SUBSTANCE EVALUATION REPORT

Public name: **4-hydroxybenzoic acid**

EC Number(s): **202-804-9**

CAS Numbers: **99-96-7**

Submitting Member State Competent Authority:

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Year of evaluation: 2014

Date: 7 June 2016

Conclusions of the most recent evaluation step*	
Concern not clarified; Need to request further information from the Registrant(s) with the draft decision	
Concern clarified; No need of further risk management measures	X
Concern clarified; Need for risk management measures; RMO analysis to be performed	

DISCLAIMER

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Executive summary

Grounds for concern

4-hydroxybenzoic acid was originally selected for substance evaluation in order to clarify initial grounds for concern:

- suspected endocrine disruptor

- consumer use

- high (aggregated) tonnage

During the evaluation also other concerns were identified. The additional concerns were:

reproductive toxicity, developmental and repeated dose toxicity

Procedure

The substance evaluation is based on information in the registration dossiers, including the Chemical Safety Report as well as the other available information relevant to the concern up to 11th March 2015. Literature data have been considered with respect to the investigation of 4-hydroxybenzoic acid (4-HBA) itself however taking into account information about parabens and other relevant substances. The eMSCA was in contact with a representative of the registrants by e-mail, who provided materials available to them.

The endocrine activity of the evaluated substance was evaluated with respect to the concerns stated in the Justification document for the selection of the substance to CoRAP. Related to the concern is the wide dispersive use of parabens as preservatives.

The concern in relation to endocrine disruption as main concern was not confirmed within evaluation and this was presented in the initial SEv report submitted with Draft Decision on 26th March 2015. However further information was required due to high tonnage as the information for some endpoints seemed to be missing.

Within the commenting period a dossier update has been submitted together with comments and further information as read-across document data matrix for methylparaben extended to ethylparaben and more information about uses and relevant amounts used in EU. Comments were delivered to the eMSCA on 14 October 2015. As a result, the information is sufficient for eMSCA to conclude and not to address additional concerns in a SEv decision. Thus decision making process could be terminated.

Conclusions

Based on unpublished and published available information it can be concluded that for the evaluated substance concerns about the endocrine activity are unjustified. The inclusion of 4-HBA in the Priority list (database of substances with ED potential) can be attributed to precautionary criteria and unavailability of the unpublished study with negative results already existing at the time of the inclusion.

The estrogenic activity of 4-HBA is insignificant considering that the positive result from first UT assay from Priority list is isolated. This is concluded with regard to later results from UT assays

together with negative results in binding assay on ERRγ (unlike parabens in the same assay). This is also supported e.g. by no activity (comparing to parabens) in toxicity study by yolk protein induction addressing hazard towards environment. All available data suggested that 4-HBA as such is inactive in connection with the endocrine activity in contrast to alkyl parabens in general.

The concern in relation with endocrine disruption as main concern was not confirmed within this evaluation and 4-HBA is not regarded as having estrogenic activity. Information about uses and relevant amounts used in EU confirmed that there is no consumer or professional use for concerned registrants or high tonnage of 4-HBA used as substance itself. As the evaluated substance is used as intermediate and the concern connected with endocrine disruption was not confirmed, the information as a whole, was decisive for this conclusion.

CONTENTS

TABLES

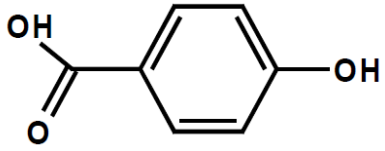
1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

1.1 Name and other identifiers of the substance

Table 1: Substance identity

Public Name:	4-hydroxybenzoic acid
EC number:	202-804-9
EC name:	4-hydroxybenzoic acid
CAS number:	99-96-7
CAS name:	Benzoic acid, 4-hydroxy-
IUPAC name:	4-hydroxybenzoic acid
Index number in Annex VI of the CLP Regulation	---
Molecular formula:	C7H6O3
Molecular weight range:	138.12
Synonyms:	p-Hydroxybenzoesäure para-hydroxybenzoic acid PHBA p-HBA 4-hydroxybenzoic acid PHBS p-Salicylsäure 4-Carboxyphenol p-salicylic acid p-Hydroxybenzoic acid 4-Hydroxybenzoesäure para-Hydroxybenzoesäure p-HBS

