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Datum | Date
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Comment: Proposal for harmonized classification for Dibutyltin dilaurate

Dear Sirs,

The MSCA of Norway has proposed the following harmonized classification for Dibutyltin dilaurate (CAS#: 77-58-7):

- Muta. 2; H341
- Repr. 1B; H360FD
- STOT-RE 1; H372

In our opinion it is not sufficed justified to classify Dibutyltin dilaurate as toxic for reproduction category 1b.

Most of the studies used for reasoning of this proposal showed a specific toxicity of Tributyltin and not of Dibutyltin:

- Mechanistic evaluation of old reproduction / developmental studies
- New knowledge of the mechanistic differences in immunotoxicity of Dibutyltin and Tributyltin (old immunotoxicity studies on Dibutyltin dichloride determined only the adverse effects of Tributyltin not of Dibutyltin (e.g. Pieters, 1994)

Summary of literature:

Dibutyltin is responsible for lack of deactivation of cortico steroide. This results in a suppression of immune system and a transfer of maternal cortico steroide to fetus.

Tributyltin increases the level of cortico steroide (suppression of the immune system) and disrupts the placenta barrier (protection of the fetus against maternal cortico steroide results in foetal growth, postimplantation lost, malformation and programme responses leading to later disease). Furthermore Tributyltin causes changes in osteoclasts and osteoblasts resulting in malformation. The outcome of inhibition of aromatase by Tributyltin caused the endocrine properties.

For the adverse effects of Dibutyltin there is a clear dose response, but for the adverse effects of Tributyltin it seems, there is no threshold. Even at low levels, Tributyltin causes adverse effects.

The above named adverse effects are primary effects, but there are still secondary adverse effects from suppression of the immune system, resulting in delayed development of fetus. Under consideration of the strange of the primary effects the secondary effect via a decrease of zinc in plasma is not discussed here.

The following studies were used for reasoning classification and labeling of Dibutyltin compounds as toxic for reproduction in the past:

1. Waalkens-Berendsen DH, Dibutyldichlorostannane (CAS # 683-18-1): Reproduction / developmental toxicity screening test in rats, 2003
2. Osterburg, I., Dibutyltin dichloride oral (gavage) teratogenicity study in the rat, 1994
3. Ema, M., Itami, T., & Kawasaki, H., Susceptible period for the teratogenicity of di-n-butyltin dichloride in rats, 1992
4. Noda, T., Morita, S. and Baba, A., Teratogenic effects of various di-n-butyltins with different anions and butyl(3-hydroxybutyl)tin dilaurate in rats, 1993
5. Ema, M. and Harazono, A., Adverse effects of dibutyltin dichloride on initiation and maintenance of rat pregnancy, 2000

6. Ema, M., Itami, T. and Kawasaki, H., Teratogenicity of di-n-butyltin dichloride in rats, 1991
7. Ema, M., Kurosaka, R., Amano, H. and Ogawa, Y., Comparative Developmental Toxicity of Butyltin Trichloride, Dibutyltin Dichloride and Tributyltin Chloride in Rats, 1995
8. Ema, M., Kurosaka, R., Amano, H. and Ogawa, Y, Comparative Developmental Toxicity of Di-, Tri- and Tetrabutyltin Compounds after Administration during Late Organogenesis in Rats, 1996

The studies form Osterburg et al., 1992, Ema et al., 1992, Noda 1993, Ema et al., 1991, Ema et al., 1995 and Ema et al., 1996, noted malformation.
In 2002 there was an agreement by the producers of Dibutyltin compounds to reduce the Tributyltin content to concentrations 0.67 w/w % or less. Studies performed after this agreement (Waalkens-Berendsen, et al., 2003 and Ema et al., 2000) did not show malformations any longer. For the Waalkens-Berendsen, et al., 2003,x Dibutyltin with 0.3 w/w % impurity Tributyltin was used (**DeWolf, 2003**).

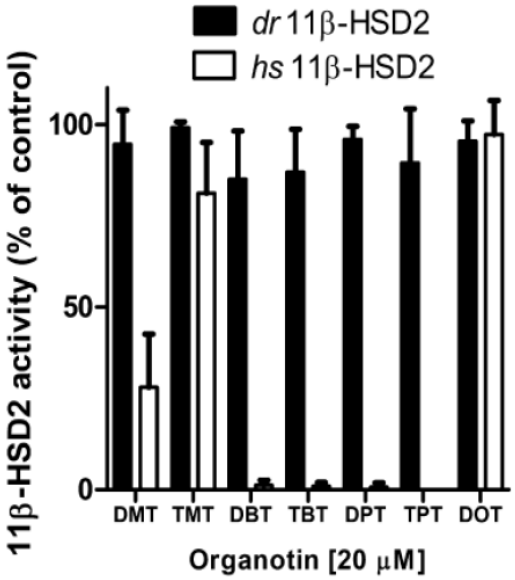
Abstracts of the named studies are in Annex I.

