Chlorophene (CAS 120-32-1)

Assessment on endocrine activity (toxicity/human health)

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1. Introduction - OECD Conceptual Framework (CF) for Testing and Assessment of Endocrine Disrupters

"The OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupters (as revised in 2012) lists the OECD Test Guidelines and standardized test methods available, under development or proposed that can be used to evaluate chemicals for endocrine disruption. The Conceptual Framework is intended to provide a guide to the tests available which can provide information for endocrine disrupters' assessment but is not intended to be a testing strategy. Furthermore, this Conceptual Framework does not include evaluation of exposure; however this should be included when deciding whether further testing is needed. Further information regarding the use and interpretation of these tests is available in Guidance Document No. 150."
(https://www.oecd.org/env/ehs/testing/OECD%20Conceptual%20Framework%20for%20Testing%20and%20Assessment%20of%20Endocrine%20Disrupters%20for%20the%20public%20website.pdf)

As mentioned in the OECD guidance document 150
(http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2012)22&doclanguage=en)

"It is important to bear in mind that the CF **is not a testing strategy** to be followed linearly from Level 1 through to Level 5, although in cases where little or no information is available (*i.e.* for new chemicals) it could provide ideas about where to start testing. … The purpose of this GD is therefore to assist assessors of endocrine-relevant tests with data interpretation in the light of information that may already exist, and to provide **optional** suggestions for obtaining additional data, if required, to increase confidence in conclusions on the endocrine disrupting possibilities of a particular chemical….As stated earlier, this process of data interpretation and assessment involves the need for a **weight of evidence approach** that considers both mechanistic and apical information, and it is selfevident that the more data which support a particular conclusion, the more reliable that conclusion will be."

2. Publicly available screening data with focus on endocrine activity

ToxCast® is a research activity launched by US EPA in 2007 that uses automated chemical screening technologies (called "high-throughput screening assays") to expose living cells or isolated proteins to chemicals. Details on the program, the context and results are publicly available. In the screening system cells or proteins are examined for changes in biological activity that may suggest potential toxic effects and eventually potential adverse health effects (for more detail see https://www.epa.gov/chemical-research/toxicity-forecasting)

As mentioned: "ToxCast screens chemicals in over 700 high-throughput assays that cover a range of high-level cell responses and approximately 300 signaling pathways".

Additional assays are mentioned by Rotroff et al. 2014 (Environmental Science & Technology 48, 8706-8716, 2014) and are coming e.g. from Tox21 activities (NTP, NCGC as contributors) (http://www.epa.gov/ncct/Tox21/)

Peer reviewed publications in the context of ToxCast are available, including publications dealing with endocrine activity:

https://cfpub.epa.gov/si/si_lab_search_results.cfm?fed_org_id=1267&SIType=PR&TIMSType=Journal&showCriteria=0&address=ncct%252Fpublications.html&view=citation&sortBy=pubDateYear&keyword=ToxCast

E.g. the two publications by Rotroff et al. give insight in the endocrine related assays (Rotroff et al. ENVIRONMENTAL HEALTH PERSPECTIVES. Open Access(Epub):1-39, 2012) and outlined a way how to integrate the information from a subset of assays to develop an in this case Estrogen Receptor Interaction Score (Rotroff et al. Environmental Science & Technology 48, 8706-8716, 2014).

The assays under ToxCast are also included in the **EDSP21 Dashboard**. As mentioned in the internet (https://www.epa.gov/chemical-research/endocrine-disruption-screening-program-21st-century-edsp21) "EPA researchers developed the Endocrine Disruption Screening Program for the 21st Century Dashboard (EDSP21 Dashboard) to provide access to new chemical data on over 1,800 chemicals of interest." and "The purpose of the EDSP21 Dashboard is to help the Endocrine Disruptor Screening Program evaluate chemicals for endocrine-related activity".