Structural formula:



1.2 Composition of the substance

Stated in Confidential Annex

1.3 Physico-chemical properties

4-hydroxybenzoic acid, or p-hydroxybenzoic acid, is a phenolic derivative of benzoic acid. It is a white crystalline solid that is slightly soluble in water and chloroform, but well soluble in alcohols, ether, and acetone. [72]

logKow (non-ionised form) = 0.878 (22°C, pH=3.5), logKow (ionised form) < 0.3 [68];

1.37 at 25 °C (acc. OECD TG 107) [70];

Water solubility: 4.87 g/L at 20 °C [68]; 6 g/L at 25 °C (acc. OECD TG 105) [70]

Vapour pressure: 1.05×10^{-5} Pa at 20 °C (acc. OECD TG 104) [68]

Particle size distribution: ca. 50% < 85.5 µm; 3% < 20 µm; practically no particles < 10 µm (acc. OECD TG 110) [70]

Dissociation constants: K1=3.3x10-5and K2=4.8x10-1 at 19°C [68]

2 MANUFACTURE AND USES

2.1 Quantities

Aggregated tonnage (per year)

>1000 tonnes

2.1.1 Manufacturing processes

Not relevant for this evaluation

2.2 Identified uses

2.2.1 Uses by workers in industrial settings

Intermediate.

2.2.2 Use by professional workers

No professional uses.

2.2.3 Uses by consumers

No consumer uses.

2.3 Uses advised against

2.3.1 Uses by workers in industrial settings advised against

No information available.

2.3.2 Use by professional workers advised against

No information available.

2.3.3 Uses by consumers advised against

No information available.

3 CLASSIFICATION AND LABELLING

3.1 Harmonised Classification in Annex VI of the CLP Regulation

The substance does not have a harmonized classification.

3.2 Self-classification

CLP classification:
Eye Damage 1, H318 (Causes serious eye damage)
STOT SE 3, H335 (May cause respiratory irritation)
Route of exposure: Oral (for extremely high dosages)
Inhalation (Depending on the physical state, the risk of inhalation can vary.)
DSD:
Xi; R37 Irritating to respiratory system
Xi; R41 Risk of serious damage to eyes

The Classification and Labelling Inventory:
In addition to the self-classification given above, the following are notified:
Eye Irrit. 2; H319: Causes serious eye irritation.
Skin Irrit. 2; H315: Causes skin irritation.
Acute Tox. 4; H302: Harmful if swallowed

4 ENVIRONMENTAL FATE PROPERTIES

The substance is readily biodegradable with very low potential for adsorption and bioaccumulation and naturally occurs in the environment. In case of industrial release to water practically all amount of substance is detained in water. An eventual release to other compartments is subsequently distributed as ca one fourth to water and rest to soil. However, all such releases from production site are prevented by technical measures.

5 HUMAN HEALTH HAZARD ASSESSMENT

Conclusions related to the concern

Introduction

4-HBA is a natural constituent of food and a metabolite of many dietary phenols. The substance is metabolized and eliminated as unchanged plus glycine conjugate 4-HHA (there is information from older human assays which confirms that), but 4-HBA is subject also to ethereal conjugation with sulphate and decarboxylation by the gut flora.

According to a study published in 2000 an estimated dietary burden of 4-HBA is ca 1.7 mg/d (e.g. by processed berries - otherwise as general rule is naturally present as conjugates), which do not fully consider the burden of hydroxybenzoates added to foods as preservatives or all conjugates (e.g. for salicylic acid that value is 0.11±10.27mg). An estimated average daily burden of parabens themselves was low, only 0.1mg for MeP, EtP and ProP. Excretion products of parabens are mainly 4-HBA, further the glycine conjugate 4-HHA and smaller amounts of glucuronide and sulphate conjugates.