Immune suppression of Di- and Tributyltin and gene expression

11β- hydroxysteroid dehydrogenase type 1 (11β-HSD1) catalyses the conversion of inactive glucocorticoid (GC) in the active species, the type 2 the different way. Thereby the access of GC to the glucocorticoid receptor (GR) and the mineralocorticoid receptors (MR) is regulated, additional to the rendering specificity of MR for aldosterone (**Odermatt, 2012**).

Ohshima, 2005, showed that Tributyltin interacts with 11β-HSD1. A direct consequence of this interaction is the increase of the glucocorticoid steroid in rats respective cortisol in human.
11β-HSD2 is interrupted by Dibutyl- and Tributyl tin (**Meyer, 2012**).

Figure 1 – Inhibition of 11β-HSD2 by Organotins (adopted form Meyer, 2012)



Effect of various organotins on human and zebrafish 11β-HSD2 activity. Human (white bars) and zebrafish 11β-HSD2 (black bars) activity was measured with 50 nM cortisol as substrate in the presence of vehicle (0.05% DMSO) or 20 nM of the corresponding organotin for 10 min at 37 ◦ C using cell lysates. Data were normalized to vehicle control and represent mean ± SD from three independent experiments.

Gumy, 2008, demonstrates that dibutyltin disrupts glucocorticoid receptor function. Dibutyltin blocks the glucocorticoid-induced expression of hepatic PEPCK and TAT, two enzymes with a key role in energy metabolism of the immune system and disrupts the glucocorticoid receptor-mediated regulation of gene transcription at the initial step of receptor activation by abolishing ligand binding to the receptor. Furthermore Dibutyltin is able to abolish the suppressive effect of glucocorticoids on the synthesis of the pro-inflammatory cytokines TNF-α and IL-6 by reducing the GR-dependent trans-repression of NF-κB activation.

Tomiyama, 2009, describes the different mechanisms of the cell death of T-lymphocytes induced by Dibutyltin and Tributyltin; Dibutyltin does not affect the membrane directly, but instead rapidly passes through, Tributyltin directly affects and destroys their structure.

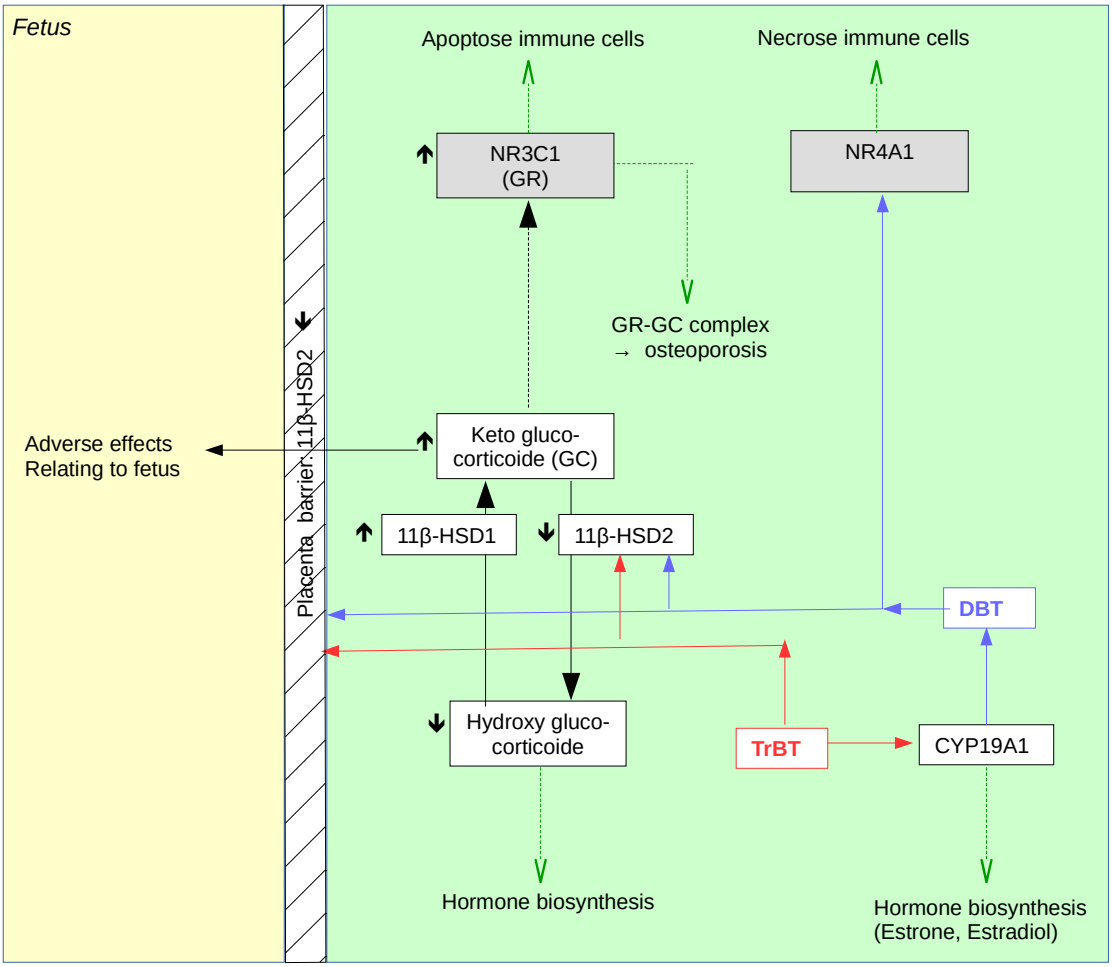
The cell death will be introduced by Tributyltin trough apoptosis:

- reduction of the mitochondrial function an membrane potential
- release of cytochrom c into the cytoplasm
- activation of capases
- activation of CAD by ICAD decrease

The activations of inactive capase by Tributyltin enhanced a dead signal response. For Dibutyltin the founded apoptosis response is too weak to cause cell death via apoptosis.

A reason for these variations in ability to induce caspases / apoptotic pathways in the lymphocytes may be due to the difference in the intracellular point of action between Dibutyl- and Tributyltin (differences in reactivities at the site of action due to inherent differences in each alkyltin´s structure). These differences in properties rise to critical variations in the intracellular distrubution of the agents.

Figure 2 – Survey action of DBT and TrBT to glucocorticoid system and consequences



Gennari, 2006, marks, that the cell death of T-lymphocytes induced by Dibutyltin through necrosis and by Tributyltin through apoptosis.

Person, 2010, addresses the effects of Butyltin exposure on the transcription factors c-Jun, Fos and Elk-1 and also investigated the effects of Butyltin exposure on the binding activity of AP-1.

The data show that Dibutyltin and Tributyltin provide important details regarding the regulatory effects these have on transcription. These results indicate that

1. following a brief 10 min treatment with Tributyltin the phosphorylation state of c-Jun is altered
2. Alterations are seen in the phosphorylation state of c-Jun and the total levels of c-Jun at 10 min and 1 h exposures with Tributyltin reduction in AP-1 binding to its DNA element may be a result of the Tributyltin-induced effects on c-Jun activation and total levels.
3. Dibutyltin exposures did not cause changes in the phosphorylation state of the transcription factors or their total levels in NK cells.
4. The effects of Tributyltin on AP-1 were no longer seen after 6 hour.

Glucocorticoid level – active and inactive glucocorticoid, placenta barrier

Seckl, 2004, studies the glucocorticoid administration during pregnancy and found reduces offspring birth weight and alteration in organs.

The transfer form maternal glucocorticoid steroid to the foetus causes adverse effects in the foetus directly or in the later life. The placenta cannot stop lipophilic steroids crossing to the foetus, but uses placental 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) catalyzes the conversion of active 11β- hydroxyglucocorticoids into their inactive 11-keto derivatives. In the placenta, 11β-HSD-2 forms a potent barrier to maternal glucocorticoids. A relative deficiency of 11β-HSD-2, with consequent reduced placental inactivation of maternal steroids, lead to overexposure of the foetus to glucocorticoids, retard foetal growth and programme responses leading to later disease. This was shown for example by **Klemke, 1995**, in pigs, too. **Holmes, 2006**, 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. In contrast to rats, the adult mouse forebrain has no 11β-HSD2. Conversely, plentiful 11β-HSD2 is present in the fetal CNS until the end of midgestation with the subsequent patterns of loss of 11β-HSD2 paralleling terminal differentiation of particular regions. Thus, any CNS programming likely reflects developmental and neurological effects.

Atanasov, 2005, made further investigation of the interaction of organotin and the 11β-hydroxysteroid dehydrogenase type 2. The results form Seckl were confirmed. Atanasov founds, that inhibition of 11β-HSD2 by organotin compounds is reversible. 11β-HSD enzymes play a pivotal role in regulating proliferation and differentiation in various tissues. 11β-HSD1 generates active glucocorticoids and promotes differentiation, and 11β-HSD2 inactivates glucocorticoids, thereby promoting proliferation. 11β-HSD1 and 11β-HSD2. Organotin-dependent inhibition of 11β-HSD2 may cause antiproliferative effects on immature thymocytes by increasing locally the ratio of active to inactive glucocorticoids or, alternatively, by increasing systemic glucocorticoid levels. Both organotin-induced inhibition of 11β-HSD2 and thymotoxicity are reversible.