The data for the EDSP Dashboard comes from various sources, including ToxCast data. Regarding ToxCast "data use considerations" it is highlighted (https://actor.epa.gov/edsp21/)

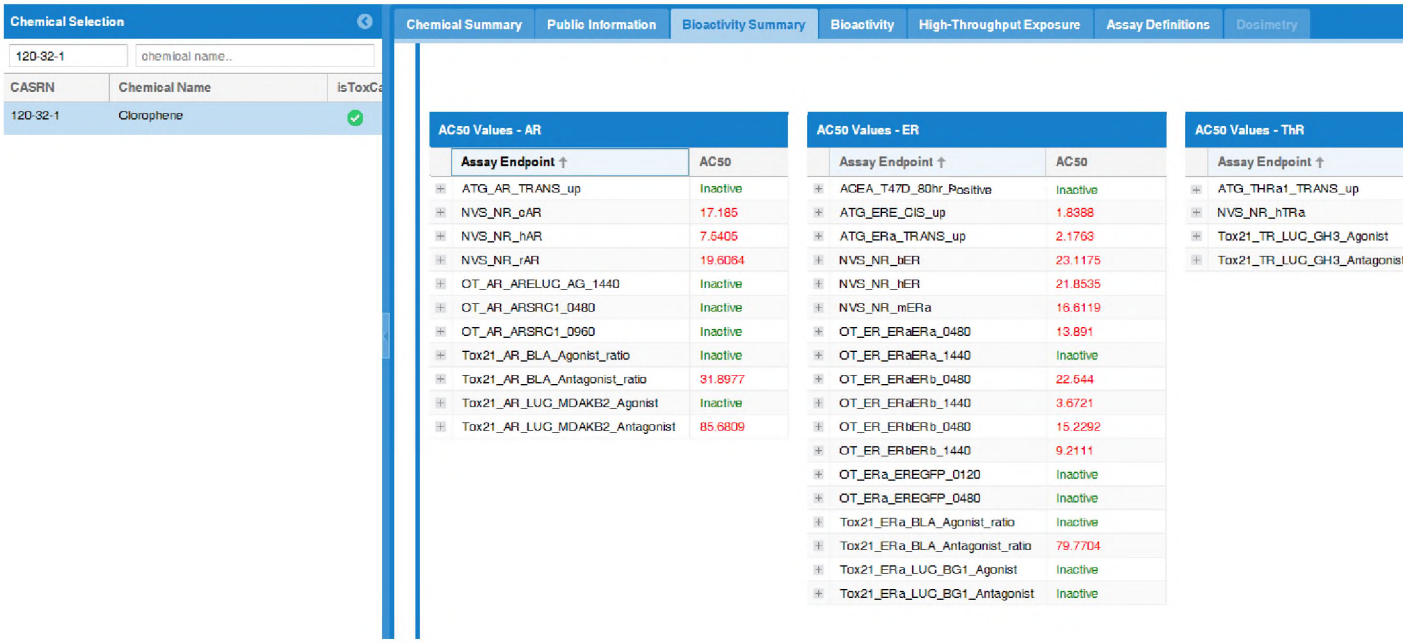
that “The activity of a chemical in a specific assay does not necessarily mean that it will cause toxicity or an adverse health outcome. There are many factors that determine whether a chemical will cause a specific adverse health outcome. Careful review is required to determine the use of the data in a particular decision context.”

As the EDSP21 Dashboard includes ToxCast data it provides the most recent and complete overview on the result of screening activities relevant for the assessment of endocrine activity.

3. Available screening data on chlorophene with focus on endocrine activity

The EDSP21 Dashboard provides is the most recent and complete overview on the result of screening activities relevant for the assessment of endocrine activity. Therefore in the following the result of the EDSP21 Dashboard search for Chlorophene from November 2016 is given. Regarding ToxCast the information as retrieved in 2014 is shown in Annex 1 as background information.

EDSP21 Dashboard search for Chlorophene from November 2016:



The EDSP21 data cover androgen (AR), estrogen (ER) and thyroid (ThR) related assays.

- Of the 11 **androgen** receptor related assays 5 revealed some activity at concentrations in the micromolar range of 7.5 to 85.7 µM.
- Some but not all assays (11 of 18) related to **estrogenic** parameters showed activity. The respective assays indicated activity at concentrations in the micromolar range (AC50 of 1.8 to 79.8 µM).
- Of the 4 **thyroid** related assays one was positive at a high concentration of 44 µM.
- The cytotox limit in the in vitro assays is 3.13 µM (see Annex 2) therefore the positive responses were all, with the exception of one (ATG_ERE_CIS_up) above the cytotox limit.

