the 90-day study in mice ([redacted]., 2018b), statistically-significantly decreased total white blood cell (WBC), absolute neutrophil (NEUT), absolute lymphocyte (LYMPH) and eosinophil (EOS) counts were observed in top dose males. Relative changes compared to control were > 10 %. In females of the top dose, statistically-significantly decreased absolute and relative EOS counts were observed (well > 10 % change compared to control). Absolute and relative monocyte (MONO) count was statistically-significantly increased in the mid and/or top dose in males, however, no dose-response relationship was evident. Overall, treatment-related and adverse changes in some white blood cell (WBC, NEUT, LYMPH, EOS) parameters were seen in males and females at the top dose (1200 and 1304 mg/kg bw/d in males and females respectively). However, these were isolated findings which were not evident in any other repeated dose studies in mice, of different timeframes (28-day ([redacted]., 2016), 90-day ([redacted] 1983) and 18-month ([redacted] 2018d)), nor in any studies in rats (28-day, 90-day and 2-year studies). Therefore HSE does not consider these haematological changes to be indicative of immune-specific toxicity of cinmethylin.

In dogs ([redacted]1985), statistically significantly changed parameters in haematology were seen after 26 weeks of cinemthylin administration. After 26 weeks, statistically-significant increases in total WBC and absolute neutrophil counts were observed in males from the mid dose (3,000 ppm or 83.4 mg/kg bw/d). However, neutrophil cell counts were not changed dose-dependently and the WBC count means were in the same range as seen in pre-tests, whereas the control mean was quite low. In females of the mid dose group, WBC and absolute neutrophil counts were significantly increased, however, a dose-response relationship was not evident. After 52 weeks, in males of the top dose group, WBC and neutrophil counts were increased, although not statistically-significantly; in addition lymphocyte and monocyte counts were statistically-significantly increased in this group. Both a dose-response and relative changes of > 10 % compared to control were evident in these parameters in top dose males. In females, WBC and neutrophil counts were statistically-significantly increased from the mid dose group and absolute lymphocyte counts were statistically-significantly increased at the top dose. Again a dose-response and relative changes of > 10 % compared to control were evident for statistically-significant values. These findings were considered treatment-related and adverse. They reflect most probably an inflammatory reaction. Overall, treatment-related and adverse effects on white blood cell paramaters were seen from the mid dose (3,000 ppm or 83/81 mg/kg bw/d in M/F respectively) in one 1-year dog study ([redacted], 1985). However, these were isolated findings which were not evident in any other repeated dose studies in dogs (5-week ([redacted], 1984), 13-week ([redacted], 1987)) ; in particlar they were not evident in either of the other two 1-year study in dogs ([redacted], 1988a and [redacted], 1988b). Therefore HSE does not consider these haematological changes to be indicative of immune-specific toxicity of cinmethylin.

Table 6.8-32. List of effects on immune-related parameters investigated in the regulatory, GLP-compliant studies conducted with cinmethylin

		Rat 28-d		Mouse 28-d		Rat 90-d		Mouse 90-d		Dog 90-d		Dog 1-y		Rat 28-d dermal		Rat combined chronic & carc.		Mouse carc.		Rat dev. tox.		Rabbit dev. Tox.		Rat 2-gen	
OECD TG No.		407		407		408		408		409		EPA		410		453		451		414		414		416	
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Total leukocyte count (WBC)	Abs. Rel.	- -	-	-	-	-	-	↓ -	-	-	-	-	↑	-	-	-	-					-	-		
Differential blood count Lymphocytes	Abs. Rel.	-	-	-	-	-	-	↓ -	-	-	-	↑ -	↑ -	- -	- ↓	-	-	↓	-			-	-		
Neutrophils	Abs. Rel.	-	-	-	-	-	-	↓ -	-	-	-	- -	↑ -	- -	- ↑	-	-	↑	-			-	-		
Large unstained cells	Abs. Rel.	-	-	-	-	↑ -	- ↑	-	-					-	-	- -	↓ -					-	-		
Eosinophils	Abs. Rel.	-	-	-	-	- ↓	↓ ↓	↓ -	↓ ↓	-	-			-	-	-	-	↓	↓			-	-		
Basophils	Abs. Rel.	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-			-	-		
Monocytes	Abs. Rel.	↑ ↑	-	-	-	-	-	↑ ↑	-	-	-	↑ -	- ↓	-	-	-	-	↓	↓			-	-		
Spleen weight	Abs. Rel.	-	-	-	-	- ↑	- -	-	-	-	-	-	-	-	-	- -	↓ -	- -	↓ -	-	-	-	-	-	-
Spleen histopathology		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-						
Cellularity of PALS, lymphoid follicles, marginal zone, red pulp		-	-	-	-																				
Cellular composition of follicles		-	-	-	-																				
No. of germinal centers		-	-	-	-																				
Thymus weight	Abs. Rel.	-	-	-	-	↓ -	↓ -	- -	↑ -					-	-										
Thymus histopathology		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-						
Grade of cortico-medullar ratio		-	-	-	-																				
Increase of starry sky cells		-	-	-	-																				
Cellular density in the cortex		-	-	-	-																				
Cellular density in the medulla		-	-	-	-																				
Lymph node histopathology		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-						
Cellularity of follicles, interfollicular area, paracortical area, medulla		-	-	-	-																				
Cellular composition of		-	-	-	-																				

paracortex																									
No. of germinal centers		-	-	-	-																				
Hyperplasia of high endothelial venules		-	-	-	-																				
Payer's patches (of the jejunum)		-	-	-	-	-	-	-	-					-	-										
Cellularity of follicles		-	-	-	-																				
Cellularity of interfollicular area		-	-	-	-																				
Bone marrow histopathology		-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-						
Cellularity		-	-	-	-																				
Myeloid/erythroid ratio		-	-	-	-																				

Blue fields mark investigation;
- no effect.

In the 28-day dermal study in rats [redacted] 2018c), a statistically-significantly decreased mean corpuscular haemoglobin (MCH) and relative lymphocyte count in high dose females as well as statistically-significantly increased relative neutrophil counts in mid and high dose females was recorded (Table 6.3-63). These parameters were within the HCD range; only neutrophil counts showed a relative change > 10 % compared to controls. However, they were not affected in males and were not corroborated by changes in other parameters (total red blood and white blood counts). Therefore they were considered unrelated to treatment. Overall, no treatment-related effects on haematology were seen up to the top dose (1000 mg/kg bw/d) in this 28-day dermal study.

In the combined chronic toxicity/carcinogenicity study in rats ([redacted] 2018), there were no treatment-related haematology findings in both the chronic and the carcinogenic phases.

In the carcinogenicity study in mice ([redacted] 2018d), there were no treatment-related adverse effects on haematological parameters at either 12- or 18-months.

Spleen weight and spleen gross necropsy / histopathology was investigated in all subchronic to chronic studies in rats, mice and dogs as well as in rat reproductive toxicity studies and in the rabbit developmental study. Only sporadic effects were seen (see below).

In the rat, effects on spleen weight and histopathology were generally unremarkable in all dietary studies except for some body weight-related, secondary changes. In the 90 day study ([redacted] 2018a), increased relative spleen weights were seen in males of the top dose group (+17 % ($p \leq 0.05$)), which was secondary to decreased body weight (-17 % ($p \leq 0.01$)). However, changes were not considered to be treatment-related based on a lack of correlated histopathological changes. In the combined chronic toxicity and carcinogenicity study in rats ([redacted] 2018), a statistically-significant decrease in absolute spleen weight (-2 % ($p \leq 0.05$)) was seen at 24 months in females of the top dose; however, change compared to controls was < 10 % and no concomitant histopathology was found. This finding was not considered to be treatment-related. No significant changes of spleen weight were seen in the 28-day dermal study ([redacted] 2018c). In parental animals and pups of the 2-generation study in rats ([redacted] 2018a ; [redacted], 2018), due to the lack of dose-response, changes in spleen weight were also not considered to be treatment-related. In the teratogenicity study in rats ([redacted] 1984), two top dose dams died intercurrently on GD 15 and were noted as showing decreased spleen size. However, similar findings were not observed in rats which survived. Therefore, this finding was not considered to be treatment-related. Overall, there were no specific weight or histopathological changes in spleen observed in any rat study.

In mice, spleen weights and spleen histopathology were unremarkable in all dietary studies (28-day, 90-day, carcinogenicity study), except for reduced absolute spleen weights in females after exposure for 18 months [redacted] 2018d) (- 25 % ($p \leq 0.01$) and – 40 % ($p \leq 0.01$) in the mid and high dose groups). However, these findings were not considered treatment-related due to a lack of dose-response and/or concomitant histopathological findings. In dogs, spleen was unaffected after 90-day and 1-year substance administration. In the teratogenicity study in rabbits ([redacted] 2018b), an isolated finding of spleen torsion in one low dose group doe was reported as spontaneous finding, while spleen weights and histopathology in parental animals were unaffected. Five fetuses out of one litter of the high mid dose group (250 mg/kg bw) showed discolored spleens. However, this isolated finding was considered a spontaneous finding based on absence of dose-response.

Thymus weight and/or histopathology was investigated in all subacute to chronic studies in rats, mice and dogs. No treatment-related changes were seen in any species. In the 28-day study in the rat ([redacted] 2015), no significant changes in thymus weight and/or histopathology (including evaluation of alterations of grade of cortico-medullar ratio (related only to area), starry sky cells, cellular density in the cortex and cellular density in the medulla) were observed. In the 90-day study in rats ([redacted] 2018a), absolute thymus weight was reduced by -19 % ($p \leq 0.05$) to -26 % ($p \leq 0.01$) in all treated males. In high dose females, a reduced absolute thymus weight (-22 % ($p \leq 0.01$)) was recorded. However, these findings were not considered treatment-related based on values falling within the range of the historical control data and due to a lack of correlated histopathological changes. Chronic administration in rats ([redacted]., 2018) did not lead to any remarkable histopathological findings in the thymus. No effects on the thymus were seen in the 28-day dermal study in rats ([redacted]., 2018c).

In mice, thymus weight was evaluated in the 28-day study ([redacted] 2016) and in the 90-day ([redacted] 2018b) study; no treatment-related changes were observed. The increased absolute thymus weight of 13 %

($p \leq 0.05$) in females of the highest dose (5000 ppm) after 90 days was not considered to be treatment-related due to the lack of dose-response and lack of correlated macro- or microscopic findings. No histopathological changes were seen in any study in mice, including the carcinogenicity study.

In dogs, measurement of thymus weights was not part of the study protocols for the 90-day and 1-year studies; however, due to the lack of treatment-related finding in thymus histopathology, the thymus was not considered to be a target organ of toxicity in dogs.

Histopathology of lymph nodes (mesenteric and axillary lymph nodes), peyer's patches of the jejunum and bone marrow was unremarkable in all studies.

It can therefore be concluded that cinmethylin does not affect the immune system, and a specific *in vivo* immunotoxicity study is not required.

B.6.9. MEDICAL DATA AND INFORMATION

Cinmethylin is a new herbicide active ingredient, which has not yet been sold commercially and aside from pilot scale preparations, has been handled by only a limited number of employees or contract scientists involved in regulatory and field biological testing. Therefore, human data is limited at this time. Three searches were conducted.

1. A search in the databases listed below - restricted to "human" - was performed on March 22nd, 2018 for the following terms:

Terms used:
'BAS 684*'
'CAS 87818-31-3'
'Cinmethylin'

Datebases used:
PubMed
Embase 74 (Elsevier)
Embase alert (Elsevier)
Current Contents
Cochrane Library-Central
Biosys (Thomson Reuters)
gms (German Medical Science)
Livivo (ZBMed)

2. Crosscheck via ChemIDplus (https://chem.nlm.nih.gov/chemidplus/chemidlite.jsp)

3. Crosscheck via Toxnet (http://toxnet.nlm.nih.gov/)

These searches revealed no relevant documents.

HSE is satisfied that relevant serches were conducted and, a this time, no medical data is available.

B.6.9.1. Medical surveillance on manufacturing plant personnel and monitoring studies

All persons handling crop protection products are surveyed by regular medical examinations. There are no specific parameters available for effect monitoring of cinmethylin. Thus, the medical monitoring program is designed as a general health check-up, with special interest in the primary target organs presumed to be relevant by analogy from animal experiments.