Belkacemi , 2011, came to the same results like Atanaov (activation of 11β-HSD1 (inrease in active glucocorticoids), disruption of 11β-HSD2 (disruption of the placenta barrier converting active in inactive glucocorticoids)). The reduced placental a pivotal role in regulating proliferation catabolic capacity was accompanied by downregulation of SLC2A3, SLC38A1, and SLC38A2 expression, and by increased SLC38A4 expression, in labyrinth zones from the mid- and proximal-horns. In marked contrast to the labyrinth zone, the basal zone, which is the site of hormone production, did not show significant changes in any of these enzymes or transporters. These results suggest that dysregulation of the labyrinth zone GC “barrier”, and more importantly decreased nutrient supply resulting from downregulation of some of the amino acid system A transporters, may contribute to suboptimal fetal growth under maternal under-nutrition.

The interaction of Tributyltin with 11β-HSD1 (increase of glucocorticoid steroid) and Tributyl- and Dibutyltin with 11β-HSD2 (disruption of the placenta transformation from the glucocorticoid hydroxy into the keto form) results in reproduction and development effects at nanomolar concentrations of the Tributyltin. **Cooke, 2004**, found adverse concerning the reproduction and development of rats at a dose of 0.025 mg TrBT / kg bw/day. From this study were reported adverse effects in pups weight gain, triglyceride in blood, food consumption, hepacotyten and the liver. Studies with pure Dibutyltin does not show longer this drastic effects.

Furthermore there was found Dibutyltin in the stomach of suckling pups, while the dams feed with Tributyltin. From the scientific base it is known that in the animal is degrade the Tributyltin via the Dibutyltin into the Monobutyltin. With this knowledge there is an evidence the Dibutyltin is transferred from the dams to the suckling pups via lactation.

Adeeko, 2003, found in a reproduction toxicity study effects caused by interaction of Tributyltin with 11β-HSD1 and 11β-HSD2, too. The effects of tributyltin chloride exposure on pregnancy were an increase in the number of non-pregnant females. Dams gained significantly less weight during pregnancy and also appeared to be less physically active. Although the incidence of preimplantation loss was not increased in any of the tributyltin chloride treatment groups, there was a significant increase in postimplantation loss among dams exposed tributyltin chloride from GD 0 –19. As expected, litter size was also decreased and an increased number of dead fetuses. The incidence of bipartite sternebrae, presenting as ossified loci in the sternebrae, was significantly higher in fetuses of dams adminstered with tributyltin chloride from days 0 –19 compared to controls. Reduced ossification was also seen in the pelvic girdle, skull, and limbs of fetuses of dams exposed to tributyltin chloride, however, this did not reach statistical significance. Two fetuses in this group and in the 10 mg/kg tributyltin chloride group had a misaligned or split sternum (sternoschisis).

Asakawa, 2000, also demonstrated significant decreases in body weight in female F1 rats exposed to Tributyltin via the placenta and their dams' milk.

The exposure to Tributyltin during developmental stages of rats after weaning induced the inhibition in body weight gain and behavior (**Ikeuchi, 2012**). From the results of body weight, the exposure via the placenta and their dams' milk has a stronger inhibitory effect than does that in the developmental stages. The mechanisms of inhibition of development and behavior changes by the exposure of Tributyltin chloride via the placenta and their dams' milk and/or oral exposure via food were not clear. Especially, for the rats exposed to Tributyltin via food, the reason the exposure after weaning enhanced inhibitory effects remains unclear. In addition, the possible sexual difference in sensitivity to Tributyltin exposure via the placenta and their dams' milk and/or oral exposure via food also requires further clarification. For the mechanism of development, examination of the alteration in metabolism related to body weight gain such as lipid metabolism may be useful. To elucidate

the neurotoxic mechanism of Tributyltin, and the difference due to timing of exposure via food or to that of the sexes, it may be useful to examine alterations in gene expressions.

Furthermore it was shown **by Gennari, 2000**, that DBT interacts with GR antagonist NR4A1. The activation of NR4A1 causes a necrosis in immune cells. For a more detailed description of the molecular mechanism of the immune suppression see **Hansen, 2014**.

Endocrine properties:

Tributyl- and Triphenyltin compounds are potent endocrine disruptors in mammals through the activation of PPARγ or RXR. It had been demonstrated that Tributyl- and Triphenyltin directly bind to the retinoid X receptor (RXR) and the peroxisome proliferator-activated receptor (PPARγ) with high affinity and function as transcriptional activators. These compounds promoted adipocyte differentiation, which is triggered by the PPARγ /RXR signaling pathway (**Nakanishi , 2006**). In the publication is reported that Tributyltin and Triphenyltin are effective aromatase (CYP19A) inhibitors (catalyzes the biosynthesis of C18-estrogens (17 -estradiol, estrone, and estradiol) from C19-steroids (testosterone, androstenedione, and 16 -hydroxyandrostenedione)) and was further demonstrated that the gene expression of human aromatase is regulated by the activation of PPAR or RXR with citation of several third source next to his own studies. **Omura, 2001**, found the same effects concerning 17β-estradiol and a adverse effect on the male reproductive (quality and quantity of sperm) at concentrations of 25 ppm.