The study authors stated that the biological effects associated with dietary sources are likely to be weak but they also mentioned the need for investigating of the role of the gut microflora to find out the extent to which degradation of other classes of phenols (e.g. flavonoids) contributes to the burden of HBAs such as 4-HBA and others. These data had been published in the year 2000 however burden surely had increased from various products within the subsequent decade [38].

In published literature in 2013 a total average exposure to parabens is anticipated of about 76 mg/d as an overall estimate from different sources, when significant part of this value are pharmaceuticals which include oral administration (cosmetics and similar products 50 mg, pharmaceuticals 25 mg, food 1 mg). [39]

Another study for instance only for MeP alone estimated following daily intake values DI based on urinary concentrations. The geometric mean DI was estimated to be 0.5−0.7 mg/day for U.S. and Chinese children, but much higher values were estimated for Chinese adult females (5.1 mg/day) and adult males (1.6 mg/day). The maximum DI was estimated at ca 6 and ca 7 mg/day for U.S. girls and boys, respectively. The maximum DI estimated for Chinese girls, boys, adult males, and adult females were 18.7, 9.2, 18.7, and 34.8 mg/day, respectively [40].

Thus regular exposure occurs, however relation to potential endocrine activity is low as estrogenic activity of 4-HBA was not confirmed and the substance can be regarded as inactive (see section Endocrine properties).

The presence of 4-HBA in the human body is essential in natural processes for the formation of ubiquinones (e.g. Ubiquinone 6) and it is also present in urine due to the decomposition of the amino acid.

A recent review from 2013 has presented various biological properties of 4-HBA and eventually its derivates

- antimicrobial (against Gram +ve and Gram –ve bacteria),
- antialgal,
- antimutagenic (mentioned derivates as caffeic acid, gallic acid, etc.),
- antiestrogenic (4-HBA mentioned in study only in relation as metabolite of parabens),
- hypoglycemic (4-HBA might have an insulin-like activity, causing the hypoglycemic effect in the diabetic rats; decreases plasma glucose level in dose dependent manner by increasing peripheral glucose consumption)
- nematicidal (4-HBA exerts paralytic effect against second stage juveniles of nematode *Meloidogyne incognita*)
- antiviral (only derivates mentioned again and note that antiviral activity of a specific hydroxybenzoic acid ester is higher than that of its corresponding acid)
- antioxidant
- anti-platelet aggregating (gallic acid ester derivate was mentioned to act better than aspirin)
- anti-inflammatory (4-HBA isolated from ethanolic extract of Vitex glabrata - smooth chaste tree - had effect comparable to diclofenac sodium on rat models)

Authors had reviewed parabens: “1000 (BuP) to 1,000,000 (MeP) times less estrogenic than estradiol, absorbed through skin quickly and undergo hepatic metabolism to form 4-HBA which is detected in blood and urine of mammals (however small amount of parabens may remain intact and was discovered in human breast tissue and milk). Exposure to parabens increased uterus weight via ER- dependent pathway” [41].

However, there is also the possibility, that regular uptake may cause adverse effects like those described for benzoic acid. The conversion of benzoic acid to hippuric acid is a saturable process relating to availability of glycine. There may be consequences for metabolism at sufficiently high doses even with rapid excretion of the acid itself (ADI of up to 5mg/kg bw).

Another example for the possibility for prolonged metabolism: salicylic acid, as an acetylsalicylic acid metabolite is excreted together with conjugates corresponding to 4-HBA conjugates and

elimination of salicyluric acid and salicylic acid in plasma are slower for underweight children, who excreted less free acid and more salicyluric acid in comparison to normal-weight children. [38]

4-HHA was identified as one of the major component of uremic serum [42].

The confirmatory marker of glycine deficiency for detoxification conjugation reactions is elevated benzoate. Abnormal values of urinary benzoate and hippurate may indicate clinically significant detoxification or microbial imbalance in the digestive tract [43].

This eventualities were considered in regard to initial thoughts when registrants have sought to apply an analogue approach also to salicylic acid and acetylsalicylic acid besides MeP.