The surveillance program includes a general physical examination including neurological status, red and white blood cell counts, liver enzymes. Adverse health effects suspected to be related to cinmethylin exposure have not been observed.

B.6.9.2. Data collected on humans

No reports of adverse effects were identified during routine monitoring of production plant workers and among personnel involved in the experimental biological testing or field trials with cinmethylin or cinmethylin containing products. There is no evidence or data available to support any findings in relation to poisoning with cinmethylin.

B.6.9.3. Direct observation

No human cases of intoxication or poisoning deriving from cinmethylin are known to BASF SE.

B.6.9.4. Epidemiological studies

Neither data on exposure of the general public nor epidemiologic studies are available for BASF SE, nor is BASF SE aware of any epidemiologic studies performed by third parties. As such, no observations regarding health effects after exposure of the general public are known to HSE.

B.6.9.5. Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical test

An analytical method for the determination of cinmethylin in blood plasma of rats and mice is available. A a fully validated method for the determination of of cinmethylin and the metabolite M684H011 in body fluids (whole blood and urine) is available (see Volume 3 CA B5, section B.5.2.6). Clinical tests are not known. No specific symptoms of poisoning are seen or have been identified in animal studies.

B.6.9.6. Proposed treatment: first aid measures, antidotes, medical treatment

See safety data sheet / precautions; symptomatic and supportive treatment, no specific antidote known.

B.6.9.7. Expected effects of poisoning

Specific clinical signs of poisoning are not expected, based on results of acute and subacute studies in animals. In these studies, clinical observations consisted of unspecific signs like impaired body weight development. In an acute neurotoxicity study in rats treatment-related unspecific neurobehavioral changes were observed after bolus administration at a dose level of 1,000 mg/kg bw/day in females (retarded righting response and motor activity). At 2,000 mg/kg bw/day, additional unspecific clinical findings were noted in males and females (labored respiration, piloerection, slightly closed eyelids, limply hanging in hand during handling, slight impairment of the gait and reduced exploration of the area). These observations were transient as they were only noted on the day of application.

B.6.10. REFERENCES RELIED ON

LITERATURE SEARCH

A literature search on cinmethylin was performed by the applicant (BASF). Cinmethylin was originally developed by Shell in the 1980's and marketed in some Asian countries mostly as a rice herbicide. The literature search report on cinmethylin (BASF DocID 2018/1099008) described the general search and evaluation process as well as details on search profiles, search histories and summary tables.

The search was performed in July 2017, followed by updates in February and April 2018. All available CAS numbers of stereoisomers of cinmethylin and metabolites were included in the search profile.

The first step of the search result i.e. the processing of publications based on summary records was done by the Information Center and involved the separation into "hits" and "ballast" (i.e. obviously irrelevant records). The "ballast" was not further processed. The "hits" were further evaluated by the scientific experts and categorised into "not relevant", "not reliable", and "used for dossier".

From the Consumer Safety literature search results, one paper (on 1,4-cineole) was included in the dossier as supplemental information on the comparison of metabolism in rat and humans (Miyazawa *et al.*, 2008); however HSE did not consider this paper relevant to the metabolism of cinmethylin as 1,4-cineole is not the active, nor is it structurally-related to the active. Two further papers contributed to the topic "toxicity of metabolites" (Maguin *et al.,* 2006; Masereeuw *et al.,* 1995) and were included in Volume 3 CA B6 (part II), section 6.8.1.

As a result of the searches, a total of six publications were regarded relevant and reliable (one with limited reliability). These publications were included in the dossier and are summarised and discussed above in section 'B.6.8 Other Toxicological Studies' of this document.

A list of studies, which were performed by Shell Agriculture in the 1980's to investigate the toxicity of cinmethylin, was also submitted. Most of the studies were discussed in relevant sections of this docuemnt; however, some of the studies suffer distinct deficiencies that prevent a comprehensive evaluation or are not required for submission within the EU.

DATABASES searched

STN-Databases:

Agricola	1979 – to present
ANABSTR - Analytical Abstracts	1980 – to present
BIOSIS	1926 – to present
CABA - CAB Abstracts	1973 – to present
CAPLUS - Chemical Abstracts Plus	1907 – to present
CAS REGISTRY	1907 – to present
CSNB - Chemical Safety NewsBase	1981 – to 2016
EMBASE	1947 – to present
MEDLINE	1946 – to present
PQScitech	1962 – to present
TOXCENTER	1907 – to present

CD-ROM-Database:

PESTICIDE MANUAL current information
15. Edition, Vers. 5.2 2011/2012

Internet-Databases licenced by BASF:

REAXYS - Organic compounds, formerly Beilstein 1771 – to present
https://www.reaxys.com/ for registered BASF employees

Public Internet:

GESTIS - Database on hazardous substances of the German Social Accident Insurance
current information
http://biade.itrust.de/biade/lpext.dll?f=templates&fn=main-h.htm

General Search and Evaluation Process
Literature searches were done by information professionals (chemists, biologists) of the *BASF Scientific Information*. Search profiles for literature searches needed for registration of crop protection agents were developed and optimised during the last ten years. Current requirements for the present literature search for cinmethylin were defined in close co-operation between the cinmethylin scientific expert team and Agro information professionals. Main searches were done on 7th and 27th July 2017. Later update searches were done on 20th February 2018 and 23th-26th April 2018. Duplicates search results from different databases in a respective section were removed in the STN databases by the "*duplicate remove*" command. The search process is documented in detail with search profiles, search histories and summary tables according to the guidance of EFSA – 'Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009, EFSA Journal 2011;9(2):2092'. The process of selection of relevant scientific peer-reviewed open literature was done in two steps:
The first selection step for relevance based on summary records (e.g. titles, abstracts, index terms, keywords) was done by the Agro information professionals.

- Obviously irrelevant records were tagged as "Ballast". This ballast was controlled by scientific experts in the corresponding subject areas, but was not further processed.
- Summary records which appeared to be relevant and those of unclear relevance were tagged as "Hit" and went to the next level of evaluation.

The second detailed assessment was done by the scientific experts in the corresponding areas. Records tagged as "Hit" were further evaluated in depth. To facilitate a comprehensible listing of the "Hits" in the different regulatory areas, an Excel file was generated for each section with three typical registers, namely:

- "no relevant endpoint"
- "evaluated - not-relevant"
- "used for dossier"

In a first step (rapid assessment), the "Hits" were reviewed based on the information given in the **title and the abstract** with regard to relevance for the regulatory endpoints in the respective regulatory area. Those records which were clearly judged as not assignable to any regulatory endpoint were shifted into the register **"no relevant endpoint"** with an explanation of rationale.

In a second step (detailed assessment), all remaining records were assessed in detail by the respective expert(s) based on the **complete report** and thus, separated into relevant reports for further discussion and those clearly not relevant.

Criteria to assign a record to the register **"evaluated - not-relevant"** were:

- Those records which provided information supporting the existing regulatory data package without any new relevant data or information were classified as "confirmatory data".
- Those records which were not assignable to the substance of interest (for example records regarding mixtures, not about the test substance or other relevant substance).
- Secondary literature linking to primary literature which was already discussed under relevant records.
- Records with limited reliability of grade 3 or 4 based on the 'Klimisch' scoring system.
- Records which were judged as not relevant due to other reasons, with a respective justification.

Criteria to assign a record to the register **"used for dossier"** were:

- Records providing information about additional/new/unknown/potentially contradictory effects or data which might impact the hazard assessment endpoints or the risk assessments parameters and which, in addition, have a high grade of reliability, i.e., grade 1 or 2 based on the 'Klimisch' scoring system.

Those records assigned to the category "used for dossier" were provided with a Doc ID and discussed in detail in the respective dossier chapter.

SUBSTANCES
Cinmethylin (CAS Reg No 87818-31-3)

Substance-Search in CAS Registry File, 20150705/UP:
Family Search (Fam) with CAS Reg-No 87818-31-3

Resulting CAS Registry numbers for search:

Cinmethylin including isomers and isotopes:
(87818-31-3 or 87819-60-1 or 87818-61-9 or 112502-84-8 or 99827-45-9 or 87818-68-6)

Cinmethylin metabolites:
For categories: Toxicity Animal & Human, Operator Exposure, Mode of Action, QSAR:
(87819-14-5 or 99765-53-4 or 99765-60-3 or 130772-87-1 or 1334643-80-9 or 42013-20-7 or 612-20-4)
(119973-33-0 or 119973-33-0 or 119973-35-2)

For all other categories:
(22555-57-3 or 22621-68-7 or 38630-76-1 or 50302-07-3 or 87172-89-2 or 87819-14-5 or 96645-97-5 or 99765-53-4 or 99765-60-3 or 103834-29-3 or 110901-97-8 or 119973-51-2 or 120053-26-1 or 120053-27-2 or 130772-87-1 or 134461-72-6 or 134461-73-7 or 119973-33-0 or 119973-33-0 or 119973-35-2))
(134527-97-2 or 134527-98-3 or 134527-99-4 or 134528-00-0 or 134528-01-1 or 134528-02-2 or 152453-46-8 or 152453-51-5 or 152453-52-6 or 152453-53-7 or 152519-96-5 or 152519-97-6 or 152519-98-7 or 152519-99-8 or 1334643-80-9 or 1933681-69-6)

For final update new metabolites (CAS Registry 2018-02-14/UP) were searched additionally:

For categories: Toxicity Animal & Human, Operator Exposure, Mode of Action, QSAR:
(87819-14-5 or 99765-53-4 or 99765-60-3 or 130772-87-1 or 1334643-80-9 or 42013-20-7 or 612-20-4)

For categories Metabolism and Residues in Animals and Animal Products, Metabolism and Residues in Plants:
(87-41-2 or 612-20-4 or 42013-20-7 or 87129-26-8 or 98857-39-7 or 119973-42-1 or 119973-44-3 or 119973-46-5 or 119973-50-1 or 119973-52-3 or 119997-23-8 or 161168-84-9 or 176896-66-5 or 1932066-49-3 or 1932367-62-8 or 1932534-82-1 or 1932543-20-8)

Search of common and trade names:
Cinmethylin#
SD95481 or WL95481 or (SD or WL)(w)(95481 or 95(w)481)
BAS(w)684# or BAS684#

Toxicology Animals and Human – search

Databases:
Toxcenter and Embase

Search strategy for substances:

Toxcenter:
all CAS Registry numbers, common/trade names, no mixtures

Embase:
all CAS Registry numbers, common/trade names, no mixtures
Cinmethylin+nt/ct

Search strategy for data: (further details, including the full list of search terms used is available in section 8, page 47 of the 'Literature Searcg Report, BASF DocID 2018/1099008)
Substances AND
****ACUT SUBCHRONIC CHRONIC****
------OR-----
****SKIN SENSITIZATION****
------OR-----
****CANCER****
------OR-----
****TERATOGEN REPROTOX****
------OR-----
****NEUROTOX****
------OR-----

****IMMUNOTOX****
-----All former Items and----
****ANIMALS****
------OR-----
ENDOCRIN
------OR-----
****MUTAGEN****
------OR-----
****METABOLISMUS****
------OR-----
****ALTERNATIVE METHODS****

Number of records after first selection step for Section 8 (Human and animal toxicity):

Database:	**Embase - Excerpta Medica Database**	**Toxcenter - Toxicology Center Database**
Provider:	STN International	STN International
Justification for choosing the source: - for STN databases referring to STN database summary sheets	Embase covers the most important international biomedical, drug-related and clinical literature, with a particular focus on adverse drug reactions and on Evidence Based Medicine. Sources are more than 7,600 peer-reviewed journals. All MEDLINE records are included. Records contain bibliographic information, controlled terms, in-depth indexing, drug trade names, abstracts and CAS Registry Numbers	Toxcenter covers all aspects of occupational hazards, adverse drug reactions, environmental ollution, chemically induced diseases, food contamination, pesticides and herbicides. Records contain bibliographic data, abstracts, indexing terms and CAS Registry Numbers
Coverage of Sub-databases:	ANEUPL, BIOSIS, CAplus, CIS, CRISP, DART, EMIC, EPIDEM, ETIC, FEDRIP, HAPAB, HMTC, IPA, MEDLINE, PESTAB, PPBIB, RISKLINE, TSCATS	
Date span of the source:	1947 – to present	1970 – to present
Date of the search:	2017-07-07	2017-07-07
Date span of the search:	Until update	Until update
Date of the latest database update included in the search:	20170706	20170703
Search strategies used for this data requirement (including any limits)	see above Search Report Cinmethylin	see above Search Report Cinmethylin
Total number of summary records for Cinmethylin and Metabolites:	17	72
Total number of summary records after removing duplicates:	10	62
Total number of summary records retrieved after first selection step:	4	24

Update Search on 2018-02-20 (Toxcenter: 20180220/UP, Embase 20180219/UP) for the section Toxicology Animal retrieved 2 additional results.