Baken, 2006, examined the change in gene expression of mice and rats exposed to Tributyltin. She found as well as Nakanishi changes in Retinoid X receptor (RXR) and the peroxisome proliferator-activated receptor γ (PPARγ) expression in both species. Another important point in this study is the difference in the genetic pathway expression between the different animal species, resulting in different toxicity. For example, whereas in the mouse thymus oxidation of fatty acids was increased, in the rat liver lipid synthesis was inhibited by down-regulation of Srebp1. Another is example that there in the mouse no 11β-HSD2 forebrain 11β-HSD2 barrier in contrast to rats.

Le Maire, 2009, illustrated the Tributyltin interaction with the Retinoid X receptor α and peroxisome proliferator-activated receptor γ and the mechanism causing the endocrine properties:

Tributyltin is able to activate RXR–PPAR- γ through RXR because this heterodimer interacts poorly with corepressors in vivo and belongs to the group of so-called 'permissive' heterodimers, which can be stimulated by RXR ligands on their own. Accordingly, significant activation of other permissive heterodimers such as RXR–LXR or RXR–NURR1 has been reported. Using stably transfected HGELN Gal4-PPAR- α and HGELN Gal4-PPAR-δ cell lines, we showed that TBT is able to activate all three RXR–PPAR-α, - γ and - δ heterodimers. As RXR- α Cys 432 has a crucial function in the mechanisms of binding and activation by Tributyltin, we looked at whether this residue is conserved in other nuclear receptors and found that the presence of a cysteine residue at this particular position is unique to RXR-α, -β and -γ . Conversely, the PPAR- γ LBP contains a cysteine (Cys 285) that couples covalently with conjugated oxo fatty acids and might act as an anchoring point for Tributyltin. However, in contrast to Cys 432, which is located in RXR- α helix H11, Cys 285 of PPAR-γ resides in helix H3. Hence, Tributyltin could bind to PPAR-γ in a region of the LBP, which does not allow efficient stabilization of the active receptor conformation. Finally, it was recently reported that dibutyltin acts as a potent antagonist of the glucocorticoid receptor. Similarly to other oxo-steroid receptors, glucocorticoid receptor contains cysteine residues that could help fix dibutyltin in the hormonebinding site. The involvement of cysteine residues in the binding of organotins to receptors other than RXR remains to be established. Nevertheless, our data suggest that tin compounds could use the specific Sn-S interaction to modulate the transcriptional activity of several nuclear receptors, the functional outcome being dictated by the structure of the organotin and the position of the anchoring cysteine in the LBP.

Nakanishi, 2002, demonstrates that these findings of the mechanism are relevant in human.

Lee, 2012, identified, that ovarian weight was significantly decreased in rats administered 10 mg/kg Tributyltin compared to that in control rats. As determined by the TUNEL assay, the number of apoptotic follicles in ovary was significantly increased in rats administered 10 mg/kg Tributyltin. The real-time PCR results showed that the expression of adipogenesis-related genes such as PPAR γ, aP2, CD36, and PEPCK was increased after Tributyltin

administration. In addition, apoptosis-related genes such as TNFα and TNFR1 were expressed more in the Tributyltin-administered rats compared with the control rats.

The relation between the mode of action of tributyltin, aromatse, ovaries and hormone is clarified by **Chen, 2002**. and **Saioh, 2001**, In principle, Aromatase, a cytochrome P450, catalyzes three consecutive hydroxylation reactions converting C19 androgens to aromatic C18 estrogenic steroids. Upon receiving electrons from NADPH-cytochrome P450 reductase, aromatase converts androstenedione and testosterone to estrone and estradiol, respectively. Aromatase is expressed in a tissue-specific manner. This enzyme is mainly expressed in the ovaries of premenopausal women. The ovarian aromatase produces mainly estradiol which plays essential roles in many reproductive and metabolic processes. A high level of aromatase is expressed in the placenta of pregnant women.

The major estrogen produced by placenta is estriol. In postmenopausal women and men, adipose tissue and skin cells are the major sources of estrogen production. However, the aromatase activity in these tissues is significantly lower than that in ovaries and the levels of circulating estrogen are much lower in men and postmenopausal women than in premenopausal and pregnant women. In men, testis, testicular germ cells, and epididymal sperm all express aromatase. In addition, during the fetal development, aromatase is expressed in the pituitary gland. The fetal expression of aromatase in the brain is believed to determine male or female metabolic patterns expressed during adult development.

Si, 2011, describes a delayed development of testes of mouse offspring from dams dosed with 100µg/kg bw Tributyltin via gestation. Further behavioral test showed a significant delay in cliff drop aversion response on offspring of dams dosed with 10 µg/kg bw Tributyltin.

Cho, 2011, point out, that Tributyltin act as an antagonist on human estrogen receptors via interaction with nuclear receptors such as RXRs and PPARγ in nanomolecular levels.