  For the assay "ATG_ERE_CIS_up" also a majority of the single samples showed results above the cytotox limit:

ATG ERE CIS up HITCALL	
Sample	AC50 in µM
TX000858	1.82
TV000362	0.89
TX509149	4.17
TX409149	3.24
TX309149	5.75
TX209149	6.17
TX109149	5.62
TX009149	7.94
overall	1.8388

- For comparison: known endocrine substances showed a respective acitivity at much lower concentrations. E.g. with estradiol the AC50 for estrogenic activity started with concentrations as low as 0.0009 µM, whereas the cytotox limit for that substance was 161.9 µM.

In summary, chlorophene shows positive results in some of the screening assays, but the activity was in the micromolar range and with isolated exceptions well above the cytotox limit. Therefore based on this information there is no indication of a specific endocrine activity.

4. In vivo studies with chlorophene – is there evidence of endocrine activity?

There is a wide variety of regulatory guideline studies available on the toxicity of chlorophene, covering also potential sensitive live stages. As defined in the OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupters the comprehensive guideline studies, namely chronic toxicity studies in rats and mice, development toxicity studies in rats and rabbits (OECD Framework Level 4) as well as the two generation reproduction toxicity study in rats (OECD Framework Level 5) are of highest importance for the assessment of a potential endocrine activity for Human Health.

The comprehensive data base available for chlorophene consists of e.g. two 2 year repeated dose toxicity feeding studies in rats and mice, a 1 year dermal skin painting study, 90 day repeated dose toxicity studies in dogs, rats and mice, developmental toxicity studies in rats and rabbits, 2-generation reproduction toxicity study in rats. An assessment of all available in vivo data was done by the Risk Assessment Committee (RAC) in 2015 (see Opinion proposing harmonised classification and labelling at EU level of Chlorophene; adopted 12 March 2015, CLH-O-0000001412-86-58/F)

Chlorophene is a **nephrotoxicant** in experimental animals. This property is seen in throughout all repeated dose toxicity studies performed with chlorophene.
RAC concluded on repeated dose toxicity: "*On the basis of increased incidence of nephropathy and increased kidney weight in rodents after oral administration, and in rabbits after dermal administration of chlorophene, RAC concludes that chlorophene should be classified as STOT RE 2 (H372: May cause damage to kidneys through prolonged exposure).* "
RAC concluded on carcinogenicity: "*In conclusion, RAC agrees with the DS that the rare transitional cell carcinoma observed in rats and the renal neoplasms occurring in male mice fulfil the criteria for classification as Carc. 2. This is also supported by the lack of a mode of action that would dismiss the relevance to humans.*"

Regarding **reproductive toxicity/fertility** some deviations from control reproduction parameters were observed (fertility/fecundity index and oestrus cycle length) in a two-generation reproductive toxicity study on rats. The relevance of the responses was questioned in the discussion of the classification (see RAC opinion 2015) , e.g. based on historical control data from the laboratory where the study was conducted that suggested that the effects on fertility index, fecundity and oestrous cycle length were due to biological variability rather than treatment related. Changes observed were proposed to be secondary

to maternal toxicity, e.g. reduced female weight gain during gestation and suggested nephrotoxicity. However, RAC concluded: *"In addition to the evaluation of the CLH report and the information received during public consultation, RAC has also considered the information provided in the 2-generation study report itself. In this study, the authors concluded that there were no adverse effects on reproduction or fertility. However, RAC notes that the reduction in fertility index was found to occur in a dose-dependent manner which was reproducible in both P and F1 generations. Historical control data were provided by the testing laboratory for 9 studies between the years 2002 – 2011. The range for historical control female rat fertility index was 80-100% and the value derived for P females at 540 mg/kg bw/day in the current study was outside of this (76.7%). RAC agrees that this value was not marked when compared to the historical observations, but considers the concurrent control values to provide the most relevant comparison. There was a clear reduction in both generations when compared to historical control data. RAC is of the opinion that the slightly reduced fertility index observed in P and F1 generation rats treated with chlorophene in the 2-generation study were indicative of a weak adverse effect on fertility. Pre-mating body weight of females were unaffected by chlorophene treatment. Kidney toxicity, whilst not explicitly stated in this study, was not considered severe at similar doses in a 95-day study in rats. A decrease in body weight gain (-12% at 540 mg/kg bw/day) occurred only during the gestation period and so was not considered relevant to the period during which fertilisation may be affected. As stated by the DS, there is no established relationship between fertility effects and less marked systemic toxicity. Therefore, it should be assumed that effects on fertility seen at dose levels causing less marked systemic toxicity were not a secondary consequence of this toxicity.*