Hits after first selection step: 2

An additional search for new Cinmethylin metabolites (CAS numbers 87-41-2, 87129-26-8, 98857-39-7, 119973-42-1, 119973-44-3, 119973-46-5, 119973-50-1, 119973-52-3, 119997-23-8, 161168-84-9, 176896-66-5, 1932066-49-3, 1932367-62-8, 1932534-82-1, 1932543-20-8) has been performed, covering time range until update; In file HCAPLUS CAS numbers have been searched by linking with CAS-specific descriptors (roles): all CAS numbers (L)(BIOL OR FORM OR OCCU OR BCP OR BPR OR PYP OR PRP)/RL
For the section Toxicology Animal 63 additional results were retrieved for these metabolites.

Hits after first selection step: 21

3 additional results were retrieved in category Operator Exposure and have been categorised and evaluated as Tox Animal & Human.

Hits after first selection step for 3 results categorised as Tox Animal and Human : 2

Mode of Action Search
Databases:
CAPLUS, BIOSIS, Medline and Embase

Search strategy for substances:
In CAPLUS, BIOSIS, Medline:
all CAS Registry numbers, common/trade names, no mixtures

In Embase:
all CAS Registry numbers, common/trade names, no mixtures
Cinmethylin+nt/ct

Search strategy for data:
Substances AND

Mode of Action
• (mode# or mechanism#)(2w)action# or MoA
---AND---
Organismen
---or---
in BIOSIS: Organismen, mammalia+nt/orgn
in Medline: Organismen, mammals+nt/ct
in Embase: Organismen, mammal+nt/ct
in CAPLUS: Organismen, primates+nt/ct or rattus+nt/ct or muridae+nt/ct

Database:	BIOSIS	CAPLUS Chemical Abstracts Plus	Embase - Excerpta Medica Database	Medline
Provider:	STN International	STN International	STN International	STN International
Justification for choosing the source: - for STN databases referring to STN database summary sheets	BIOSIS Previews® is the largest and most comprehensive life science database in the world. Amongst others, subject coverage includes Agriculture, Biochemistry, Biophysics, Botany, Environmental Biology, Physiology, Toxicology. Sources include periodicals, journals, conference proceedings, reviews, reports, patents, and short communications. Nearly 6,000 life source journals, 1,500 international meetings as well as review articles, books, and monographs are reviewed for inclusion. Bibliographic information, indexing terms, abstracts, and CAS Registry Numbers are all searchable.	The Chemical Abstracts (CA) database covers all areas of Biochemistry, Chemistry and Chemical engineering, and related sciences. Sources include over 8,000 journals, patents from 38 national patent offices and two international patent organizations, technical reports, books, conference proceedings, and dissertations. Electronic only journals and Web preprints are also covered. Bibliographic terms, indexing terms, roles, CAS Registry Numbers, International Patent Classification, and abstracts are searchable.	Embase covers the most important international biomedical, drug-related and clinical literature, with a particular focus on adverse drug reactions and on Evidence Based Medicine. Sources are more than 7,600 peer-reviewed journals. All MEDLINE records are included. Records contain bibliographic information, controlled terms, in-depth indexing, drug trade names, abstracts and CAS Registry Numbers	Medline covers all areas in the field of biomedicine. Sources are Index Medicus, Index to Dental Literature, HealthSTAR database and International Nursing Index Over 50% of MEDLINE's citations have abstracts, CAS Registry Numbers are present.
Coverage of Sub-databases:				
Date span of the source:	1926 – to present	1907 – to present	1947 – to present	1946 – to present
Date of the main search:	2017-07-07	2017-07-07	2017-07-07	2017-07-07
Date span of the search:	Until update	Until update	Until update	Until update
Date of the latest database update included in the search:	20170705	20170706	20170706	20170706
Search strategies used for this data requirement (including any limits)	See above	See above	See above	See above
Total number of summary records for Cinmethylin	1	19	0	0

and Metabolites:				
Total number of summary records after removing duplicates:	1	19	0	0
Total number of summary records retrieved after first selection step:	1	9	0	0

Update Search on 2018-02-20 (BIOSIS: 20180214/UP; CAPLUS: 20180219/UP; Embase: 20180219/UP; Medline 20180219/UP) for the section Mode of action retrieved 1 additional results.

Hits after first selection step: 1

An additional search for new Cinmethylin metabolites (CAS numbers 87-41-2, 87129-26-8, 98857-39-7, 119973-42-1, 119973-44-3, 119973-46-5, 119973-50-1, 119973-52-3, 119997-23-8, 161168-84-9, 176896-66-5, 1932066-49-3, 1932367-62-8, 1932534-82-1, 1932543-20-8) has been performed, covering time range until update, for the section Mode of Action; 20 additional results were retrieved for these metabolites.

Hits after first selection step: 6

QSAR search
Databases:
CAPLUS

Search strategy for substances:
all CAS Registry numbers, common/trade names, no mixtures

Search strategy for data:
Substances AND
• que QSAR or QBAR or QSPR or QSTR
• que (quant? or qual?)(2w)structur?(2w)(activit? or propert? or toxic?)(2w)relation?
• que (quant? or qual?)(2w)biol?(w)activit?(2w)relation?)
• que derek or Lhasa(w)structur? or in(w)silico or insilico

---or---
Substances Link
• (structur?(w)activit?(w)relation?)

Database:	**CAPLUS Chemical Abstracts Plus**
Provider:	STN International
Justification for choosing the source: - for STN databases referring to STN database summary sheets	The Chemical Abstracts (CA) database covers all areas of Biochemistry, Chemistry and Chemical engineering, and related sciences. Sources include over 8,000 journals, patents from 38 national patent offices and two international patent organizations, technical reports, books, conference proceedings, and dissertations. Electronic only journals and Web preprints are also covered. Bibliographic terms, indexing terms, roles, CAS Registry Numbers, International Patent Classification, and abstracts are searchable.
Date span of the source:	1907 – to present
Date of the main search:	2017-07-07
Date span of the search:	Until update
Date of the latest database update included in the search:	20150706
Search strategies used for this data requirement (including any limits)	see above Search Report Cinmethylin
Total number of summary records for Cinmethylin and Metabolites:	5
Total number of summary records retrieved after first selection step:	2

Update Search on 2018-02-20 (CAPLUS: 20180219/UP) for the section QSAR retrieved no additional results.

An additional search for new Cinmethylin metabolites (CAS numbers 87-41-2, 87129-26-8, 98857-39-7, 119973-42-1, 119973-44-3, 119973-46-5, 119973-50-1, 119973-52-3, 119997-23-8, 161168-84-9, 176896-66-5, 1932066-49-3, 1932367-62-8, 1932534-82-1, 1932543-20-8) has been performed, covering time range until update, for the section QSAR; 11 additional results were retrieved for this metabolite.

Hits after first selection step: 3.

Studies checked for reliability (after relevance check)

Title	Abstracts	Category	Author	Source/Patent No	Reliability Evaluation
O-Acylation as a novel conjugation pathway for cinmethylin in rats	A complex degrdn. pattern of phenyl-14C-labeled cinmethylin (I) rats following oral administration has been reported earlier. In addn. to the undegraded I, 10 metabolites were detected. In the urinary org. extractable fraction, 2 minor metabolites (each accounting for administered radioactivity) were identified as o-(acetoxymethyl)benzoic acid and 9-(acetoxymethyl)-.alpha.-carboxycinmethylin. They were the corresponding O-acetyl analogs of o-(hydroxymethyl)benzoic acid and 9-hydroxy-.alpha.-carboxycinmethylin, the principal metabolites of I. A detailed description of the isolation and identified of these 2 novel O-acylation conjugates is presented.	Tox Animal + Human	Lee, Philip W. Woodward, Michael D. Stearns, Stephen M.	Journal of Agricultural and Food Chemistry (1988), 36(1), 95-7 CODEN: JAFCAU; ISSN: 0021-8561	Supplementary; Included in section B.6.1.5.
Metabolic fate of cinmethylin in rats	The metabolic fate of 14C-labeled cinmethylin (I) [87818-31-3], a novel cineole herbicide, in lab. rats following a single oral dose of 15 or 450 mg/kg was examd. The major route of elimination was via urinary excretion, and .apprx.75-85% of the administered radioactivity was eliminated during the initial 48 h post-dosing. No 14CO2 or other radioactive volatile material was detected in the respired air. A complex degrdn. pattern of I was obsd. in the animal excreta. In addn., to the undegraded I (recovered only in the fecal excreta), at least 10 metabolites were isolated and identified from the urinary and fecal excreta as org.-extractable and conjugated products. The proposed metabolic pathways of I involved hydroxylation and oxidn. at the benzyl and cineole portions of the parent mol., conjugation (with glucuronic acid and glycine), and ether cleavage. Pharmacokinetic patterns indicated the rapid disposition of I and its metabolites from the treated animals. No toxicol. relevant level of residues was detected in tissues other than the liver.	Tox Animal + Human	Lee, Philip W. Stearns, Stephen M. Powell, Walter R. Stoutamire, Donald W. Payne, George B. Woodward, Michael D. Burton, William B. Silveira, Edward J. Ehmann, Axel	Journal of Agricultural and Food Chemistry (1986), 34(2), 162-70 CODEN: JAFCAU; ISSN: 0021-8561	Supplementary; Information covered in toxicokinetic studies evaluated in section B.6.1.5.

Predictive Endocrine Testing in the 21st Century Using in Vitro Assays of Estrogen Receptor Signaling Responses	Thousands of environmental chems. are subject to regulatory review for their potential to be endocrine disruptors (ED). In vitro high-throughput screening (HTS) assays have emerged as a potential tool for prioritizing chems. for ED-related whole-animal tests. In this study, 1814 chems. including pesticide active and inert ingredients, industrial chems., food additives, and pharmaceuticals were evaluated in a panel of 13 in vitro HTS assays. The panel of in vitro assays interrogated multiple end points related to estrogen receptor (ER) signaling, namely binding, agonist, antagonist, and cell growth responses. The results from the in vitro assays were used to create an ER Interaction Score. For 36 ref. chems., an ER Interaction Score >0 showed 100% sensitivity and 87.5% specificity for classifying potential ER activity. The magnitude of the ER Interaction Score was significantly related to the potency classification of the ref. chems. ER.alpha./ER.beta. selectivity was also evaluated, but relatively few chems. showed significant selectivity for a specific isoform. When applied to a broader set of chems. with in vivo uterotrophic data, the ER Interaction Scores showed 91% sensitivity and 65% specificity. Overall, this study provides a novel method for combining in vitro concn. response data from multiple assays and, when applied to a large set of ER data, accurately predicted estrogenic responses and demonstrated its utility for chem. prioritization.	Tox Animal + Human	Rotroff, Daniel M. Martin, Matt T. Dix, David J. Filer, Dayne L. Houck, Keith A. Knudsen, Thomas B. Sipes, Nisha S. Reif, David M. Xia, Menghang Huang, Ruili Judson, Richard S.	Environmental Science + Technology (2014), 48(15), 8706-8716 CODEN: ESTHAG; ISSN: 0013-936X	Reliable Included in section B.6.8.2 (mechanistic information). 2010/1233112
Profiling 976 ToxCast Chemicals across 331 Enzymatic and Receptor Signaling Assays	Understanding potential health risks is a significant challenge due to the large nos. of diverse chems. with poorly characterized exposures and mechanisms of toxicities. The present study analyzes 976 chems. (including failed pharmaceuticals, alternative plasticizers, food additives, and pesticides) in Phases I and II of the U.S. EPA's ToxCast project across 331 cell-free enzymic and ligand-binding high-throughput screening (HTS) assays. Half-maximal activity concns. (AC50) were identified for 729 chems. in 256 assays (7135 chem.-assay pairs). Some of the most commonly affected assays were CYPs (CYP2C9 and CYP2C19), transporters (mitochondrial TSPO, norepinephrine, and dopaminergic), and GPCRs (aminergic). Heavy metals, surfactants, and dithiocarbamate fungicides showed promiscuous but distinctly different patterns of activity, whereas many of the pharmaceutical compds. showed promiscuous activity across GPCRs. Literature anal. confirmed >50% of the activities for	Tox Animal + Human	Sipes, Nisha S. Martin, Matthew T. Kothiya, Parth Reif, David M. Judson, Richard S. Richard, Ann M. Houck, Keith A. Dix, David	Chemical Research in Toxicology (2013), 26(6), 878-895 CODEN: CRTOEC; ISSN: 0893-228X	Reliable Included in section B.6.8.2 (mechanistic information). 2013/1371960