Malformation – skeletal effects

Yonezawa, 2007, investigated the effects of organotin compounds on den osteoclast differentiation, RANKL-stimulated RAW264.7 cells were treated with MBT, DBT, TBT or TPT. MBT and DBT had no effects at nontoxic concentrations, whereas TBT and TPT significantly inhibit TRAP+ osteoclast formation at very low concentrations. TBT and TPT dose-

dependently inhibit the increase in RANKL-induced TRAP activity (osteoblasts) . The interaction of Tributyltin with retinoicacid receptor RAR and retinoid X receptor RXR cause malformations. The malformations were localized primarily in the central nervous system, the branchial arches, and their derivatives These terata included excessive folding and elevation of the neural tube floor plate, exencephaly (with detachment of the cephalic neuroepithelium and rarefied cephalic mesenchyme), persistent patency of Rathke's pouch, small trigeminal ganglia, neural diverticula (chiefly from the spinal cord), and/or various optic and otic defect, (**Pauken, 1999**). **Verhaegen, 2011**, determined that Tributyltin changed the expression of CrCEcR and CrcRXR especially in ovaries. By alteration of this expression the T-box genes changed. The disorders in the T-box genes are responsible for other malformations Ema found (**Packham, 2003**). These findings are supported for example by **Castro, 2007**. **Atanasov, 2005**, pointed out, that triorganotins and are potent activators of the nuclear hormone receptors RXR and PPAR γ and that they promote adipocyte differentiation, suggesting that these organotins might contribute to the development of metabolic diseases, interestingly, the diorganotins were found to be inactive toward RXR and PPAR γ and so the diorganotins are not able to cause malformations. Another evidence for malformation in the skeletal system of animals is the Tributyltin impairs the dentin mineralization and the enamel formation in mouse teeth (**Salmela, 2008**); other studies show that the is a problem in the Calcium metabolism of the body caused by Tributyltin.

Discussion and Summary:

The studies show the radical effects relating reproduction toxicity. The initial doses for these effects are very low. Via the pointed out effects, the mechanisms in the genetic expression pathways and dose-response-effects the findings from the old studies on Dibutyltin with Tributyltin impurities are explainable.

The Tributyltin impurities, which have been present and tested in the past, were underestimated in their effects. It was not considered that the observed adverse effects could be caused by the impurities present in the test material.

From state of the scientific and technical knowledge it is to assume, that adverse effects observed in the samples tested exclusively or at least contribute to a significant extend to the observed effects arise for Tributyltin impurities; the fact that after the reduction of the

Tributyltin impurity around 2000 there was no longer malformation in animal found, supports this.

Since it is today technically feasible in a state of the art production process to manufacture Dibutyltin in a quality that assures Tributyltin impurity levels of lower than 0.1 w/w %; in our opinion supported by the explained mechanisms and the studies new studies in animal with Dibutyltin compounds with very low or no impurities, it is recommend to perform new screening studies in animal with Tributyltin free Dibutyltin compounds and to determine the mechanism of Dibutyltin toxicity on genetic basis, instead to classify the substance as toxic for reproduction category 1b in lack of information and doubt on the evidence of the mechanism.

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Annex I – Studies reasoning classification and labeling of Dibutyltin dichloride as Repr. 1b

- Waalkens-Berendsen DH, Dibutyldichlorostannane (CAS # 683-18-1): Reproduction / developmental toxicity screening test in rats, 2003

  In the Reproduction/developmental toxicity screening test in rats (TNO study number: V 4906) the test material was determined to have a NOAEL for general toxicity established on the low-dose level of 5 mg/kg diet and the NOAEL for reproductive toxicity was established at the mid-dose level of 30 mg/kg diet.

  The study was performed in accordance with the OECD Guideline for Testing of Chemicals no. 421 (adopted 27 July 1995). and was carried out in accordance with the OECD Principles of Good Laboratory Practice (as revised in 1997). No mortalities were observed. No clinical signs were observed in the male and female animals from the start of the study until sacrifice. Examination of the thymus revealed severe to very severe lymphoid depletion in 12/12 high-dose females, and moderate to severe lymphoid depletion in 6/12 (pregnant) mid-dose females.

  Three females showed late resorptions (autolytic fetuses) in the uterus during necropsy.

- Osterburg, I., Dibutyltin dichloride oral (gavage) teratogenicity study in the rat, 1994

  I*n the oral (gavage) teratogenicity study in the rat (HD project number: 380 -211) the test material was determined to have a NOAEL of 1.0 mg/kg bw/day for maternal toxicity and 5.0 mg/kg bw/day for teratogenicity.*

  No mortalities were observed in the study groups.

  No treatment-related clinical signs were observed.

  Minor changes such as thinness, bloody incrusted nose, injury at snout, extremities, trunk or skull/ear or hair loss were seen in a few animals of all dose groups on single days.

At 10 mg/kg body weight gain was clearly reduced during the treatment period, in particular from day 9 to 16 of gestation.

At 5.0 mg/kg, body weight gain was slightly lower than in the control group from day 9 to 12 of gestation.