RAC therefore concludes that classification for effects on fertility is warranted. As there is no human evidence to suggest that chlorophene is a known reproductive toxicant, category 1A is not appropriate. In consideration of category 1B, it is noted that the effect was weak and only observed in females, i.e. there was no evidence of testicular toxicity or other relevant effects in males. There were no changes to other reproductive parameters, no gross or microscopic findings to the reproductive system and litter sizes also remained unaffected. There is no indication of a mechanistic explanation for the effect observed on fertility. No effects on fertility were observed in a less robust 1-generation study. Taking all this into account the strength of evidence appears too weak to require a classification as Repr. 1B. On the basis of dose-related changes to fertility index observed in female rats treated with chlorophene, occurring in the absence of marked systemic toxicity and to an extent that was outside of the relevant historical control range, RAC therefore agrees with the DS that

chlorophene should be classified Repr. 2 (H361f – suspected of damaging fertility). "

Overall, as also concluded by RAC, the effect observed in the two generation study was weak, only observed in females and there were no changes to other reproductive parameters, no gross or microscopic findings to the reproductive system and litter sizes also remained unaffected. Therefore there is no evidence of a specific and biologically relevant endocrine activity.

Based on all available in vivo data kidney toxicity is the most sensitive toxicological effect.

5. Summary and Conclusion

Chlorophene shows positive results in some of the screening assays on endocrine activity, but the activity was in the micromolar range and with isolated exceptions well above the cytotox limit. Therefore based on this information there is no indication of a specific endocrine activity.

In vivo a two generation reproductive toxicity study in rats showed some deviations from control reproduction parameters (fertility/fecundity index and oestrus cycle length). The effect was weak, only observed in females and there were no changes to other reproductive parameters, no gross or microscopic findings to the reproductive system and litter sizes also remained unaffected. The relevance of the responses was questioned in the discussion of the classification, but the observations finally were considered leading to a classification Repr. 2 (H361f – suspected of damaging fertility).

Based on all available in vivo data kidney toxicity is the most sensitive toxicological property. The respective observations at the kidney were the basis for the agreed classification as STOT RE 2 (H372: May cause damage to kidneys through prolonged exposure) as well as Carc. 2.

Overall, whereas the screening assays on endocrine activity showed some positive results the respective activity was consistently weak and therefore does not indicate a specific endocrine activity. This conclusion is in line with the available in vivo toxicological studies, which indicated only weak (or even questionable) isolated observations in a two generation reproductive toxicity study without evidence of a specific and relevant endocrine activity. The kidney is the main target organ of toxicity.

The recently agreed classification confirms this view:

- STOT RE 2 (related to kidney damage).
- Carc 2 (based on with transitional cell carcinoma in rats and the renal neoplasms in male mice)
- Repr. 2 (H361f – suspected of damaging fertility; no indication of a mechanistic explanation)

Thus, based on all available toxicity data chlorophene is not an endocrine disruptor.