	the most potent chem.-assay pairs (54) but also revealed 10 missed interactions. Twenty-two chems. with known estrogenic activity were correctly identified for the majority (77%), missing only the weaker interactions. In many cases, novel findings for previously unreported chem.-target combinations clustered with known chem.-target interactions. Results from this large inventory of chem.-biol. interactions can inform read-across methods as well as link potential targets to mol. initiating events in adverse outcome pathways for diverse toxicities.		J. Kavlock, Robert J. Knudsen, Thomas B.		
In Vitro Screening of Environmental Chemicals for Targeted Testing Prioritization: The ToxCast Project	Background: Chem. toxicity testing is being transformed by advances in biol. and computer modeling, concerns over animal use, and the thousands of environmental chems. lacking toxicity data. The U.S. Environmental Protection Agency's ToxCast program aims to address these concerns by screening and prioritizing chems. for potential human toxicity using in vitro assays and in silico approaches. Objectives: This project aims to evaluate the use of in vitro assays for understanding the types of mol. and pathway perturbations caused by environmental chems. and to build initial prioritization models of in vivo toxicity. Methods: We tested 309 mostly pesticide active chems. in 467 assays across nine technologies, including high-throughput cell-free assays and cell-based assays, in multiple human primary cells and cell lines plus rat primary hepatocytes. Both individual and composite scores for effects on genes and pathways were analyzed. Results: Chems. displayed a broad spectrum of activity at the mol. and pathway levels. We saw many expected interactions, including endocrine and xenobiotic metab. enzyme activity. Chems. ranged in promiscuity across pathways, from no activity to affecting dozens of pathways. We found a statistically significant inverse assocn. between the no. of pathways perturbed by a chem. at low in vitro concns. and the lowest in vivo dose at which a chem. causes toxicity. We also found assocns. between a small set of in vitro assays and rodent liver lesion formation. Conclusions: This approach promises to provide meaningful data on the thousands of untested environmental chems. and to guide targeted testing of environmental contaminants.	Tox Animal + Human	Judson, Richard S. Houck, Keith A. Kavlock, Robert J. Knudsen, Thomas B. Martin, Matthew T. Mortensen, Holly M. Reif, David M. Rotroff, Daniel M. Shah, Imran Richard, Ann M. Dix, David J.	Environmental Health Perspectives (2010), 118(4), 485-492 CODEN: EVHPAZ; ISSN: 0091-6765	Reliable Included in section B.6.8.2 (mechanistic information). 2016/1227708

Ototoxicity of the three xylene isomers in the rat	Numerous expts. have shown that the arom. solvents can affect the auditory system in the rat, the cochlea being targeted first. Solvents differ in cochleotoxic potency: for example, styrene is more ototoxic than toluene or xylenes. The goal of this study was to det. the relative ototoxicity of the 3 isomers of xylene (o-, m-, or p-xylene). Moreover, by dosing with the 2 urinary metabolites of xylene, methylhippuric (MHAs) and mercapturic acids (MBAs), this study points toward a causal relation between the cochleotoxic effects and potential reactive intermediates arising from the biotransformation of the parent mols. Sep. groups of rats were exposed by inhalation to 1 isomer following this schedule: 1800 ppm, 6 h/d, 5 d/wk for 3 wk. Auditory thresholds were detd. with brainstem-auditory evoked potentials. Morphol. anal. of the organ of Corti was performed by counting both sensory and spiral ganglion cells. Among the 3 isomers, only p-xylene was cochleotoxic. A 39-dB permanent threshold shift was obtained over the tested frequencies range from 8 to 20 kHz. Whereas outer hair cells were largely injured, no significant morphol. change was obsd. within spiral ganglia. The concns. of urinary p-, o-, or m-MHA were greater (p-MHA: 33.2 g/g; o-MHA: 7.8 g/g; m-MHA: 20.4 g/g) than those obtained for MBAs (p-MBA: 0.04 g/g; o-MBA: 6.2 g/g; m-MBA: 0.03 g/g). Besides, there is a large difference between o-MBA (6.2 g/g) and p-MBA (0.04 g/g). As a result, since the cysteine conjugates are not determinant in the ototoxic process of xylenes, the location of the Me groups around the benzene nucleus could play a key role.	Tox Animal + Human Ballast	Maguin, Katy Lataye, Robert Campo, Pierre Cossec, Benoit Burgart, Manuella Waniusiow, Delphine	Neurotoxicology and Teratology (2006), 28(6), 648-656 CODEN: NETEEC; ISSN: 0892-0362	Reliable Included in Section B.6.8.1 (Metabolites).

Xenobiotic-Metabolizing Enzyme and Transporter Gene Expression in Primary Cultures of Human Hepatocytes Modulated by Toxcast Chemicals	Primary human hepatocyte cultures are useful in vitro model systems of human liver because when cultured under appropriate conditions the hepatocytes retain liver-like functionality such as metab., transport, and cell signaling. This model system was used to characterize the concn.- and time-response of the 320 ToxCast chems. for changes in expression of genes regulated by nuclear receptors. Fourteen gene targets were monitored in quant. nuclease protection assays: six representative cytochromes P 450, four hepatic transporters, three Phase II conjugating enzymes, and one endogenous metab. gene involved in cholesterol synthesis. These gene targets are sentinels of five major signaling pathways: AhR, CAR, PXR, FXR, and PPAR.alpha.. Besides gene expression, the relative potency and efficacy for these chems. to modulate cellular health and enzymic activity were assessed. Results demonstrated that the culture system was an effective model of chem.-induced responses by prototypical inducers such as phenobarbital and rifampicin. Gene expression results identified various ToxCast chems. that were potent or efficacious inducers of one or more of the 14 genes, and by inference the 5 nuclear receptor signaling pathways. Significant relative risk assocns. with rodent in vivo chronic toxicity effects are reported for the five major receptor pathways. These gene expression data are being incorporated into the larger ToxCast predictive modeling effort.	Mode of Action	Rotroff, Daniel M. Beam, Andrew L. Dix, David J. Farmer, Adam Freeman, Kimberly M. Houck, Keith A. Judson, Richard S. LeCluyse, Edward L. Martin, Matthew T. Reif, David M. Ferguson, Stephen S.	Journal of Toxicology and Environmental Health, Part B: Critical Reviews (2010), 13(2-4), 329-346 CODEN: JTECFR; ISSN: 1093-7404	Reliable Included in section B.6.8.2 (Mechanistic information). 2010/1233112

Screening for androgen receptor activities in 253 industrial chemicals by *in vitro* reporter gene assays using AR-EcoScreen cells	Recently, there has been great concern about the potential of industrial chems. to act as endocrine disrupters. In this report, we conducted a pilot study to validate the use of AR-EcoScreen cells for tier 1 screening of androgen receptor (AR) agonist and antagonist activities. From 253 test compds., we identified two AR agonists and nine antagonists. The two agonists, 2-tert-butylanthraquinone and benzoanthrone, were relatively weak (10% maximal activation of the pos. control, 5.alpha.-dihydrotestosterone, at 2.54.times.10-7 and 4.46.times.10-6 M, resp.). The most potent antagonist was 3,3'-dichlorobenzidine dihydrochloride (IC50 = 2.28.times.10-7 M). The order of the anti-androgenic activities was 3,3'-dichlorobenzidine dihydrochloride > 4-diethylaminobenzaldehyde > 4,4'-[1-[4-[1-(4-hydroxyphenyl)-1-methylethyl]phenyl]ethylidene]bis[phenol] > 2,4,6-trichlorophenylhydrazine = 4-(phenylpropyl)pyridine > 2-hydroxy-4-methoxybenzophenone > 2,2-bis(4-cyanophenyl)propane > 4-methoxy-2-methyldiphenylamine = 2,4-diphenyl-4-methylpentene-1. These results suggest that AR-EcoScreen cell line has the potential to be used as a tool for the large scale tier 1 screening of chems. for androgen receptor agonist and antagonist activity.	Tox Animal + Human	Araki, Naohiro Ohno, Ken Nakai, Makoto Takeyoshi, Masahiro Iida, Mitsuru	Toxicology *in Vitro* (2005), 19(6), 831-842 CODEN: TIVIEQ; ISSN: 0887-2333	Limited. Included in section B.6.8.1.1, information on M684H059.

Introduction: The studies listed below were performed by Shell Agriculture in the 1980's to investigate the toxicity of cinmethylin. Most of the studies were discussed in this DAR; however, some of the studies suffer distinct deficiencies that prevent a comprehensive evaluation or are not required for submission within the EU. Therefore, the studies which were not further considered in this DAR are shortly summarised with a justification for non-submission.

Report ID	Authors	Title	Abstract in case that the study is not further considered in the dossier, otherwise link to dossier	Justification for no consideration in the evaluation
CI-411-002	[redacted] and [redacted]	Acute Oral Toxicity of SD 95481 in the Mouse	Evaluated in the DAR, section B.6.2.1.	
CI-411-001	[redacted] and [redacted]	Acute Oral Toxicity of SD 95481 in the Rat	Evaluated in the DAR, section B.6.2.1.	
CI-412-001	[redacted]	Acute Toxicity Studies with Technical SD 95481 including Acute Dermal Toxicity of Technical SD 95481; Rabbit Eye Irritation; Primary Skin Irritation of Technical SD 95481	Evaluated in the DAR, section B.6.2.	
CI-413-001	[redacted]	Acute 4-Hour Inhalation Study in Rats with CINCH Technical Herbicide	Evaluated in the DAR, section B.6.2.3.	
CI-416-001	[redacted] and [redacted]	Guinea Pig Skin Sensitisation Study of SD 95481	Evaluated in the DARr, section B.6.2.1.	
CI-416-002	[redacted]	WL95481: Skin Sensitizing Potential	Evaluated in the DAR, section B.6.2.6.	

CI-440-012	[redacted]	Dermal Absorption of [^{14}C] Cinmethylin in the Rat	Not evaluated in the DAR Studies of the dermal absorption of cinmethylin were carried out at doses of 10, 1 and 0.1 mg of [14C]cinmethylin per rat on October 2, 7, and 13, 1986 respectively. Doses were applied over 12 cm^2 areas of the animals' backs from which the fur had been clipped. Protective, non-occluding de¬vices were attached to the rats to isolate the dosed area. Groups of four animals were sacrificed at time points of 0.5, 1, 2, 4, 10, and 24 h after dosing. The remaining unabsorbed dose was then washed off the skin. Excreta, carcass, blood and the skin in the dose area were assayed for absorbed radioactivity. The skin wash and protective coverings were assayed for nonabsorbed radioactivity. The recovery of the administered radio¬activity from all sources averaged 94.5 ± 4.8(SD)%. Absorption of the dose was most rapid during the first 0.5 h, ranging from 8.5% for the highest dose to 5.5% for the lowest. The absorption rates during the initial 0.5 h at the dose levels of 0.1, 1 and 10 mg/animal were 0.9, 11 and 140 mg/cm 2/h, respectively; and the absorption rates for the 10 h period were 0.15, 1.7 and 12 mg/cm 2/h, respectively. By 4 h, roughly equivalent percentages (11-12%) of all dose levels had been absorbed. Ab¬sorption at the 24-h time point averaged 26-28% for all dose levels and appeared to be linear within the dose levels tested. The major route of elimination of the absorbed [14C]cinmethylin equivalents over the first 24 h was urinary excretion, with lesser amounts excreted in feces. The maximum urinary and fecal excretions occurred during the 10-24 h sampling interval.	*In vivo* data with the radiolabelled TGAI are not the preferred or required data. Therefore, *in vitro* data with the representative formulation BAS 684 03 H were evaluated.