At 10.0 mg/kg, mean daily food consumption was reduced during the treatment period, in particular from day 9 to 16 of gestation.

Necropsy did not reveal any treatment-related findings. Minor findings in the kidneys or liver were observed in a few animals of all study groups.

The mean weight of the thymus in dams was clearly reduced in group 5 (10.0 mg/kg) and slightly reduced in group 4 (5.0 mg/kg).

At histopathological examination, an increased number of dams showed a thymus atrophy at 10 mg/kg. A slightly increased incidence of thymus atrophy was also seen in group 3 (2.5 mg/kg) and group 4 (5.0 mg/kg), therefore, an effect of treatment with the test article cannot be excluded.

No effect of treatment was observed on implantation.

Post-implantation loss was not affected by treatment.

The mean number of fetuses per litter, mean fetal weights and fetal sex distribution, as well as the mean placental weight was not affected by treatment.

Although the incidence of fetuses with malformations was slightly increased at 10.0 mg/kg, this was due to three fetuses in two litters. This low incidence and the lack of a consistent malformation render this finding of equivocal toxicological significance.

- Ema, M., Itami, T., & Kawasaki, H., Susceptible period for the teratogenicity of di-n-butyltin dichloride in rats, 1992

  Pregnant rats were given di-n-butyltin dichloride (DBT) by gastric intubation at a dose of 20 mg/kg on days 7-9, 10-12 or 13-15 of pregnancy or at a dose of 20 or 40 mg/kg on day 6, 7, 8 or 9 of pregnancy. While treatment with DBT on days 7-9 was significantly and highly teratogenic, no evidence of teratogenicity was detected when DBT

was given on days 10-12 or 13-15. Treatment on day 7 or 8 with both doses of DBT, but neither on day 6 or 9, resulted in an increased incidence of fetuses with malformations. The highest incidence of malformed fetuses occurred after treatment on day 8. The incidence of malformed fetuses was proportional to the dose of DBT. Anomaly of tail, anal atresia, club foot, omphalocele, deformity of the vertebral column, defect of the ribs and anophthalmia or microphthalmia were predominantly observed. It could be concluded that, following maternal exposure to DBT in rats, developing offspring are not susceptible to teratogenic effects of DBT on day 6 and that day 7 is the earliest susceptible period, day 8 is the highest susceptible period and day 9 is no longer a susceptible period for teratogenesis of DBT.

- Noda, T., Morita, S. and Baba, A., Teratogenic effects of various di-n-butyltins with different anions and butyl(3-hydroxybutyl)tin dilaurate in rats, 1993

  In our previous study, di-n-butyltin diacetate has been shown to cause malformations such as cleft mandible, ankyloglossia, fused ribs, etc. in rat fetuses after oral treatment of maternal rats on day 8 of gestation. In this study, teratogenic effects of five di-n-butyltin compounds with different anions and also butyl(3-hydroxybutyl)tin dilaurate (3-OHDBTL) were examined in Wistar rats. Anion groups of these compounds were as follows: diacetate (DBTA), dichloride (DBTC), maleate (DBTM), oxide (DBTO) and dilaurate (DBTL). On day 8 of gestation, pregnant rats were treated orally with these compounds at 80 mumol/kg, or with 3-OHDBTL at 80 or 160 mumol/kg. Cesarean sections were performed on day 20 of gestation and fetuses were examined for their external and skeletal anomalies. Types of the observed malformations were similar to those in the previous study with DBTA except 3-OHDBTL, though the incidence of fetuses with malformations was different. In the DBTC-treated group, skeletal anomalies were predominant to external ones, especially with the higher incidence of fused ribs than in the other groups. In the 3-

OHDBTL-treated group, no external and skeletal anomalies were observed at 80 mumol/kg and cleft lower lip and ankyloglossia were observed in only one fetus, but peaked mandible (the tip of lower jaw with acute angle) was specific at 160 mumol/kg. From these results, the di-n-butyl group rather than anionic group was found to be important in the teratogenicity of di-n-butyltin compounds as well as in the other kinds of toxicities and 3-OHDBTL, one of the main metabolites of DBTL, is not the critical substance of teratogenicity because of a very low teratogenic potency.

- Ema, M. and Harazono, A., Adverse effects of dibutyltin dichloride on initiation and maintenance of rat pregnancy, 2000