Annexes:

Annex 1: ToxCast data as retrieved in 2014:

Data on Chlorophene related to endocrine activity:

In the following the available data for Chlorophene as retrieved in 2014 are given for the ToxCast list of genes with assays related to "endocrine". The "active" responses are highlighted in bold (AC50 = half-maximal response in micromolar):

Estrogen Receptor (ER) related:

- **NVS_NR_bER: Bovine estrogen receptor from uterine membrane (bER); Target: Estrogen Receptor: 2 Percent Activity; AC50 21.5 µM**
- **NVS_NR_hER: Human estrogen receptor from MCF-7 cells (hER); Target: Estrogen Receptor: 2 Percent Activity; AC50 21.7 µM**
- **NVS_NR_mERa: Mouse estrogen receptor, recombinant; Target: Estrogen Receptor alpha: 2 Percent Activity; AC50 13.8µM**
- NCGC_ERalpha_Agonist: Human reporter gene assay with HEK293H; Target: Estrogen Receptor alpha: no data
- ATG_ERa_TRANS: Human reporter gene assay with HepG2, Target: Estrogen Receptor alpha: inactive
- NCGC_ERalpha_Antagonist: Human reporter gene assay with HEK293H; Target: Estrogen Receptor alpha: no data
- **ATG_ERE_CIS: Human receptor gene assay with HepG2, Target: Estrogen Receptor Response Element: 1.48 fold induction; AC50 5.9 µM**
- ATG_ERRa_Trans: Human receptor gene assay with HepG2; Target: Estrogen Related Receptor alpha: inactive
- ATG_ERRg_Trans: Human receptor gene assay with HepG2; Target: Estrogen Related Receptor gamma: inactive
- **OT_ER_ERaERa_0480: 3.47 Percent Activity; AC50 > 12.5 µM**
- **OT_ER_ERaERb_0480: 3.36 Percent Activity; AC50 > 12.7 µM**
- **OT_ER_ERbERb_0480: 2.67 Percent Activity; AC50 11.6 µM**
- OT_ER_ERaERa_1440: inactive
- OT_ER_ERaERa_1440_agonist: Human protein-fragment complementation assay with HEK293T; Target: Estrogen Receptor alpha: inactive
- **OT_ER_ERaERb_1440: Human protein-fragment complementation assay with HEK293T; Target: Estrogen Receptor alpha and beta: 8 Percent Activity; AC50 > 3.58 µM**
- OT_ER_ERbERb_1440: inactive
- OT_ER_ERbERb_1440_agonist: Human protein-fragment complementation assay with HEK293T; Target: Estrogen Receptor beta: inactive
- *OT_ERa_EREGFP_0120; OT_ERa_EREGFP_0480; OT_ERa_ERELUC_AG_1440: inactive*
- Tox21_ERa_BLA_Agonist_ratio: reporter gene assay: human reporter gene assay in HEK-293 cells; Target: Estrogen Receptor alpha: inactive
- Tox21_ERa_LUC_BG1_Agonist: human reporter gene assay in BG-1 ovarian cells; Target: Estrogen Receptor alpha: inactive
- Tox21_ERa_BLA_Antagonist_ratio: human reporter gene assay in HEK-293 cells; Target: Estrogen Receptor alpha: inactive
- **Tox21_ERa_BLA_Antagonist_viability: 8 Percent Activity; AC50 > 45.4 µM**
- Tox21_Era_LUC_BG1_Antagonist: human reporter gene assay in BG-1 ovarian cells; Target: Estrogen Receptor alpha; inactive

- **Tox21_ERa_LUC_BG1_Antagonist_viability: human reporter gene assay in BG-1 ovarian cells; Target: Estrogen Receptor alpha: 8 Percent Activity; AC50 < 0.00118 µM**
- **ACEA_T47D_80hr_Negative: Human T-47D mammary cell growth; Target: estrogen receptor: 8 Percent Activity; AC50 22.2 µM**
- ACEA_T47D_80hr_Positive: Human T-47D mammary cell growth; Target: estrogen receptor: inactive (Literature: Rotroff et al. 2014 Supplementary file 7)

Androgen Receptor (AR) related:

- ATG_AR_TRANS: Human reporter gene assay with HepG2; Target: Androgen Receptor: inactive
- NCGC_AR_Agonist: Human reporter gene assay with HEK293H; Target: Androgen Receptor: no data
- **NVS_NR_rAR: Rat androgen receptor; recombinant protein containing ligand binding domain only; Target: Androgen Receptor: 2 Percent Activity; AC50 13 µM**
- **NVS_NR_hAR: 6. Human androgen receptor from LnCAP cells; Target: Androgen Receptor: 1 Percent Activity; AC50 7.35 µM**
- NCGC_AR_Antagonist: Human reporter gene assay with HEK293H; Target: Androgen Receptor: no data
- *OT_AR_ARELUC_AG_1440: inactive*
- *OT_AR_ARSRC1_0480: inactive*
- *OT_AR_ARSRC1_0960: inactive*

