Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

to the Opinion proposing harmonised classification and labelling at EU level of

Dibutyltin dilaurate

EC Number: 201-039-8
CAS Number: 77-58-7

CLH-O-0000001412-86-59/F

Adopted

05 June 2015

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in this table as submitted by the webform. Please note that some attachments received may have been copied in the table below. The attachments received have been provided in full to the dossier submitter and RAC.

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Substance name: dibutyltin dilaurate
CAS number: 77-58-7
EC number: 201-039-8
Dossier submitter: Norway

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
10/11/2014	Germany		Individual	1
Comment received				
As classification of DBTL mainly relies on the classificaton of DBTL, which is also explained by the orally bioavailable form after gastric passage (>>DBTC is a metabolite formed rapidly in the stomach after oral exposure to DBTDL due to HCl-mediated (low pH) hydrolysis of DBTDL.<<) it is prudent to consider specific concentration limits for DBTL and other DBT-Compounds to correct for this fact.				
Dossier Submitter's Response				
During simulated reaction studies at conditions resembling the mammalian gastric system, DBTDL hydrolysed into DBTC by 87.8% after 2 hours. Since the hydrolysis is relatively fast and almost all DBTDL is hydrolysed into DBTC we do not consider a SCL to be relevant for the hazard classes evaluated in this dossier.				
RAC's response				
Given that DBTDL will, at least in part, be hydrolysed in the stomach upon oral exposure, producing DBTC, RAC assessed data of both DBTDL and DBTC when evaluating the relevance for setting Specific Concentration Limits. RAC considers setting of Specific Concentration Limits as not necessary given that cf. CLP-guidance: - for STOT RE, the margin between the effective dose levels and the guidance values are small. - for reproductive toxicity, the ED_{10}-values fall within the ranges of a medium potency group (i.e. 4 mg/kg bw/d < ED_{10} < 400 mg/kg bw/d) and modifying factors which might changing the potency group are considered not needed, resulting in the GCL of 0.3% for both effects on fertility and sexual function as well as effects on development.				

Date	Country	Organisation	Type of Organisation	Comment number
10.11.2014	Germany	Galata Chemicals GmbH	BehalfOfAnOrganisation	2
Comment received				

Tributyl based impurities in the dibutyltin dilaurate (DBTL) studies likely have driven the unfavorable results or at the very least contributed to them. Tributyltin is well known to be reprotoxic and immunotoxic at very low levels, and thus it would not be valid to classify DBTL as reprotoxic category 1B or STOT RE (immunotoxic) category 1 based on studies where there were there were tributyltin based impurities. The attached document provides more evidence to this point with references to the applicable studies:

ECHA comment: the text of the attachment is inserted in this comment box:

Comments about the proposed Harmonised Classification and Labelling for Dibutyltin dilaurate

The Norwegian Environment Agency submitted on 15/09/2014 a Proposal for Harmonized Classification and Labeling for Dibutyltin dilaurate (CAS# 77-58-7; EC# 201-039-8) The substance is identified as a monoconstituent substance with a purity of > 95 %. Impurities are not expressively mentioned and not considered as relevant for the classification.

Actual literature shows, that the role of the process caused impurity tributyltin (laurate or chloride) in conjunction with dibutyltin (laurate or chloride) has been and still is underestimated in its contribution to the reprotoxicity of the substance in concern. Based on the following comments we believe that a DBTL manufactured in a purity of a state of the art production process, containing <=0.1 % TBTL needs not to classified as Repr. 1B.

Seckl et. al. 2004[1] shows that concentrations of the active glucocorticoid cortisol are high in maternal blood during pregnancy. The placenta cannot stop lipophilic steroids crossing to the fetus, but uses placental 11 β-hydroxysteroid dehydrogenase type 2 (11 β -HSD2) to rapidly inactivate cortisol to inert cortisone. Thus fetal exposure and adverse effects in the fetus directly or in the later life are minimized.

Based on this mechanism **Atanasov et al. 2005**[2] could explain organotin dependent toxicity in thymus and placenta by enhanced glucocorticoid concentrations due to inhibition of 11 β-HSD2 activity by TBT.

Holmes, 2006[3], summarized that loss of 11β-HSD2 activity results in an early life exposure to high maternal glucocorticoids, resulting in low birth weight and a programmed behavioral phenotype of increased anxiety.

Gumy, 2008[4], demonstrates that dibutyltin selectively inhibits the glucocorticoid receptor transactivation activity.