CI-420-001	[redacted]	Fourteen Day Feeding Study of SD 95481 in the Rat	Not evaluated in the DAR A fourteen day feeding study of SD 95481 was conducted in the rat. Six dose levels (0, 300, 1000, 3000, 10000, and 30000 ppm) were tested in groups of ten (5 male, 5 female) Fischer 344 rats. SD 95481 was incorporated into the basal diet of Purina Certified Rodent Chow 5002 at appropriate concentrations to provide the designated ppm dose level; prepared use batches were fed ad libitum. No lethalities were observed in either the control or treated rats. The treatment-related clinical sign of toxicity observed in the study was piloerection in male and female rats at 30000 ppm SD 95481. A significant decrease in food consumption was observed in male rats at weeks 1 and 2, and in female rats at week 1 only for animals receiving 10000 and 30000 ppm SD 95481 diets. Remarkable treatment-associated macroscopic observations during necropsy included: enlarged livers, roughened hair coat and yellow stained fur on the inguinal area in the 10000 and 30000 ppm animals. Decreased body weight in the 10000 (male only) and 30000 ppm dose groups resulted in statistically significant decreases in brain, kidney and testes weights at 30000 ppm and increased relative brain, kidneys and testes weights at 10000 and 30000 ppm in male rats and increased relative kidney weights in female rats at 30000 ppm SD 95481. Absolute brain weights were significantly decreased in female rats at doses of 1000, 3000 and 30000 ppm SD 95481. Statistically significant increases in absolute and relative liver weights were observed in male rats at dietary doses of >3000 and >1000 ppm respectively and female rats at >10000 and >3000 ppm respectively.	Screening study- non GLP- no data requirement.
CI-420-002	[redacted]	Fourteen Day Feeding Study of SD 95481 in the Mouse	Not evaluated in the DAR A fourteen day feeding study of SD 95481 was conducted in the mouse. Six dose levels (0, 300, 1000, 3000, 10000, and 30000 ppm) were tested in groups of ten (5 male, 5 female) B6C3F1 mice. SD 95481 was incorporated into the basal diet of Purina Certified Rodent Chow 5002 at appropriate concentrations to provide the designated ppm dose level; prepared use batches were fed ad libitum. No lethalities were observed in either the control or treated mice. Prominent clinical signs of toxicity included infrequent and inconsistent observations of polyuria, hypoactivity, and hyperactivity. Significant body weight depres¬sion was observed in all mice of the 10000 and 30000 ppm dose levels. A significant decrease in food consumption was observed in male mice receiving 10000 and 30000 ppm and female mice receiving 30000 ppm SD 95481 diets. Treatment-associated macroscopic observations during necropsy included: hepatomegaly, thymic atrophy, and genital hypoplasia	Screening study- non GLP- no data requirement.

			(immaturity) in the 10000 and 30000 ppm animals. Mean kidney weights were decreased in mice fed 30000 ppm compared to controls. Mean relative brain weights were increased in female mice at doses of 10000 and 30000 ppm SD 95481. A dose related increase in absolute and relative liver weights was observed in male and female mice at doses of 1000-30000 ppm SD 95481. These values were generally statistically significant at doses >3000 ppm.	
CI-420-004	[redacted]	Five Week Dietary Feeding Study in Dogs - SD 95481 Technical	Evaluated in the DAR, section B.6.3.1.	
CI-420-003	[redacted]	21 Day Repeated Dermal Application Study in Rabbits with CINCH ® Herbicide	Not evaluated in the DAR A study was conducted to evaluate the potential toxicity of technical CINCH (R) Herbicide resulting from repeated dermal applications to New Zealand White rabbits. Doses of CINCH were applied for 21 consecutive days to skin sites clipped free of hair. Doses of CINCH were made on the basis of dilutions in 2% methyl cellulose; all male and female rabbits received daily doses of 1.0 ml/kg of the following dilutions: undiluted, 50%, 12.5%, or 2.5%. A vehicle control group received only 1.0 ml/kg of 2% methyl cellulose diluent. Skin sites were not occluded following treatment and sites were washed with mild soap end water 6 hours following daily dosing. Throughout the 21 in-life days of the study, **6 rabbits were found dead (two vehicle control females; one 12.5% CINCH treated female ; two female and one male treated with 2.5 % CINCH)**. Because of the lack of a dose related trend, these deaths are not considered to be related to CINCH treatment. The study pathologist considered the deaths, as well as the significant abnormal clinical signs, to be the result of an enteritis condition (mucoid) in some of the rabbits. The disease is not uncommon in the laboratory rabbit. Daily dermal exposure to CINCH resulted in significant, dose-related skin irritation. Undiluted CINCH end 50 % CINCH resulted in moderate to severe skin irritation; 12.5 % CINCH resulted in slight to severe irritation; 2.5% CINCH resulted in slight skin irritation. These grossly observable reactions were associated with lesions observed microscopically. The skin reactions were dose related and only one rabbit dosed with 2.5 % CINCH showed significant pathology to skin, observed microscopically (mild acanthosis). When compared to vehicle control rabbits, body weight and food consumption values were significantly lower at 1 week for animals treated with undiluted or 50% (males only) CINCH.	The study shows several deficiencies that prevent a meaningful interpretation: 1: The substance is administered as % dilution, so that the dose is unclear. 2. The application site was uncovered so that ingestion of the test substance was possible. 3. Several animals died as consequence of an enteritis without relation to the substance.

			Treatment with CINCH did not affect haematology or blood chemistry parameters and did not alter liver, kidney or testes weights, when compared to values obtained from vehicle control rabbits. Microscopic evaluation of liver and kidney of rabbits treated with undiluted CINCH revealed no toxicity, when compared to tissues from vehicle control rabbits. It can be concluded that, with the exception of skin reactions, the no-effect-level (NOEL) of CINCH, administered to the skin of rabbits repeatedly, is 12.5 %. This NOEL is selected on the basis of significantly lower body weight and food consumption values in rabbits exposed to undiluted or 50 % CINCH.	Conclusion: Based on these circumstances, the study is considered invalid and was not further considered for submission. A new study up to limit dose was conducted.
CI-425-001	[redacted]	Subchronic Feeding Study of SD 95481 in the Rat	Evaluated in the DAR, section B.6.3.2.	
CI-425-002	[redacted] and [redacted]	Subchronic Feeding Study of SD 95481 in the Mouse	Evaluated in the DAR, section B.6.3.2.	
CI-425-003	[redacted]	Thirteen Week Dietary Feeding Study in Beagle Dogs CINCH Herbicide (Technical)	Evaluated in the DAR, section B.6.3.2.	
CI-427-007		A 2 Year Feeding Study with SD 95481 in Rats Corrigendum to SBGR.85.084	Evaluated in the DAR, section B.6.5.1.	
CI-427-001	[redacted]	A 2 Year Feeding Study with SD 95481 in Rats (Report No.: SBGR.85.084)		

CI-427-008	[redacted] and [redacted]	Corrigendum: Preparation of Supplement for Submission to the Japanese Ministry of Agriculture, Forestry and Fisheries from Shell Group Research report SBGR.85.084 (A 2 Year Feeding Study with SD 95481 in Rats) Corrigendum		
CI-427-006	[redacted] and [redacted]	Preparation of Supplement for Submission to the Japanese Ministry of Agriculture, Forestry and Fisheries from Shell Group Research report SBGR.85.084 (A 2 Year Feeding Study with SD 95481 in Rats)		
CI-427-002	[redacted]	One Year Dietary Feeding Study in Dogs -SD 95481 Technical	Evaluated in the DAR, section B.6.3.3.	
CI-427-003	[redacted]	One-Year Dietary Feeding Study in Beagle Dogs: CINCH Herbicide (Technical)	Evaluated in the DAR, section B.6.3.3.	
CI-427-004	[redacted]	CINCH Herbicide (Technical): Reversibility of Toxicity in Beagle Dogs (A 12 Month Feeding Study with 6 Months Reversibility)	Evaluated in the DAR, section B.6.3.3.	

CI-427-005	[redacted] and [redacted]	One-Year Dietary Feeding Study in Beagle Dogs: CINCH Herbicide (Technical) Supplemental Appendix to Du Pont Report No. SRO-614-87 (WTP-365)	Evaluated in the DAR, section B.6.3.3, as part of [redacted] (1988a) (CI-427-003).	
CI-428-001	[redacted] and [redacted]	Oncogenicity Study of SD 95481 in the Mouse	Evaluated in the DAR, section B.6.5.2.	
CI-428-002	[redacted] and [redacted]	Preparation of Supplement for Submission to the Japanese Ministry of Agriculture, Forestry and Fisheries from Regulatory Information Record No. WRC RIR-424 (Oncogenicity Study with SD 95481 in Mice)	Evaluated in the DAR, section B.6.5.2.	
CI-430-001	[redacted]	Two Generation Reproduction Study of CINCH Herbicide (SD 95481) in Rats	Evaluated in the DAR, section B.6.6.1.	
CI-432-001	[redacted], [redacted] and [redacted]	CINCH Herbicide (SD 95481) Teratology, Study in Sprague Dawley Rats	Evaluated in the DAR, section B.6.6.2.	
CI-432-002	[redacted].	Teratology Study of CINCH Herbicide (Technical SD 95481) Administered Orally Via Stomach Tube to New Zealand White (NZW) Rabbits	Evaluated in the DAR, section B.6.6.2.	
CI-432-003	[redacted]	Teratogenicity Study of IN-YA168 in Rabbits	Evaluated in the DAR, section B.6.6.3.	

CI-435-001	Glueck, D.M. and Sawin, V.L.	Assay of SD 95481 for Gene Mutation in Salmonella typhimurium	Not evaluated in the DAR The potential of technical SD 95481 to induce gene mutations in Salmonella typhimurium was assessed in a preincubation plate incorporation assay in strains TA98, TA100, TA1535, TA1537, and TA1538 in the absence and in the presence of S-9. Positive controls (TA98 and TA1538: 2-nitrofluorene; TA100 and TA1535: N-methyl-N'-nitro-N-nitrosoguanidine; TA1537: 9-amino-acridine; metabolic activation fraction: 2-aminoanthracene) and solvent controls for the test material series (absolute ethyl alcohol) and positive control mate¬rials (dimethyl sulfoxide) were run concurrently with each mutagenicity assay. Sterility checks and strain verification checks also were run with each mutagenicity assay. SD 95481 was tested first in TA98 and TA100 at concentra¬tions from 1.6x10-6 to 5.0x10-3 mg/plate. There was no evidence of precipitation at any concentration; however, toxicity was evident in TA98 in the absence of S-9 in two non-consecutive concentrations and in TA100 in the presence of S-9 at three consecutive concentration from 5.0x10-4 to 5.0x10-3 mg/plate. There was no statistically significant increase in the number of revertant colonies with TA98 either in the absence or in the presence of S-9 and with TA100 in the presence of S-9. In TA100, the number of revertant colonies was significantly higher than in the control at a single concentration, 1.6x10-5 mg/plate in the absence of S-9. To determine whether this single point represented a spurious event or was reproducible, a repeat mutagenicity assay using only TA100 in the absence of S-9 was conducted at concentrations from 5.0x10-6 to 1.6x10-4 mg/plate. There was no evidence of precipitate formation or of toxicity and there was no increase in the number of revertant colonies that was significantly higher than the control. SD 95481 was then tested in strains TA1535, TA1537, and TA1538 at concentra¬tions from 1.6x10-6 to 5.0x10-3 mg/plate. There was no evidence of precipitate formation or toxicity at any concentration tested, and there was no increase in the number of revertant colonies either in the absence or in the presence of S-9 that was significantly higher than the control. It was concluded that technical SD 95481 did not induce gene mutations in Salmonella typhimurium, strains TA98, TA100, TA1535, TA1537 and TA1538, in the absence or presence of a metabolizing enzyme fraction derived from rat liver.	Homogeneity of cinmethylin in DMSO has been investigated microscopically by confocal laser scanning microscopy (CLSM), showing that the solubility limit of cinmethylin in DMSO is already exceeded at concentrations as low as 12 mg/ml. This insolubility is not visible with the unaided eye. As consequence m the used stock solutions in the genotoxicity studies had insufficient homogeneity and any