Progesterone Receptor (PR) related:

- **NVS_NR_bPR: Bovine progesterone receptor from uterine membrane; Target: Progesterone Receptor: 1.39 Percent Activity; AC50 11 µM**
- **NVS_NR_hPR: Human progesterone receptor from T47-D cells; Target: Progesterone Receptor: 1.64 Percent Activity; AC50 11.6 µM**

Thyroid Hormone Receptor (TR) related:

- NCGC_TRbeta_Agonist: Human reporter gene assay with HEK293H; Target: Thyroid Receptor beta: no data
- NCGC_TRbeta_Antagonist: Human reporter gene assay with HEK293H; Target: Thyroid Receptor beta: no data
- ATG_THRa1_TRANS: Human receptor gene assay with HepG2; Target: Thyroid Receptor alpha: inactive
- NVS_NR_hTRa: Human thyroid hormone receptor-alpha; Target: Thyroid Receptor alpha: inactive
- NVS_GPCR_rTHR: Rat thyrotropin-releasing hormone receptor from forebrain membranes (rTRH); Target: Thyrotropin releasing hormone receptor: inactive

Inhibition of the Aromatase Enzyme:

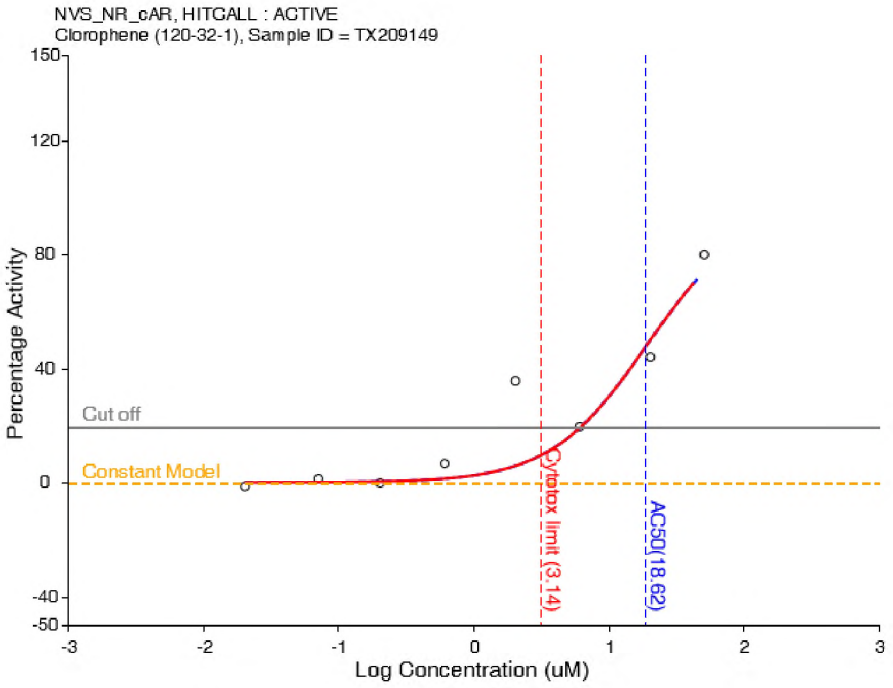
- **NVS_ADME_hCYP19A1: Human Cyp19A1; enzyme inhibition; Target: Aromatase (see Rotroff et al. 2012) : 2 Percent Activity; AC50 2.66 µM**
- Tox21_Aromatase_Inhibition_viability: inactive
- **Tox21_Aromatase_Inhibition: 8 Percent Activity; AC50 21.2 µM**