This mechanism of blocking the GR together with the TBT induced increased level of active corticosteroids via 11β-HSD1 (**Belkacemi, 2011**[5]) contributes to an additional higher concentration of active corticosteroids. As already mentioned TBT inhibits as well 11 β-HSD2 resulting in a blocked protection of the glucocorticoid sensitive placental tissue.

Nakanishi, 2005[6] reported that Tributyltin is an effective aromatase inhibitors. Aromatase catalyzes the biosynthesis of C18-estrogens (17-estradiol, estrone, and estradiol) from C19-steroids (testosterone, androstenedione, and 16 -hydroxyandrostenedione)) and thus can be responsible for the known endocrine disruptive e properties of TBT. Furthermore it was demonstrated that the gene expression of human aromatase (responsible for a key step in the biosynthesis of estrogens) is regulated by the activation of the retinoid X receptor (RXR) and the peroxisome proliferator-activated receptor (PPAR-γ), which both are activated by TBT.

The relation between the mode of action of tributyltin, aromatase, ovaries and hormones is elucidated in studies by **Chen, 2002**[7] and **Saitoh, 2001**[8]**.**

Le Maire, 2009[9] confirms the interaction of TBT with RXR–PPAR-γ. Cys 432 in RXR-α and Cys 285 in PPAR-γ have been identified as crucial binding positions for TBT and thus for the activation of these receptors. This occurs at concentrations < 0.1 %

Lee et al. 2012[10] demonstrate that TBT induces the expression of adipogenesis- and apoptosisrelated genes in the ovary suggesting a loss of ovarian function . The TBT induced expression of adipogenesis-related genes such as PPAR γ, aP2, CD36, and PEPCK and apoptosis-related genes such as TNFα and TNFR1 play a key role for the reported effects.

Si, 2011[11]. describes a delayed development of testes of mouse offspring from dams dosed with 100 µg/kg/bw TBT during gestation. Further behavioral test showed a

significant delay in cliff drop aversion response on offspring of dams dosed with 10 µg/kg/bw TBT.
Yonezawa, 2007[12] reports that TBT inhibits osteoclast differentiation through a retinoic acid receptor-dependent signaling pathway. MBT and DBT do not show these effects.
Already in 1999 (**Pauken, 1999**[13]) it has been reported that the interaction of TBT with retinoic acid receptor RAR and retinoid X receptor RXR cause malformations.
Verhaegen, 2011[14], determined that TBT changes the expression of CrCEcR and CrcRXR especially in ovaries leading to a disorder of T-box genes.
Packham 2003[15], links mutations in T-box genes to human disorders.
Salmela 2008[16], reports the decreased pre-dentin mineralization and enamel formation of mouse embryonic teeth, by TBT with a clear dose response.
The citied studies show the dramatic repro- and immunotoxic properties of TBTcompounds even in very low doses. TBT-impurities have been present in (concentration of > 0,3 %) in DBT-substances which have been tested in the past in order to assess the risk of those chemicals (**OECD 2006**[17] **/ RPA 2007**[18]). At this time it was not considered that the observed adverse effects could have been caused by the impurities present in the test material.
We today believe that the impurities caused the adverse effects observed in DBT samples exclusively or at least contribute to a significant extend to the observed adverse effects. Since it is today technically feasible in a state of the art production process to manufacture DBTC in a quality that assures TBT levels of < 0.1 % we strongly believe that such a pure material needs not to be classified as Repr 1B.

Citations:

1) Seckl, J. (2004). Prenatal glucocorticoids and long-term programming. *European Journal of Endocrinology*.
2) Atanasov, A. G., Nashev, L. G., Tam, S., Baker, M. E., & Odermatt, A. (2005). Organotins Disrupt the 11β-Hydroxysteroid Dehydrogenase Type 2–Dependent Local Inactivation of Glucocorticoids. *Environmental Health Perspectives, VOLUME 113 | NUMBER 11 | November 2005 ,* 1600-1606.
3) Holmes, M. C., Abrahamsen, C. T., French, K. L., Paterson, J. M., Mullins, J. J., & Seckl, J. R. (2006). The Mother or the Fetus? 11ß-Hydroxysteroid Dehydrogenase Type 2 Null Mice Provide Evidence for Direct Fetal Programming of Behavior by Endogenous Glucocorticoids. *The Journal of Neuroscience, April 5, 2006 • 26(14),* 3840 –3844.
4) Gumy, C., Chandsawangbhuwana, C., Dzyakanchuk, A. A., Kratschmar, D. V., Baker, M. E., & Odermatt, A. (2008). Dibutyltin Disrupts Glucocorticoid Receptor Function and Impairs Glucocorticoid-Induced Suppression of Cytokine Production. *PLoS ONE 3(10): e3545; October 2008*.
5) Belkacemi, L., Jelks, A., Chen, C.-H., Ross, M. G., & Desai, M. (2001). Altered placental development in undernourished rats: role of maternal glucocorticoids. *Reproductive Biology and Endocrinology 2011, 9:105*.
6)Nakanishi, T., Nishikawa, J.-i., & Tanaka, K. (2006). Molekular targest of Organotin compounds in endocrin dirsuption: Do organotin compounds function as aromatase inhibitors in mammals. *Environmental Science, 13, 2 (2006) 089-100,* 12.
7) Chen, S. (2002). MODULATION OF AROMATASE ACTIVITY AND EXPRESSION BY ENVIRONMENTAL CHEMICALS. *Frontiers in Bioscience 7, d1712-1719, August 1, 2002,* 8.
8) Saitoh, M., Yanase, T., Morinaga, H., Tanabe, M., Mu, Y.-M., Nishi, Y., et al. (2001). Tributyltin or Triphenyltin Inhibits Aromatase Activity in the Human Granulosa-like Tumor Cell Line KGN. *Biochemical and Biophysical Research Communications 289, 198–204 (2001,* 198-204.
9)le Maire, A., Grimald, M., Roecklin, D., Dagnino, S., Vivat-Hannah, V., Balaguer, P., et al. (2009). Activation of RXR–PPAR heterodimers by organotin environmental endocrine disruptors. *EMBO reports VOL 10 | NO 4 | 2009*.
Atanasov, A. G., Nashev, L. G., Tam, S., Baker, M. E., & Odermatt, A. (2005). Organotins Disrupt the 11β-Hydroxysteroid Dehydrogenase Type 2–Dependent Local Inactivation of Glucocorticoids. *Environmental Health Perspectives, VOLUME 113 | NUMBER 11 | November 2005 ,* 1600-1606.
Belkacemi, L., Jelks, A., Chen, C.-H., Ross, M. G., & Desai, M. (2001). Altered placental

development in undernourished rats: role of maternal glucocorticoids. *Reproductive Biology and Endocrinology 2011, 9:105*.
Chen, S. (2002). MODULATION OF AROMATASE ACTIVITY AND EXPRESSION BY ENVIRONMENTAL CHEMICALS. *Frontiers in Bioscience 7, d1712-1719, August 1, 2002*, 8.
Gumy, C., Chandsawangbhuwana, C., Dzyakanchuk, A. A., Kratschmar, D. V., Baker, M. E., & Odermatt, A. (2008). Dibutyltin Disrupts Glucocorticoid Receptor Function and Impairs Glucocorticoid-Induced Suppression of Cytokine Production. *PLoS ONE 3(10): e3545; October 2008*.
Holmes, M. C., Abrahamsen, C. T., French, K. L., Paterson, J. M., Mullins, J. J., & Seckl, J. R. (2006). The Mother or the Fetus? 11ß-Hydroxysteroid Dehydrogenase Type 2 Null Mice Provide Evidence for Direct Fetal Programming of Behavior by Endogenous Glucocorticoids. *The Journal of Neuroscience, April 5, 2006 • 26(14)*, 3840 –3844.
Lee, H., Lim, S., Yun, S., Yoon, A., Park, G., & Yang, H. (2012). Tributyltin increases the expression of apoptosis and adipogenesis-related genes in rat ovaries. *Clin Exp Reprod Med 2012;39(1):15-21*, 7.
Nakanishi, T., Nishikawa, J.-i., & Tanaka, K. (2006). Molekular targest of Organotin compounds in endocrin dirsuption: Do organotin compounds function as aromatase inhibitors in mammals. *Environmental Science, 13, 2 (2006) 089-100*, 12.
Packham, E. A., & Brook, D. J. (2003). T-box genes in human disorders. *Human Molecular Genetics, 2003, Vol. 12, Review Issue 1*, 8.
Pauken, C. M., LaBorde, J. B., & Bolon, B. (1999). Retinoic acid acts during peri-implantational development to alter axial and brain formation. *Anatomy and Embryology, Volume 200, issue 6 (November 4, 1999)*, 645 - 655.
Saitoh, M., Yanase, T., Morinaga, H., Tanabe, M., Mu, Y.-M., Nishi, Y., et al. (2001). Tributyltin or Triphenyltin Inhibits Aromatase Activity in the Human Granulosa-like Tumor Cell Line KGN. *Biochemical and Biophysical Research Communications 289, 198–204 (2001,* 198-204.
Salmela, E., Sahlberg, C., Alaluusua, S., & Lukinmaa, P.-L. (2008). Tributyltin Impairs Dentin Mineralization and Enamel Formation in Cultured Mouse Embryonic Molar Teeth. *TOXICOLOGICAL SCIENCES 106(1), 214–222 (2008)*, 8.
Seckl, J. (2004). Prenatal glucocorticoids and long-term programming. *European Journal of Endocrinology*.
Si, J., Li, J., Zhang, F., Li, G., Xin, Q., & Da, B. (2012). Effects of perinatal exposure to low doses of tributyltin chloride on pregnancy outcome and postnatal development in mouse offspring. *Environmental toxicology, (2012) 27(10)*, 605-612.
Verhaegen, Y., Parmentier, K., Sweve, L., Renders, E., Rougé, P., De Coen, W., et al. (2011). The heterodimeric ecdysteroid receptor complex in the brown shrimp Crangon crangon: EcR and RXR isoform characteristics and sensitivity towards the marine pollutant tributyltin. *General and Comparative Endocrinology 172 (2011)*, 11.
Yonezawa, T., Hasegawa, S.-i., Ahn, J.-Y., Cha, B.-Y., Teruya, T., Hagiwara, H., et al. (2007). Tributyltin and Triphenyltin inhibit osteoclast differentiation throug a retinoic acid receptor-signaling pathway. *Biochemical and Biophysical Research Communications 355 (2007) 10-15*, 6.
18) RPA, 2007. Risk & Policy Analysts. Impact Assessment of Potential Restrictions on the Marketing and Use of Certain Organotin Compounds. Final Report, October 2007. Prepared for European Commission Directorate-General Enterprise and Industry.