CI-435-005	Brooks, T.M and Wiggins, D.E.	Bacterial Mutagenicity Studies with WL 95481	Not evaluated in the DAR S. typhimurium strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538, and a strain of E. coli WP2 uvrA (pKM101) were exposed to WL95481 (batch: 513F, purity: mind. 91.9%) in the presence and absence of metabolic activation for 48 - 72 hours. Vehicle (DMSO) and positive controls were included in two independent experiment. In each Ames standard plate test (SPT), the test item was tested in triplicates of eight concentrations in a range of 31.25 to 5000 µg/plate with and without S9 mix (Aroclor 1254-induced rat liver S9 fraction). The stability of the test item preparation in the vehicle DMSO was verified analytically for a period of 2 days at room temperature. The test item formed an oily smear on the surface of the top agar layer at 250 µg/plate onwards, and precipitation was observed at the top dose of 5000 µg/plate. No bacteriotoxic effect was observed up to 5000 µg/plate, the highest concentration evaluated. A relevant increase in the number of his+ and trp+ revertants was not observed in SPT either without S9 mix or after the addition of a metabolizing system. The positive controls induced the appropriate response in the corresponding strains, thus demonstrating the sensitivity of the test system. Based on the results of the present study, WL95481 is not mutagenic in the Ames standard plate test with and without metabolic activation under the experimental conditions chosen.	prepared dilutions thereof are questionable in their concentrations. Furthermore, the studies have several deviations from current guidelines and were not interpretable based on missing raw data , historical control data, missing data on negative and positive controls.
CI-435-002	Gingell, R.	Unscheduled DNA Synthesis Assay of CINCH Herbicide (SD 95481)	Not evaluated in the DAR The objective of this study was to evaluate the potential of CINCH® HERBICIDE (technical SD 95481) to induce unscheduled DNA synthesis (UDS) in primary cultures of rat hepatocytes. The results of this assay may be used to indicate the potential of CINCH to cause repairable DNA damage, and contribute to an evaluation of its potential genotoxicity. Primary cultures of rat hepatocytes were prepared from an adult male Fischer 344 rat and incubated with various concentrations of CINCH in dimethylsulfoxide. Concentrations in medium of 100 ug/ml and above were lethal to the hepatocytes; concentrations of 3.2 to 32 ug/ml were toxic, but no toxicity was observed at concentrations of 1 ug/ml and below. No evidence for increased UDS activity, as determined by mean nuclear grain count or number of cells with increased nuclear labeling, was obtained for CINCH at nonlethal concentrations (0.1 to 32 ug/ml). The positive control compound 2-	

			acetylaminofluorene produced a marked dose-dependent increase in UDS. Chemical analysis indicated that the tested sample of CINCH was stable over the experimental period, and that representative concen¬trations of CINCH in dimethylsulfoxide were close to the nominal values. Thus CINCH HERBICIDE (SD 95481) was inactive at all nonlethal concentrations in a valid rat primary hepatocyte unscheduled DNA synthesis assay.	
CI-435-003	Sawin, V.L, *et al*	Assay of Technical Grade SD 95481 for Gene Mutation in Mouse Lymphoma Cells	Not evaluated in the DAR SD 95481 (batch: 513H, purity: mind. 95.0%) was tested in vitro for its ability to induce forward mutations in mammalian cells by assessing the mutation of the TK locus in Mouse Lymphoma L5178Y TK+/-cells (clone 3.7.2c). Two independent experiments were conducted in the presence (Aroclor 1254-induced rat liver S9) or absence of metabolic activation with two parallel cultures each. Based on the cytotoxicity results, concentrations of up to 170 µg/mL were used in the main experiments. The treatment intervals for both experiments in the presence and absence of metabolic activation were generally 4 hours (1.0 x 106 cells/mL). Ethyl methane sulfonate (EMS) served as positive controls in the experiments without metabolic activation and Benzo[a]pyrene (B[a]P) served as positive controls in the experiments with metabolic activation. After the incubation period treatment media were replaced by culture medium in both experiments and the cells were incubated for 48 h for expression of mutant cells (3 x 10^5 cells/mL). This was followed by incubation of cells in selection medium containing TFT for about 10 days (selecting plates: 1.0 x 10^6 cells/plate; viability count plated: 200 cells/plate). No biological relevant and reproducible as well as dose dependent increase in mutant colony numbers was observed in both main experiments with and without addition of the metabolizing system. This includes the increased mutant frequency at the respective top dose groups that revealed cytotoxicity by RTG (relative total growth) of below 20%. This also applies for the statistically significant increase at a cytotoxic concentration of 170 µg/mL with addition of S9 mix that was, however, not exceeding the threshold for the mutation frequency based on GEF (global evaluation factor). According to OECD TG 490 (2016), tested concentration had to exceed the threshold in the absence of cytotoxicity (RTG > 20%) to be judged as positive. Therefore, the statistically significant increase was considered as not biologically relevant. Appropriate reference mutagens (EMS and B[a]P) used as positive controls showed a distinct increase in induced mutant colonies, indicating the test-system to be sensitive. However, EMS was used at cytotoxic concentrations in both experiments without the addition of S9 mix. Concurrent vehicle control values were	

			within the range of the historical control data of the performing laboratory (as stated in the report, however, data not shown).	

CI-435-007	Jones, E. and Fenner, L.A.	B. subtilis rec Assay to Assess the Potential of WL 95481 to Cause Damage to DANN	Not evaluated in the DAR B. subtilis strains H17 rec+ (repair proficient) and M45 rec- (repair deficient) were exposed to WL 95481 (batch: ST 82/255; purity: 93%) at concentrations of 50, 150, 500, 1500 and 5000 µg/mL with and without metabolic activation by Aroclor 1254-induced rat liver S9 mix for 24 hours at 37°C. The difference between the two plates that differs only in B. subtilis strain used represents the differential inhibition of growth of the repair deficient strain and is therefore a measure of DNA damage. Additionally, a spot test using impregnated disks with the same test substance concentrations with and without addition of metabolizing system were placed on a lawn culture of the bacteria to detect the difference in the zone of inhibition of growth around the disk between the repair proficient and repair deficient stains for measuring of the DNA damaging effect of the test compound. For each test, positive controls consisted of 2-Aminoanthracene (2-AA, with S9) and 2-Acetylfuorine (2-AF, without S9) as well as negative controls consisted of Streptomycin (with S9) and Kanamycin (without S9) and the vehicle control (DMSO) were applied. There were no differences between the growth of the two strains in the presence of WL 95481 with and without metabolic activation neither in the spot test nor in the survival test. The positive controls gave an appropriate response, demonstrating the sensitivity of the test system used. Thus, based on the results of this study, WL 95481 does not show DNA damaging properties with and without metabolic activation under the conditions used.	

CI-435-006	Meyer, A.L. and Wiggins, D.E	Genotoxicity Studies with WL 95481: *In Vitro* Chromosome Studies with WL 95481	Not evaluated in the DAR WL 95481 (batch: 513F, purity: 93.1%) was tested in vitro for the ability to induce chromosome aberrations in Chinese Hamster Ovary (CHO) cells in the presence and absence of metabolic activation (S9 fraction of Aroclor 1254-induced rat liver). Based on the results of a cytotoxicity pre-test for dose selection, WL 95481 was tested at 4, 20, and 45 µg/mL in the absence of metabolic activation and at 8, 40, and 80 µg/mL in the presence of metabolic activation. The cells were exposed to the test item for 3 h in the presence and absence of metabolic activation and were sampled at 3 sample times at 8, 12, and 24 h after study start. Negative (medium), vehicle (DMSO) and positive controls (Cyclophosphamide (CPA) and Methyl methane sulfonate (MMS) for the experiment with and without metabolic activation, respectively) were included to demonstrate the sensitivity of the test system. Two hours prior to cell harvest, addition of colcemid (0.2 µg/mL) arrested cells in the metaphase. After slide preparation and staining of the cells, about 300 well spread metaphases per dose and treatment condition were analyzed for chromosomal aberrations. Cytotoxicity was monitored via Mitotic Index (MI) calculation based on evaluation of 1500 cells per dose group for metaphases, however, only for the 24-hour sampling time-point. Stability of the test item in the vehicle DMSO for a period of 2 days at room temperature was verified analytically. There was no evidence of a substance-related increase in chromosome damage in the presence of metabolic activation at any sample time. The positive controls MMS and CPA induced substantial chromosome damage in any experiment. Thus, under the experimental conditions chosen here, the conclusion is drawn that WL 95481 has no chromosome-damaging (clastogenic) properties under in vitro conditions using CHO cells.	
CI-435-004	[redacted] and [redacted]	*In Vivo* Chromosome Aberration Assay in Rat Bone Marrow of SD 95481 Technical Grade	Evaluated in the DAR, section B.6.4.2.	
CI-440-001	[redacted]	Biochemical Studies of SD 95481 in the Rat	Evaluated in the DAR, section B.6.8.2.	
CI-440-002	[redacted]	Biochemical Studies of SD 95481 in the Mouse	Evaluated in the DAR, section B.6.8.2.	

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA 5.1.1/1	[redacted]	2017	In-life phase of distribution and metabolism of (14C) BAS 684 H in tissues and plasma after oral single administration in male and female Wistar rats 2017/1158148 [redacted] yes Unpublished	Yes	Yes	New data eligible for data protection according to SANCO/12576/2012	BASF	None
KCA 5.1.1/2	[redacted].	2018	In-life phase of distribution and metabolism of (14C) BAS 684 H in tissues and plasma after oral single administration in male and female Wistar rats 2018/1072281 [redacted] yes Unpublished	Yes	Yes	New data eligible for data protection according to SANCO/12576/2012	BASF	None
KCA 5.1.1/3	[redacted]	2018	14C-BAS 684 H - Study on kinetics in Wistar rats after oral and intravenous administration 2017/1145830 [redacted] yes Unpublished	Yes	Yes	New data eligible for data protection according to SANCO/12576/2012	BASF	None
KCA 5.1.1/4	[redacted]	2018	Excretion and metabolism of 14C-BAS 684 H (Reg.No. 900202) after oral administration in rats 2017/1078601 [redacted] yes Unpublished	Yes	Yes	New data eligible for data protection according to SANCO/12576/2012	BASF	None
KCA	Funk-Weyer	2017	Comparative in-vitro metabolism with 14C-BAS	No	Yes	New data eligible for	BASF	None

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5.1.2/1	D., Ufer G.		684 H 2017/1172468 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished			data protection according to SANCO/12576/2012		
KCA 5.2.1/1	[redacted]	2016a	BAS 684 H (Cinmethylin) - Acute oral toxicity in rats 2016/1273410 [redacted] yes Unpublished	Yes	Yes	New data eligible for data protection according to SANCO/12576/2012	BASF	None
KCA 5.2.1/2	[redacted]	1982	Acute oral toxicity of sd95481 in the rat CI-411-001 [redacted] yes Unpublished	Yes	Yes	New data eligible for data protection according to SANCO/12576/2012	BASF	None
KCA 5.2.1/3	[redacted]	1982	Acute oral toxicity of sd95481 in the mouse CI-411-002 [redacted] yes Unpublished	Yes	Yes	New data eligible for data protection according to SANCO/12576/2012	BASF	None
KCA 5.2.2/1	[redacted]	2016a	BAS 684 H (Cinmethylin) - Acute dermal toxicity in rats 2016/1225928 [redacted] yes Unpublished	Yes	Yes	New data eligible for data protection according to SANCO/12576/2012	BASF	None
KCA 5.2.2/2	[redacted]	1981a	SD 95481: Acute dermal LD 50, eye irritation, and skin irritation, all in rabbits	Yes	No	Not applicable	<none>	None