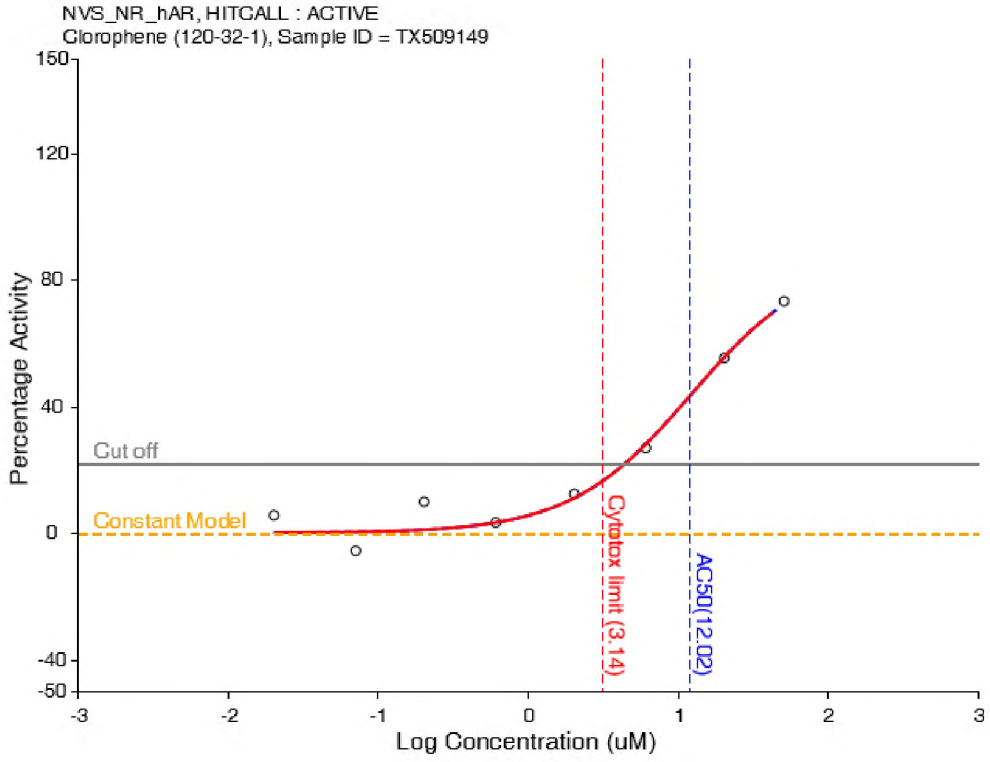
Overall, from the long list of ca. 700 assays some revealed active results but only 17 of these actives were related to endocrine parameters.

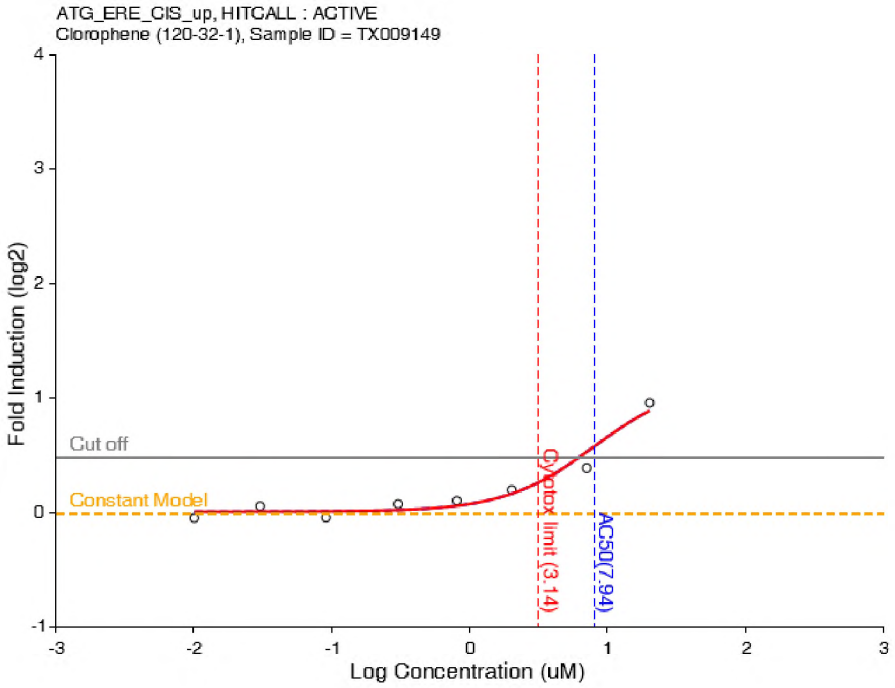
Based on these data there is no indication of a thyroid related activity.
Some but not all assays (11 of 24) related to estrogenic parameters showed activity. Of these, one single assay indicated activity at low concentration (AC50 of < 0.00118 µM). The other positive responses for estrogenic parameters showed activity at micromolar concentrations (range of 3.58 - > 45 µM).
Of the 8 androgen receptor assays two revealed some activity at concentrations of > 7 µM.
The two progesterone receptor assays bPR and hPR showed some activity, however, at high test concentrations of > 10 µM.
Some inhibition of aromatase was seen at concentrations of 2.66 µM (NVS) and 21.2 µM (Tox21), respectively.