Dossier Submitter's Response
See response to comment no. 6.
RAC's response
See RAC's response to comment no. 6.

Date	Country	Organisation	Type of Organisation	Comment number
10.11.2014	Germany	Galata Chemicals GmbH	BehalfOfAnOrganisation	3

Comment received
Galata Chemicals, GmbH – the lead registrant of DBTL – has noticed that the REACh dossier incorrectly claims the use of DBTL to be valid for professional and consumer uses. We have investigated this and have determined that the only appropriate use for DBTL is industrial use. We have updated our dossier to reflect this and encouraged the other registrants to do a similar analysis and make the same update to their REACh dossiers as well.
Dossier Submitter's Response
Thank you for the information.
RAC's response
Noted

Date	Country	Organisation	Type of Organisation	Comment number
04.11.2014	Germany		MemberState	4
Comment received				
The German CA supports the proposal of the Norwegian CA for harmonised classification and labelling of DBTDL.				
Dossier Submitter's Response				
Thank you for the support.				
RAC's response				
Noted				

Date	Country	Organisation	Type of Organisation	Comment number
07.11.2014	France		MemberState	5
Comment received				
Only few studies have evaluated the toxicity of DBTL. Toxicokinetics experiments support that DBTDL is hydrolysed in human stomach producing DBTC after oral exposure. So DBTC can be also taking into account for classification of DBTL. Mutagenicity: For both DBTL and DBTC, they are no germ cell mutagenicity available. DBTL and DBTC have genotoxic properties on somatic cells of rodents after in vivo exposures. For in vitro experiments, only DBTC has clastogenic and mutagenic properties. According to these results, FR agrees for the DBTL classification as Muta. 2 – H341. Reproductive toxicity: No one study on fertility with DBTL has been mentioned. Fertility endpoint has been studied on rodents after DBTC exposure. Results showed a significant increase of non-pregnant females after mating, a lower number of implantations, a higher incidence of pre-implantation loss, lower number of live foetuses per litter, high mortality of pregnants rats, higher number of resorption and dead foetuses per litter. DBTC reduced strongly rodent's fertility with a NOAEL of 3.8 mg/kg bw/day. Strong alterations of development (external and skeletal malformations) have been observed at 50 mg/kg and 24 mg/kg after DBTL or DBTC exposure, respectively. According to these results, FR agrees for the DBTL classification as Repr. 1B – H360FD. Specific target organ toxicity: Immune system seems to be the most sensitive organ system in rats after repeated exposure to DBTL or DBTC. Main effects are decrease of thymus weight, reduced cells count and histological alterations in thymus. LOAELs for thymus alterations are doses lower than 10 mg/kg/day, with 2.5 mg/kg/day				

bw/day for DBTC and 5.2 mg/kg bw/day for DBTDL. So FR agrees for the DBTL classification as STOT RE1-H372 (immune system).
Dossier Submitter's Response
Thank you for the support.
RAC's response
Noted