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
			CI-412-001 [redacted] no Unpublished					
KCA 5.2.3/1	[redacted] *et al.*	2017	BAS 684 H (Cinmethylin) - Acute inhalation toxicity study in Wistar rats - 4-hour liquid aerosol exposure (nose only) 2017/1068662 [redacted] yes Unpublished	Yes	Yes	New data eligible for data protection according to SANCO/12576/2012	BASF	None
KCA 5.2.3/2	[redacted]	1986	Acute four hour inhalation study in rats with cinch sd95481 technical herbicide CI-413-001 [redacted] yes Unpublished	No	Yes	New data eligible for data protection according to SANCO/12576/2012	BASF	None
KCA 5.2.4/1	Remmele M.	2017a	BAS 684 H (Cinmethylin) - In vitro skin irritation and corrosion Turnkey testing strategy 2016/1302127 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data eligible for data protection according to SANCO/12576/2012	BASF	None
KCA 5.2.4/2	[redacted]	2016b	BAS 684 H (Cinmethylin) - Acute dermal irritation / corrosion in rabbits 2016/1225929 [redacted] yes	Yes	Yes	New data eligible for data protection according to SANCO/12576/2012	BASF	None

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
			Unpublished					
KCA 5.2.4/3	[redacted]	1981b	SD 95481: Acute dermal LD 50, eye irritation, and skin irritation, all in rabbits CI-412-001 [redacted] no Unpublished	Yes	No	Not applicable	<none>	None
KCA 5.2.5/1	Remmele M.	2017b	BAS 684 H (Cinmethylin) - In vitro eye irritation Turnkey testing strategy 2016/1302128 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data eligible for data protection according to SANCO/12576/2012	BASF	None
KCA 5.2.5/2	[redacted]	2016b	BAS 684 H (Cinmethylin) - Acute eye irritation in rabbits 2016/1326828 [redacted] yes Unpublished	Yes	Yes	New data eligible for data protection according to SANCO/12576/2012	BASF	None
KCA 5.2.5/3	[redacted]	1981c	SD 95481: Acute dermal LD 50, eye irritation, and skin irritation, all in rabbits CI-412-001 [redacted] no Unpublished	Yes	No	Not applicable	BASF	None
KCA 5.2.6/1	[redacted]	2016c	BAS 684 H (Cinmethylin) - BUEHLER test in guinea pigs 2016/1330875 [redacted]	Yes	Yes	New data eligible for data protection according to SANCO/12576/2012	BASF	None

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
			[redacted] yes Unpublished					
KCA 5.2.6/2	[redacted]	1982	Guinea pig sensitization study of sd95481 CI-416-001 [redacted] yes Unpublished	Yes	Yes	New data eligible for data protection according to SANCO/12576/2012	BASF	None
KCA 5.2.6/3	[redacted]	1988	WL95481 skin sensitizing potential CI-416-002 [redacted] yes Unpublished	No	Yes	New data eligible for data protection according to SANCO/12576/2012	BASF	None
KCA 5.3.1/1	[redacted]	2015	BAS 684 H (Cinmethylin) - Repeated-dose 28-day toxicity study in Wistar rats - Administration via the diet 2015/1076329 [redacted]. yes Unpublished	Yes	Yes	New data eligible for data protection according to SANCO/12576/2012	BASF	None
KCA 5.3.1/2	[redacted]	2016	BAS 684 H (Cinmethylin) - Repeated-dose 28-day toxicity study in C57BL/6JRj mice - Administration via the diet 2014/1162710 [redacted]. yes Unpublished	Yes	Yes	New data eligible for data protection according to SANCO/12576/2012	BASF	None
KCA 5.3.1/3	[redacted]	1984	Five week dietary feeding study of sd95481 technical in dogs	Yes	No	Not applicable	BASF	None

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
			CI-420-004 [redacted] no Unpublished					
KCA 5.3.2/1	[redacted]	2018a	BAS 684 H - Repeated dose 90-day oral toxicity study in Wistar rats - Administration via the diet 2014/1228370 [redacted]. yes Unpublished	Yes	Yes	New data eligible for data protection according to SANCO/12576/2012	BASF	None
KCA 5.3.2/2	[redacted]	1983	Subchronic feeding study of sd95481 in the rat. Volume I CI-425-001 [redacted] yes Unpublished	Yes	Yes	New data eligible for data protection according to SANCO/12576/2012	BASF	None
KCA 5.3.2/3	[redacted]	2018b	BAS 684 H - Repeated dose 90-day oral toxicity in C5BL/6JRj mice - Administration via the diet 2015/1005983 [redacted]. yes Unpublished	Yes	Yes	New data eligible for data protection according to SANCO/12576/2012	BASF	None
KCA 5.3.2/4	[redacted]	1983	Subchronic feeding study of sd95481 in the mouse CI-425-002 [redacted] yes Unpublished	Yes	Yes	New data eligible for data protection according to SANCO/12576/2012	BASF	None

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA 5.3.2/5	[redacted]	1987	13 week dietary feeding study in beagle dogs of cinch herbicide technical CI-425-003 [redacted] yes Unpublished	Yes	Yes	New data eligible for data protection according to SANCO/12576/2012	BASF	None
KCA 5.3.2/6	[redacted]	1985	A one year dietars feeding study in dogs - sd95481 technical CI-427-002 [redacted] yes Unpublished	Yes	Yes	New data eligible for data protection according to SANCO/12576/2012	BASF	None
KCA 5.3.2/7	[redacted]	1988a	One year dietary feeding study in beagle dogs of cinch herbicide CI-427-003 [redacted] yes Unpublished	Yes	Yes	New data eligible for data protection according to SANCO/12576/2012	BASF	None
KCA 5.3.2/	[redacted]	1988b	Cinch herbicide: reversibility of toxicity in beagle dogs (a 12 month feeding with 6 months reversibility) CI-427-004 [redacted] yes Unpublished	Yes	Yes	New data eligible for data protection according to SANCO/12576/2012	BASF	None
KCA 5.3.2/8	Dorso L. *et al*.	2008 a	Variability in weight and histological appearance of the prostate of Beagle Dogs used in toxicology studies 2008/1104596	No	No	Not applicable	public	None

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
			no Published					
KCA 5.3.2/9	Goedken M.J. *et al*.	2008 a	Spontaneous and age-related testicular findings in beagle dogs 2018/1087293 no Published	No	No	Not applicable	public	None
KCA 5.3.2/10	Sato J.	2011 a	Histopathology of incidental findings in beagles used in toxicity studies 2018/1086610 no Published	No	No	Not applicable	public	None
KCA 5.3.3/1	[redacted]	2018c	BAS 684 H - Repeated dose 28-day dermal toxicity study in Wistar rats 2017/1094162 [redacted]. yes Unpublished	Yes	Yes	New data eligible for data protection according to SANCO/12576/2012	BASF	None
KCA 5.3.3/2	[redacted]	2018	Amendment 1: BAS 684 H - Repeated dose 28-day dermal toxicity study in Wistar rats 2018/1091459 [redacted] yes Unpublished	Yes	Yes	New data eligible for data protection according to SANCO/12576/2012	BASF	None
KCA 5.4.1/1	Woitkowiak C.	2018a	BAS 684 H + impurities (artificial batch) - Salmonella typhimurium/Escherichia coli reverse mutation assay 2018/1029052 BASF SE, Ludwigshafen, Germany Fed.Rep. yes Unpublished	No	Yes	New data eligible for data protection according to SANCO/12576/2012	BASF	None

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KCA 5.4.1/2	Woitkowiak C.	2018b	BAS 684 H with new impurity - Salmonella typhimurium/Escherichia coli reverse mutation assay 2018/1029051 BASF SE, Ludwigshafen, Germany Fed.Rep. yes Unpublished	No	Yes	New data eligible for data protection according to SANCO/12576/2012	BASF	None
KCA 5.4.1/3	Sokolowski A.	2018	BAS 684 H - Cell mutation assay at the Thymidine kinase locus (TK+/-) in mouse lymphoma L5178Y cells 2018/1066678 Envigo CRS GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished	No	Yes	New data eligible for data protection according to SANCO/12576/2012	BASF	None
KCA 5.4.1/4	Naumann S.	2018	BAS 684 H with 500 ppm 2methylbenzylchlorid: Micronucleus test in human lymphocytes in vitro 2018/1027282 Envigo CRS GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished	No	Yes	New data eligible for data protection according to SANCO/12576/2012	BASF	None
KCA 5.4.2/1	[redacted]	2018	BAS 684 H - Micronucleus test in bone marrow cells of the mouse 2018/1048783 [redacted] yes Unpublished	Yes	Yes	New data eligible for data protection according to SANCO/12576/2012	BASF	None
KCA 5.4.2/2	[redacted]	1983	In vivo chromosome aberration assay in rat bone marrow of sd95481 technical grade CI-435-004 [redacted]	No	Yes	New data eligible for data protection according to SANCO/12576/2012	BASF	None

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			yes Unpublished					
KCA 5.5/1	[redacted]	2018	BAS 684 H - Combined chronic toxicity/carcinogenicity study in Wistar rats - Administration via the diet up to 24 months 2017/1093414 [redacted] yes Unpublished	Yes	Yes	New data eligible for data protection according to SANCO/12576/2012	BASF	None
KCA 5.5/2	[redacted]	1985	A 2 year feeding study of sd95481 in rats (volume 1 of 8) CI-427-001 [redacted] no Unpublished	Yes	No	Not applicable	BASF	None
KCA 5.5/3	[redacted]	1991	A 2 year feeding study of sd95481 in rats - corrigendum CI-427-007 no Unpublished	No	No	Not applicable	BASF	None
KCA 5.5/4	[redacted]	1991a	Preparation of supplement for submission to the japanese ministry of agriculture, forestry and fisheries from shell group research report sbgr.85.084 (a 2 year feeding study with SD 95481 in rats) CI-427-008 [redacted] no Unpublished	Yes	No	Not applicable	BASF	None
KCA	[redacted]	1991b	Preparation of supplement for submission to the	Yes	Yes	New data eligible for	BASF	None

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5.5/5	[redacted]		japanese ministry of agriculture, forestry and fisheries from shell group research report SBGR.85.084 (a 2 year feeding study with SD 95481 in rats) CI-427-006 [redacted] yes Unpublished			data protection according to SANCO/12576/2012		
KCA 5.5/6	[redacted]	2018d	BAS 684 H - Carcinogenicity study in c57BL/6Rj mice - Administration via the diet up to 18 months 2017/1094161 [redacted] yes Unpublished	Yes	Yes	New data eligible for data protection according to SANCO/12576/2012	BASF	None
KCA 5.5/7	[redacted]	1986	Oncogenicity study of sd95481 in the mouse CI-428-001 [redacted] no Unpublished	Yes	No	Not applicable	BASF	None
KCA 5.5/8	[redacted]	1991	Preparation of supplement for submission to the japanese ministry of agriculture, forestry and fisheries from regulatory information record No. WRC RIR-424 (Oncogenicity study with sd95481 in mice) CI-428-002 [redacted] no Unpublished	Yes	No	Not applicable	BASF	None

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KCA 5.5/9	Hershberger L.	1991 a	EPA review of Cinmethylin studies CI-901-013 US EPA - United States Environmental Protection Agency, Washington DC, United States of America no Unpublished	No	No	Not applicable	BASF	None
KCA 5.5/10	Haseman J. *et al*.	1985 a	Neoplasms observed in untreated and corn oil gavage control groups of f344/n rats and (c57bl/6n x c3h/hen)f1 (b6c3f1) mice CI-905-002 no Published	No	No	Not applicable	public	None
KCA 5.6/1	[redacted]	1986	Two generation reproduction study of cinch herbicide sd95481 in rats CI-430-001 [redacted] yes Unpublished	Yes	Yes	New data eligible for data protection according to SANCO/12576/2012	BASF	None
KCA 5.6.1/1	[redacted]	2018a	BAS 684 H - Two-generation reproduction toxicity study in Wistar rats - Administration via the diet 2017/1094504 [redacted] yes Unpublished	Yes	Yes	New data eligible for data protection according to SANCO/12576/2012	BASF	None
KCA 5.6.1/2	[redacted]	2018	Amendment No. 1 - BAS 684 H - Two-generation reproduction toxicity study in Wistar rats - Administration via the diet 2018/1099151 [redacted]	Yes	Yes	New data eligible for data protection according to SANCO/12576/2012	BASF	None