  The present study was conducted to evaluate the adverse effects of dibutyltin dichloride (DBTCl) on initiation and maintenance of pregnancy after maternal exposure during early pregnancy in rats. After successful mating, female rats were given DBTCl by gastric intubation on Days 0 to 3 or on Days 4 to 7 of pregnancy at 0, 3.8, 7.6, or 15.2 mg/kg. Food-restricted pregnant rats were given an amount of feed equal to the feed intake of female rats treated with DBTCl at 15.2 mg/kg on Days 0 to 3 or on Days 4 to 7 of pregnancy. Female rats were sacrificed on Day 20 of pregnancy and pregnancy outcome was determined. After administration of DBTCl on Days 0 to 3, the rate of nonpregnant females and the incidence of preimplantation embryonic loss in the 7.6 mg/kg group were significantly higher than those in the control group, and those in the 15.2 mg/kg group were significantly higher than those in the control and pair-fed groups. In females with implantations, the numbers of implantations and live fetuses and the incidence of postimplantation embryonic loss in the groups given DBTCl on Days 0 to 3 were not significantly different from those in the control group. The incidence of postimplantation embryonic loss in the groups given DBTCl on Days 4 to 7 at 7.6 and 15.2 mg/kg was significantly higher than that in the control and pair-fed

groups. It can be concluded that DBTCl adversely affects initiation and maintenance of pregnancy when administered during early pregnancy and that the manifestations of the adverse effects of DBTCl vary with the gestational stage at the time of maternal exposure

- Ema, M., Itami, T. and Kawasaki, H., Teratogenicity of di-n-butyltin dichloride in rats, 1991

  Pregnant rats were given di-n-butyltin dichloride (DBT) by gastric intubation at a dose of 0, 2.5, 5.0, 7.5 or 10.0 mg/kg on days 7-15 of pregnancy. Maternal toxicity occurred in the 7.5 and 10.0 mg/kg groups as evidenced by a significant increase in maternal death and decrease in food consumption and body weight gain. The incidence of fetuses with malformations was roughly proportional to the dose of DBT, and was significantly increased in the 5.0, 7.5 and 10.0 mg/kg groups. Cleft jaw, ankyloglossia, defects of the mandible, fusion of the ribs and deformity of the vertebral column were predominantly found. It is concluded that DBT produced teratogenic effects in the absence of maternal toxicity.

- Ema, M., Kurosaka, R., Amano, H. and Ogawa, Y., Comparative Developmental Toxicity of Butyltin Trichloride, Dibutyltin Dichloride and Tributyltin Chloride in Rats, 1995

  Butyltin trichloride (BT), dibutyltin dichloride (DBT) and tributyltin chloride (TBT) were compared for their developmental toxicity including teratogenic potential following administration during the susceptible period for the teratogenesis of DBT. Pregnant rats were given either BT at a dose of 1000, 1500 or 2000 mg kg-1, DBT at a dose of 10 or 15 mg kg-1 or TBT at a dose of 40 or 80 mg kg-1 by gastric intubation on days 7 and 8 of pregnancy. Although maternal toxicity occurred, as evidenced by a significantly increased maternal lethality at 1500 and 2000 mg kg-1 and decreased maternal weight gain at 1000 and 1500 mg kg-1, no significant

increase in the incidences of postimplantation loss and malformed fetuses were observed after treatment with BT. Treatment with DBT resulted in a significantly lower maternal weight gain, lower fetal weight and higher postimplantation embryolethality. A significantly and markedly increased incidence of fetuses with malformations, such as exencephaly, cleft jaw, cleft lip, ankyloglossia, club foot, deformity of the vertebral column in the cervical and thoracic regions and of the ribs and ano- or microphthalmia, was observed in both groups treated with DBT. While treatment with TBT at 40 and 80 mg kg-1 caused a significantly decreased maternal weight gain and increased postimplantation embryolethality, no significantly increased incidence of malformed fetuses occurred. It could be concluded that BT, DBT and TBT are different in the susceptibility and spectrum of developmental toxicity.

- Ema, M., Kurosaka, R., Amano, H. and Ogawa, Y, Comparative Developmental Toxicity of Di-, Tri- and Tetrabutyltin Compounds after Administration during Late Organogenesis in Rats, 1996

  Dibutyltin dichloride (DBT), tributyltin chloride (TrBT) and tetrabutyltin (TeBT) were compared for their developmental toxicity and teratogenic potential following administration during the susceptible period for teratogenesis of TrBT. Pregnant rats were given either DBT or TrBT at a dose of 165 or 330 mumol kg-1 or TrBT at a dose of 330, 660, 1320, 2640 or 5280 mumol kg-1 on days 13-15 of pregnancy. Treatment with DBT at 165 and 330 mumol kg-1 caused a significant decrease in the maternal body weight gain. A significant decrease in the fetal weight occurred at 165 and 330 mumol kg-1. No significantly increased incidences of postimplantation loss or of fetuses with malformations were found following treatment with DBT. Treatment with TrBT at 165 and 330 mumol kg-1 resulted in a significant decrease in the maternal weight gain. A significant decrease in the fetal weight was found at 330 mumol kg-1. A significantly and markedly increased incidence of fetuses with cleft palate

was noted in both groups treated with TrBT. Treatment with TeBT caused a significantly decreased maternal body weight gain at doses of 660 mumol kg-1 and above. A significantly increased incidence of fetuses with cleft palate was observed at a dose of 5280 mumol kg-1. It could be concluded that there is a difference in the manifestation and degree of developmental toxicity between DBT, TrBT and TeBT