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
10/11/2014	Germany	TIB Chemicals AG	BehalfOfAnOrganisation	6
Comment received				
See attachment *ECHA's comment: Please refer to the attachment 1.Comment: Proposal for harmonized classification for Dibutyltin dilaurate*				
Dossier Submitter's Response				
This is a response to comment 2 and 6, which is similar. Purity of test substances: The degree of contamination of TBT in DBT test substances is unclear both in older and more recent studies. However the REACH registration does not give any information about impurities that is considered relevant for the hazard classes evaluated in this dossier. Mechanisms: Regarding the mechanism(s) behind repro/dev and immuno effects, the scientific literature provides several suggestions as mentioned in the comment from Industry, but more clarification on the mode of action (MofA) (and if TBT and DBT act through different pathways) is likely needed. <u>Aromatase enzyme (CYP19) inhibition</u> One suggested repro mechanism is through aromatase enzyme (CYP19) inhibition. In studies by Heidrich 2001 and Cooke 2002, DBT's inhibition was at least half that of TBT and inhibitory effects from DBT seems not to have been caused by TBT contamination. Also, if DBT's inhibitory effect on aromatase was due to contamination by the more potent TBT, the inhibitory effect observed by Heidrich 2001 would be expected to notably increase when the DBT concentration increased between 25-200 µM (8-fold concentration increase), but it remained constant. <u>Organotin activation of RXR/PPARγ or RXR/RXR receptor</u> Activation of RXR/PPARγ or RXR/RXR receptor dimers regulate aromatase expression and adipocyte differentiation. Aromatase may get down-regulated (or up-regulated dependent on cell type) through organotin activation of RXR/PPARγ or RXR/RXR receptor dimers (Nakanishi T. 2008). Few studies have compared TBT and DBT compounds directly, but Yanik 2011 found that <u>both</u> TBT and DBT compounds acted as adipocyte differentiators as some common RXR and PPARγ agonists also did. However, the potency of DBT appears to be lower than for TBT as was also seen by Grün 2006 who tested RXR and PPARγ receptor activation related to adipocyte differentiation. In the comment from TIB Chemicals, it is				

stated (p. 12/22) "…the diorganotins were found to be inactive toward RXR and PPARγ and so the diorganotins are not able to cause malformations." but we can not see that this statement have a proper reference in Atanasov 2005.

Glucocorticoid levels
Enhanced glucocorticoid concentrations due to inhibition of 11β-HSD2 function may contribute to the observed organotin-dependent toxicity in some glucocorticoid-sensitive tissues such as thymus and placenta. Placental 11β-HSD1 and 11β-HSD2 regulate glucocorticoid transfer into the fetus and altered enzyme levels can cause developmental effects. Please note that Ohshima 2005 observed no inhibitory effect on the enzymatic activity of 11β-HSD1 by either DBT or TBT which is supported by Atanasov 2005.
Immunosuppressive effects by apoptosis or necrosis
Tomiyama 2009 observed that death of rat T-lymphocyte was due to apoptosis for TBT and necrosis for DBT at similar doses. In general necrosis is considered more adverse than apoptosis.

Loss of thymocytes appears to involve suppression of proliferation of immature thymocytes and, at higher dosages, apoptosis of mature thymocytes (ATSDR 2005). These appear to be direct effects on the thymus as both cytotoxicity and apoptosis have been observed in thymocyte cell cultures exposed to di- or tributyltin (ATSDR 2005). Both DBT and TBT cause thymus atrophy and in a rat study DBT was more efficient than TBT. Dose levels calculated to cause 50% reduction of relative thymus weight were 18 mg DBT and 29 mg TBT per kg body wt. (Snoeij et al 1988). Hence it appears unlikely that TBT contamination was responsible for DBT's effect.

References
ATSDR 2005. U.S.H.H.S. Toxicological profile for tin and tin compounds.

Cooke GM. 2002. Effect of organotins on human aromatase activity in vitro. Toxicol Lett 126:121-130.

Grün F et al 2006. Endocrine-disrupting organotin compounds are potent inducers of adipogenesis in vertebrates. Mol Endocrinol 20: 2141-2155.