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			[redacted]. yes Unpublished					
KCA 5.6.2/1	[redacted]	1984	CINCH Herbicide (SD95481) Teratology Study in Sprague Dawley Rats CI-432-001 [redacted] no Unpublished	Yes	No	Not applicable	BASF	None
KCA 5.6.2/2	[redacted]	2018b	BAS 684 H - Prenatal developmental toxicity study in New Zealand White rabbits oral administration (Gavage) 2015/1158053 [redacted] yes Unpublished	Yes	Yes	New data eligible for data protection according to SANCO/12576/2012	BASF	None
KCA 5.6.2/3	[redacted]	1985	Teratology study of CINCH herbicide (technical SD 95481) administered orally via stomach tube to New Zealand White rabbits CI-432-002 [redacted] yes Unpublished	Yes	Yes	New data eligible for data protection according to SANCO/12576/2012	BASF	None
KCA 5.6.2/4	[redacted]	1987	Teratogenicity study of IN-YA168 in rabbits CI-432-003 [redacted] yes Unpublished	Yes	Yes	New data eligible for data protection according to SANCO/12576/2012	BASF	None
KCA	Solecki R.	2001	Harmonisation of rat fetal skeletal terminology	No	No	Not applicable	public	None

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5.6.2/5	*et al*.	b	and classification. Report of the third workshop on the terminology in developmental toxicology - Berlin, 14-16 September 2000 2001/1021583 no Published					
KCA 5.6.2/6	Solecki R. *et al*.	2003 b	Harmonization of rat fetal external and visceral terminology and classification - Report of the fourth workshop on the terminology in developmental toxicology, Berlin, 18-20 April 2002 2003/1036019 no Published	No	No	Not applicable	public	None
KCA 5.6.2/7	Feussner E.L. *et al*.	1992 a	A decade of rabbit fertility data: Study of historical control animals MK-905-020 no Published	No	No	Not applicable	public	None
KCA 5.7.1/1	[redacted]	2018e	BAS 684 H - Acute oral neurotoxicity study in Wistar rats - Administration by gavage 2016/1345328 [redacted]. yes Unpublished	Yes	Yes	New data eligible for data protection according to SANCO/12576/2012	BASF	None
KCA 5.7.1/2	Anonymous	2018 s	Historical control data for acute, oral neurotoxicity studies in rats 2018/1096177 [redacted] no Unpublished	Yes	No	Not applicable	BASF	None

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KCA 5.8.1/1	Maguin K. *et al*.	2006	Ototoxicity of the three Xylene insomers in the rat 2006/1053413 no Published	No	No	Not applicable	public	None
KCA 5.8.1/2	Masereeuw R. *et al*.	1995	Renal excretion and accumulation kinetics of 2-Methylbenzoglycine in the isolated perfused rat kidney 1996/1007340 no Published	No	No	Not applicable	public	None
KCA 5.8.1/3	Benigni R. *et al*.	2010	Structural analysis and predictive value of the rodent in vivo micronucleus assay results 2010/1233692 no Published	No	No	Not applicable	public	None
KCA 5.8.1/4	Snyder R.D. *et al*.	2006	DNA intercalative potential of marketed drugs testing positive in in vitro cytogenetics assays 2006/1051853 no Published	No	No	Not applicable	public	None
KCA 5.8.1/5	Anonymous	2018m	Case Ultra - QSAR prediction of mutagenicity (AMES) on Cinmethylin and its metabolites 2017/1158715 no Unpublished	No	No	Not applicable	BASF	None
KCA 5.8.1/6	Anonymous	2018n	Case Ultra - QSAR prediction of mutagenicity (AMES-Konsolidator) on Cinmethylin and its metabolites 2017/1158716 no Unpublished	No	No	Not applicable	BASF	None
KCA 5.8.1/7	Anonymous	2018o	Case Ultra, QSAR prediction of genotoxicity (in vivo MNT) on Cinmethylin and its metabolites	No	No	Not applicable	BASF	None

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			2017/1158717 no Unpublished					
KCA 5.8.1/8	Anonymous	2018 p	Toxtree QSAR Summary tabe (in vivo MNT): Metabolites 2017/1158718 no Unpublished	No	No	Not applicable	BASF	None
KCA 5.8.1/9	Anonymous	2015 c	CaseUltra - GT_Expert bacterial mutagenicity model 2015/1282367 no Unpublished	No	No	Not applicable	BASF	None
KCA 5.8.1/10	Anonymous	2017 b	CaseUltra: Konsolidator 2017/1066625 no Unpublished	No	No	Not applicable	BASF	None
KCA 5.8.1/11	Anonymous	2015 d	CaseUltra: GT3 MNT mouse micronucleus in vivo , mouse 2015/1282047 no Unpublished	No	No	Not applicable	BASF	None
KCA 5.8.1/12	Benigni R. *et al*.	2017 a	Development of structural alerts for the vivo micronucleus assay in rodents 2017/1158725 no Unpublished	No	No	Not applicable	BASF	None
KCA 5.8.1/13	Kemeny M.	2018a	DEREK - Genotoxicity prediction of BAS 684 H metabolites 2018/1086608 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF	None

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KCA 5.8.1/14	Araki N. *et al*.	2005	Screening for androgen receptor activities in 253 industrial chemicals by in vitro reporter gene assays using AR-EcoScreenTM cells 2005/1045820 no Published	No	No	Not applicable	public	None
KCA 5.8.1/15	Kemeny M.	2018b	DEREK - Comparative toxicity prediction of BAS 684 H Enantiomers 2018/1086609 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF	None
KCA 5.8.1/16	Anonymous	2015 e	CaseUltra: GT1 AT ECOLI E.Coli and Salmonella TA102 model 2015/1282048 no Unpublished	No	No	Not applicable	BASF	None
KCA 5.8.1/17	Anonymous	2015 f	CaseUltra: GT1 A7B bacterial mutagenicity model 2015/1282049 no Unpublished	No	No	Not applicable	BASF	None
KCA 5.8.2/1	[redacted]	1983a	Biochemical studies of sd95481 in the rat CI-440-001 [redacted], [redacted] yes Unpublished	Yes	Yes	New data eligible for data protection according to SANCO/12576/2012	BASF	None
KCA 5.8.2/2	[redacted]	1983b	Biochemical studies of sd95481 in the mouse CI-440-002 [redacted] [redacted] yes	Yes	Yes	New data eligible for data protection according to SANCO/12576/2012	BASF	None

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			Unpublished					
KCA 5.8.2/3	Rueckel M.	2018	Determination of the solubility of BAS 684 H in DMSO and Acetone 2018/1000729 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF	None
KCA 5.8.2/4	Richard A.M. *et al*.	2016	ToxCast chemical landscape: Paving the road to 21st century toxicology 2016/1352489 no Published	No	No	Not applicable	public	None
KCA 5.8.2/5	Filer D.L. *et al*.	2017	Tcpl: The ToxCast pipeline for high-throughput screening data 2017/1227067 no Published	No	No	Not applicable	public	None
KCA 5.8.2/6	Judson R.	2016	Analysis of the effects of cell stress and cytotoxicity on in vitro asssay activity across a diverse chemical and assay space 2016/1227708 no Published	No	No	Not applicable	public	None
KCA 5.8.2/7	Rotroff D.M. *et al*.	2010	Xenobiotic-metabolizing enzyme and transporter gene expression in primary cultures of human hepatocytes modulated by toxcast chemicals 2010/1233112 no Published	No	No	Not applicable	public	None
KCA 5.8.2/8	Sipes N.S. *et al*.	2013	Profiling 976 toxcast chemicals across 331 enzymatic and receptor signaling assays 2013/1371960	No	No	Not applicable	public	None

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			no Published					
KCA 5.8.2/9	Anonymous	2018 t	BAS 684 H - ToxCast output of 689 assay endpoints - Analysis and curve metrics, assay parameters, and assay annotation 2018/1090846 no Unpublished	No	No	Not applicable	BASF	None
KCA 5.8.2/10	Anonymous	2018 r	BAS 684 H - Tox Cast output of 689 assay endpoints (plots) 2018/1090847 no Unpublished	No	No	Not applicable	BASF	None
KCA 5.8.3/1	Friedman K.P. *et al*.	2016 a	Tiered high-throughput screening approach to identify Thyroperoxidase inhibitors within the ToxCast Phase I and II Chemical Libraries 2016/1351687 no Published	No	No	Not applicable	public	None
KCA 5.8.3/2	Wang J. *et al*.	2018 a	High-throughput screening and quantitative chemical ranking for sodium iodide symporter (NIS) inhibitors in ToxCast Phase I chemical library 2018/1086611 no Published	No	No	Not applicable	public	None
KCA 5.8.3/3	Hornung M.W. *et al*.	2017	Screening the ToxCast Phase 1 Chemical Library for inhibition of Deiodinase type 1 activity 2017/1225381 no Published	No	No	Not applicable	public	None
KCA 5.8.3/4	[redacted]	2011	BAS 455 H (Pendimethalin) - Developmental thyroid study in the Sprague-Dawley rat - Oral	Yes	Yes	New data eligible for data protection	BASF	None

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			administration (diet) 2011/1276730 [redacted] yes Unpublished			according to SANCO/12576/2012		
KCA 5.8.3/5	Rotroff D.M. *et al*.	2014	Predictive endocrine testing in the 21st century using in vitro assays of estrogen receptor signaling responses 2014/1323273 no Published	No	No	Not applicable	public	None
KCA 5.8.3/6	Browne P. *et al*.	2015 a	Screening chemicals for estrogen receptor bioactivity using a computational model 2015/1284313 no Published	No	No	Not applicable	public	None
KCA 5.8.3/7	Browne P. *et al*.	2017 a	Correction to screening chemicals for estrogen receptor bioactivity using a computational model 2017/1227066 no Published	No	No	Not applicable	public	None
KCA 5.8.3/8	Judson R.S. *et al*.	2015 a	Integrated model of chemical perturbations of a biological pathway using 18 in vitro high-throughput screening assays for the estrogen receptor 2015/1279970 no Published	No	No	Not applicable	public	None
KCA 5.8.3/9	Kleinstreuer N.C. *et al*.	2016 a	Development and validation of a computational model for androgen receptor activity 2017/1226848 no	No	No	Not applicable	public	None

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			Published					
KCA 5.8.3/10	Karmaus A.L. *et al*.	2016	High-Throughput Screening of chemical effects on steroidogenesis using H295R human adrenocortical carcinoma cells 2016/1119499 no Published	No	No	Not applicable	public	None
KCA 5.8.3/11	Haggard D.E. *et al*.	2018 a	High-throughput H295R steroidogenesis assay: Utility as an alternative and a statistical approach to characterize effects on steroidogenesis 2018/1086612 no Published	No	No	Not applicable	public	None
KCA 5.8.3/12	Paul K.B. *et al*.	2014 a	Development of a Thyroperoxidase inhibition assay for high-throughput screening 2014/1323274 no Published	No	No	Not applicable	public	None
KCA 5.8.3/13	Jahnke G.D.	2004	Thyroid toxicants: Assessing reproductive health effects 2004/1040995 no Published	No	No	Not applicable	public	None
KCA 5.8.3/14	Kaptein E.M. *et al*.	1994 a	Thyroid hormone metabolism - A comparative evaluation 1994/1005509 no Published	No	No	Not applicable	public	None
KCA 5.8.3/15	Lewandowski T.A. *et al*.	2004 a	Interspecies differences in susceptibility to perturbation of thyroid homeostasis: A case study with Perchlorate 2004/1040994 no	No	No	Not applicable	public	None

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			Published					
KCA 5.8.3/16	Dohler K.-D. *et al*.	1979 a	The rat as model for the study of drug effects on thyroid function: Consideration of methodological problems 1979/1001743 no Published	No	No	Not applicable	public	None
KCA 5.8.3/17	Hill R.N. *et al*.	1988 a	Thyroid follicular cell carcinogenesis 1989/1003744 no Published	No	No	Not applicable	public	None