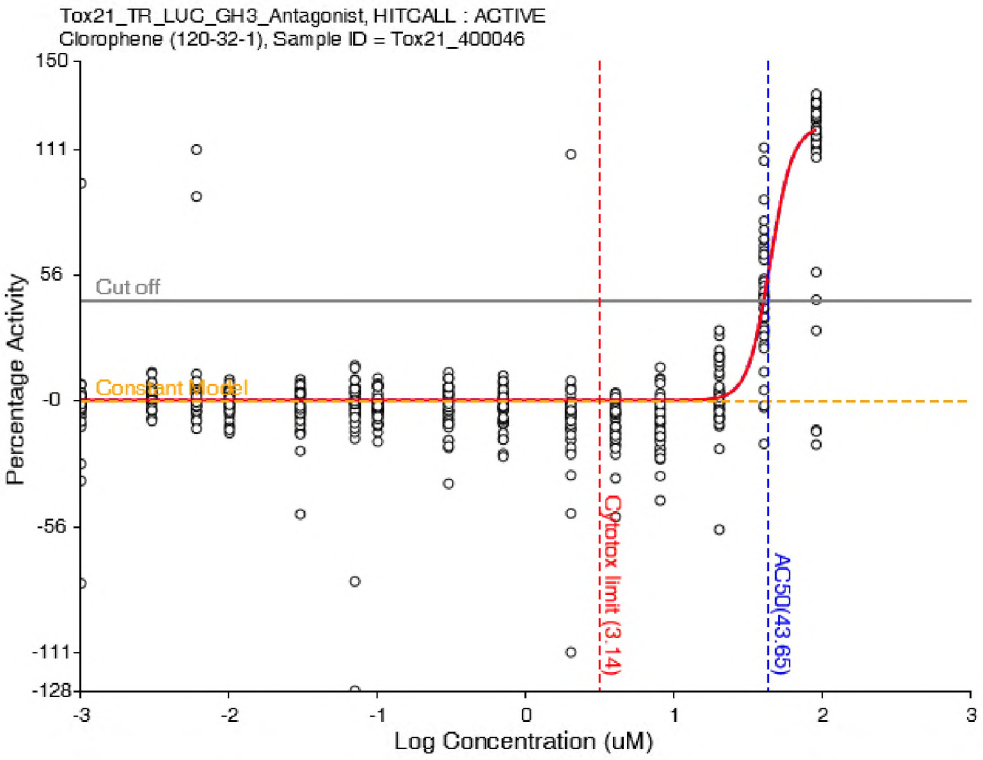
In summary, chlorophene shows no potential for thyroid related activity. For the other hormone systems described there were some positive results predominantly with activity in the in the micromolar range.

Annex 2: Examples for bioassay data in the EDSP Dashboard (retrieved November 2016)






ATG_ERE_CIS_up, HITCALL : ACTIVE
Clorophene (120-32-1), Sample ID = TX009149
Fold Induction (log2)
Cut off
Constant Model
Cytotox limit (3.14)
AC50(7.94)
Log Concentration (uM)


Tox21_TR_LUC_GH3_Antagonist, HITCALL : ACTIVE
Clorophene (120-32-1), Sample ID = Tox21_400046
Percentage Activity
Cut off
Constant Model
Cytotox limit (3.14)
AC50(43.65)
Log Concentration (uM)