Heidrich DD, Steckelbroeck S, Klingmuller D. 2001. Inhibition of human cytochrome P450 aromatase by butyltins. Steroids 66:763-769."

Nakanishi T. 2008. Endocrine disruption induced by organotin compounds; organotins functions as a powerful agonist for nuclear receptors rather than an aromatase inhibitor. J. Toxicol. Sci, 33:269-276.

Yanik SC, Baker AH, Mann KK, Schlezinger JJ. 2011. Organotins are potent activators of PPARγ and adipocyte differentiation in bone marrow multipotent mesenchymal stromal cells. Toxicol Sci, 122, 476-488.

Additional data on developmental effects of DBTDL
In a study by Moser and co-workers (2009) pregnant Sprague-Dawley rats were exposed to 0, 10, or 25 ppm DBTC in drinking water from gestational day (GD) 6 to weaning at postnatal day (PND) 21. Beginning on PND 3, half of the litters were directly dosed every 2 to 3 days via oral gavage with 0, 1, or 2.5 mg/kg DBTC such that the dose level matched the water concentration (for example, litters with 25 ppm DBTC in the water received 2.5 mg/kg). For Sn analysis, brain and blood samples were collected from culled pups on PND2 (males and females pooled), from pups (males and females separately) as well as dams at

weaning (PND21), and from adult offspring (males and females) at PND93. At all ages, brain Sn levels were higher than blood. At culling, in the directly dosed pups at weaning, and in dams at weaning, Sn levels in both tissues were linearly related to dose. Weanling pups without direct dosing showed lower levels than either culled pups or dams, indicating that lactational exposure was minimal or negligible even while maternal exposure is ongoing. In the adults, Sn levels persisted in brains of directly dosed rats, and the high-dose females had higher levels than did high-dose males. No Sn was detected in adult blood.

In conclusion: during maternal exposure to DBTC in drinking water, Sn is placentally transferred to the offspring, but lactational transfer is minimal, if any. Furthermore, Sn is concentrated in brain compared to blood, and its elimination is protracted, on the order of days to months after exposure ends.

In a study by Jenkins and co-workers (2004) neurotoxicity of monomethyltin, dimethyltin, and dibutyltin were compared to the known neurotoxicant trimethyltin by using an *in vitro* model of neuronal development in PC12 cells. Dibutyltin was the most potent neurotoxicant and was further tested *in vivo*.

In short: Dibutyltin (DBTC) significantly inhibited neurite outgrowth and caused cell death at concentrations approximately 40-fold lower than the lowest toxic concentrations of trimethyltin. Moreover, pregnant rats were dosed orally with low levels of DBTC (0, 2.5, or 5 mg/kg bw, 3 days/week) from gestational day 6 through weaning. In response, incidence of apoptotic cell death, measured by DNA fragmentation and TUNEL staining, was increased in the neocortex and hippocampus of postnatal day 38 offspring. No effect was observed at other ages examined. The present study demonstrates that DBTC is neurotoxic *in vitro* (0.1 μM (30.38 ng/ml) gave a 61% decrease in PC12 cell neurite outgrowth) at levels similar or lower than those detected in human blood samples (12-94 ng/ml) (Whalen et al., 1999) and that DBTC *in vivo* caused apoptotic cell death in neocortex and hippocampus in offspring.

The author concludes that the results suggest the possibility that chronic exposure to low levels of DBTC in the human population may be neurotoxic.

References

Jenkins SM, Ehman K, Barone S. 2004. Structure-activity comparison of organotin species: dibutyltin is a developmental neurotoxicant in vitro and in vivo. Developmental Brain Research. 151:1-12.

Moser VC, McGee JK, Ehman KD. 2009. Concentration and persistence of tin in rat brain and blood following dibutyltin exposure during development. J Toxicol Env Health. Part A. 72, 47-52.

Whalen MM, Loganathan BG, Kannan K. 1999. Immunotoxicity of environmentally relevant concentrations of butyltins on human natural killer cells in vitro. Environ. Res. 81, 108-116.

Male fertility effects of DBTC exposure:

Ananie et al., (2001) performed experiments focusing on sperm density, viability and morphology in mature Kunming male mice (7-8 /group) given a single ip injection per day for seven days. The doses were in the range of 0.025–0.40 μg/kg bw/day DBTC.

The results demonstrated that DBTC exhibited strong toxicity on sperm quality. Dosed with ≥ 0.05 μg /kg bw groups, the testes weight, sperm density and the rate of survival of sperm decreased, whereas the rate of sperm abnormalities increased significantly. In addition, treatment with 0.05 μg /kg bw resulted in increasing rate of sperm head

abnormalities, whereas administration at 0.20 and 0.40 µg /kg bw significantly increased the rate of sperm tail abnormalities. In the group treated at ≥ 0.10 µg/kg bw, the mice body weights decreased. It appeared there was a noticeable dose–response relationship between DBTC and the parameters studied.

The author concludes; "The current study revealed that DBTC might interfere with fertility in mammals by causing atrophy of the testes, including different types of abnormality and reductions in sperm viability and mortality."

Reference
Ananie D and Huang Y 2001. Effects of exposure to dibutyltin dichloride on sperm density, viability and morphology in male mice. Applied Organometallic Chemistry. 15, 727-732.

RAC's response

With respect to the mechanism, various mechanistic studies were presented both by industry and dossier submitter. RAC acknowledges that tributyltin compounds exert reprotoxic effects (RAC opinion, Tributyltin compounds, 2013). However, RAC considers that the available (mechanistic) data do not exclude dibutyltin-compounds exerting those kind effects as well.

By comparing the effective dose levels of the dibutyltin compounds DBTDL and DBTC vs effective dose-levels of tributyltin compounds, RAC noted that the observed adverse effects are comparable. Moreover, given that the effective dose levels of the tributyltin compounds are quite similar to the effective dose levels for DBTDL and DBTC, this indicates that a very high percentage tributyltin impurity would be needed to result in clear adverse effects.

All in all, RAC is of the opinion there is no robust information that demonstrates that the observed effects are due to an impurity and therefore there is no reason to discard the results of the developmental toxicity studies in the CLH report for the assessment of DBTC and DBTDL. The same applies for fertility and repeated dose toxicity.

Date	Country	Organisation	Type of Organisation	Comment number
07.11.2014	France		MemberState	7

Comment received

Only non-guideline studies have been mentioned to classify DBTD. Nevertheless, reproduction toxicity of DBTC has been evaluated on female Wistar rats according to one guideline test, OECD Screening Test 421. DBTC at about 11 mg/kg bw/day produced a significant reduction in the gestation index and increased postimplantation losses as well as pronounced foetal toxicity with increased postnatal mortality.
So please add the following reference:
Parametrix Inc (2006) Dibutyltin dichloride. IUCLID data set, 13.10.2000, update 24.07.2006, prepared for the Organotin Environmental Programme (ORTEP), Bellevue, WA, USA

Dossier Submitter's Response

Thank you for the comment. We agree that the data from the OECD 421 "Reproduction/Developmental Toxicity Screening Test" support the data on reproductive toxicity of DBTC. Hence, we have included a short review of this study below. In this study DBTC was given in the diet (0, 5, 30, 200 mg/kg) to 12 males and 12 females Wistar rats per group. The purity of DBTC is 98.57%. The content of TBTC was 0.25%. The

concentrations in the diet corresponded to: Males: 0.3-0.4, 1.9-2.3, 10.4-13.0 mg/kg bw/day, and for the females: 0.3-0.4, 1.7-2.4, 6.2-15.4 mg/kg bw/day for the 5, 30 and 200 mg/kg (Waalkens-Berendsen, 2003).

Effects on fertility and reproductive performance:

Table 1: Fertility and reproductive effects.

Concentration of DBTC in the diet (mg/kg)	***0***	***5#***	***30***	***200***
No. of pregnant with implantation, no pup	0	0	0	4*
No. of rats not pregnant	3	3	5	5
No of females with live pups	9	8	7	3*
Gestation index (%)	100	100	100	43
Post-implantation loss (%)	13.4	7.5	20.4	87.6*
No of pups delivered	11.3	10.6	11.4	6
No of live born pups	101	84	75	10
No of live pups/litter postnatal day 1	11.2	10.5	10.7	3.3*
No of live pups/litter postnatal day 4	10.7	10.4	9.6	1.7
No of stillborn pups	1	1	5	8*
Pup mortality postnatal day 4 (%)	5	1.2	11	50*
Pup weight postnatal day 1 (g)	5.6	5.8	5.5	4.4*
Pup weight postnatal day 4 (g)	8.0	8.3	7.3	6.0*
¤Runts postnatal day 1 (%)	5.9	-	-	80*
¤Runts postnatal day 4 (%)	4.2	-	-	40*

#one female was not mated, *=p<0.05,
¤ Calculated in ICLUID report ECBI/17/03 Add 5.

Several fertility parameters were significantly affected by the DBTC treatment at the highest dose level. As an example, the number of pregnant females with implantation, but no pups, was significantly higher in the high-dose group compared to the other groups, see Table 1. Also the numbers of females with live pups were significantly reduced at the highest dose, and the gestation index was less then half compared to all other groups. An increased incidence of late resorption of foetuses was seen in the uteri of three out of seven pregnant high-dose females at necropsy.

Even though the number of pregnant females in the highest group was only three, several effects on development were observed, like increased percent of runts**, reduced numbers of live pups, increased post-implantation loss are findings that support classification for development. However, in several studies as for example Noda et al., 1993 and Ema et al., 1991 in the CLH report, increased malformations like skeletal effects were observed in the foetuses. Skeletal examination of the foetuses was not performed in this study. The present study is a screening study and the number of animals and the data are limited. NOAEL for fertility and developmental effects is suggested by the author to be set at the 30 mg/kg dose (1.9-2.3 mg/kg bw/day).

**Runts are defined as pup weight less than mean pup weight of the control group minus 2 standard deviations.

Maternal effects: No mortality or clinical observations due to the treatment was observed.

1. Maternal body weight gain: All the groups gained weight, but in the highest dose group the body weight gain was 24% lower compared to the control. The number of animals in the

highest dose group is very limited. Se Table 2 below.

Table 2: Mean bodyweight gain GD0-20 compared to the control.

Concentration of DBTC in the diet (mg/kg)	***5***	***30***	***200***
Bw gain compared to control (%)	**-1**	**0**	**-24%**
Number of animals with litter	**8**	**7**	**3**

2. Effects on thymus in the females: In the dams the absolute and relative thymus weight were statistically reduced in the high-dose group. In the mid-dose group the relative but not the absolute thymus weight was statistically significantly reduced compared with the control group. The microscopic examination of the thymus revealed severe to very severe lymphoid depletion in all the high-dosed females and moderate to severe lymphoid depletion in half of the females in the mide-dose group (pregnant females).

3. Effects on ovaries: Examination of the ovaries revealed a significantly increase in the incidence of cysts in nine of the high-dosed females.

For maternal effects based on effects in thymus and body weight gain the NOAEL was suggested by the author to be set at the 5 mg/kg –dose (0.3-0.4 mg/kg bw/day).
With regard to the fertility, the Guidance on the Application of the CLP Criteria discuss maternal toxicity as follows: "Adverse effects on fertility and reproductive performance seen only at dose levels causing marked systemic toxicity (e.g. lethality, dramatic reduction in absolute body weight, coma) are not relevant for classification purposes". In this study the maternal bw gain at the highest dose was 24% lower than the control group (see Table 2 above). No reduction in absolute body weight (the animals gained weight in all dose groups), no lethality or coma related to treatment was observed at any dose levels. Hence, no marked systemic toxicity was observed according to the CLP. The CLP follows this up by: "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 are not a secondary consequence of this toxicity. However, mating behaviour can be influenced by parental effects not directly related to reproduction (e.g. sedation, paralysis), and such effects on mating behaviour may not warrant classification (Guidance on the Application of the CLP Criteria, Version 4.0 – November 2013)".

The developmental effects were observed at high-dose where also reduced body weight and effects on the thymus weight and lymphoid depletion in the dams were seen. In the CLP criteria it is stated: "Developmental effects which occur even in the presence of maternal toxicity are considered to be evidence of developmental toxicity, unless it can be unequivocally demonstrated on a case-by-case basis that the developmental effects are secondary to maternal toxicity. Moreover, classification shall be considered where there is a significant toxic effect in the offspring, e.g. irreversible effects such as structural malformations, embryo/foetal lethality, significant post-natal functional deficiencies". Hence, it is important to evaluate the relevance of the developmental effects observed. However, the study is limited and the data are considered as supportive for data already presented in the CLH report.

In conclusion, this study support that DBTC has a toxic effect on fertility and development.

Reference:

Waalkens-Berendsen, D.H., (2003). Dibutyldichlorostannane (Cas # 683-18-1): Reproduction/developmental toxicity screening test in rats. TNO Report V4906, Nutrition and Food Research, Zeist, The Netherlands.
RAC's response
Noted.

ATTACHMENTS RECEIVED:

1. **Comment: Proposal for harmonized classification for Dibutyltin dilaurate** – submitted by TIB Chemicals on 10 November 2014 (please refer to comment 6)