However the read-across rationale provided by registrants states that „salicylic acid had the highest degree of similarity to 4-HBA using the in silico ranking criteria…“ but „…salicylic acid does not meet the criteria of common precursor or breakdown products and data regarding the metabolism of the structural isomers suggest that the position of the hydroxyl group significantly influences their overall metabolism … 4-HBA can be conjugated with glycine, resulting in the formation of 4-HHA, or it can be glucuronidated through either the 4- hydroxy or 1-carboxylic acid groups (forming 4-hydroxyphenyl or 4-carboxyphenyl glucuronide, respectively). The glucuronidation is catalyzed by UDP glucuronosyltransferases.“

But 4-HBA seems to be inactive in adipogenesis, which is the area of interest currently linked to prolonged intake of parabens. Recently it has been found out, that 4-HBA (or benzoic acid) in contrast to long chain parabens, does not promote adipocyte differentiation in the in vitro murine 3T3-L1 adipogenesis model [44].

5.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

5.1.1 Non-human information

4-HBA non-human data

Oral route

Oral absorption: 100%

An older toxicokinetic study (1.0 g/kg bw, dog) by oral route compares 4-HBA and parabens absorption and excretion by examination of blood and urine samples.

This study revealed the same concentrations of free 4-HBA and the elimination kinetics in plasma after 4-HBA administration as for MeP for which only traces of the unchanged compound were found [29].

However, the main metabolite for elimination of 4-HBA in this study was a glucuronic acid-conjugate. Thus, elimination by glucuronidation via UDP-glucuronyltransferase (UGT) was a main pathway of detoxification. This is not consistent with older data on human assays or recent *in vitro* investigations with liver microsomes and UGT, when glucuranidation for 4-HBA was not a main pathway of detoxification.

Total recovery after administration of 4-HBA itself was 94% in urine, of which 40.1% as free 4-HBA, 30.0% as glucuronide, 24% other undetermined conjugates. Values after administration of

MeP were: total recovery 89% in urine, of which 21.3% as free 4-HBA, 35.1% as glucuronide, ca 33% undetermined conjugates, thus only half amount of eliminated free 4-HBA after administration of MeP in comparison to elimination after administration of 4-HBA as such.

Most of the administered substance was excreted between 6 and 24 hours after administration. However, 4-HBA was still detectable in plasma after 30 hours. Complete absorption could be shown from excretion and plasma concentration data.

After intravenous administration of 50 mg/kg bw of 4-HBA most of the dosage was excreted in 6 hours with final recovery of 83% (25% of this amount as free 4-HBA and 18 % as glucuronide conjugate), thus oral route with whole ADME cycle exhibits better but prolonged excretion.

Although levels of intact parabens in plasma were mostly below the lower limit of sensitivity, significant levels of intact parabens were determined in plasma a few hours after oral administration. It was stated that conjugation may occur before or after hydrolysis, which was not possible to distinguish by the biochemical method used in this study.

But in general parabens hydrolyse rapidly as indicated by quite similar percentage of free 4-HBA after i.v. administration of lower parabens comparing to administration 4-HBA as such by the same route [29].

The possibility of both ways of conjugation (before or after hydrolysis) is supported by other studies [65,66] when the metabolism of 4-HBA is compared with EtP in rats at the dose of 2 mg/kg by intravenous and intraduodenal administrations.

Both substances were metabolized to the same metabolites (glycine conjugate, ester type glucuronide and sulfate of 4-HBA) and excreted in the urine and bile.

It was found that for 4-HBA administrated itself the levels of the 4-HBA glycine conjugate (4-HHA 4-hydroxyhippuric acid) are lower than for EtP, which suggested a direct metabolic route from EtP to 4-HHA which also depends on route – direct conjugation was more increased within intraduodenal administration than intravenous administration.

The simultaneous administration EtP with 2-HBA was examined in a 3rd following study by the same authors [67]. The elimination of every metabolite was delayed and the interaction of salicylic acid on the biological fate of ethyl paraben was presented. The excretion of unconjugated 4-HBA metabolite increased and 4-HHA and 4-hydroxybenzoylglucuronide was decreased.

Dermal route

Dermal absorption: 4% determined (10% is default according to logKow).

The determined dermal absorption value from a toxicokinetic study with radiolabelled 4-HBA (rat, 120 hours) is only ca 4% of the dosage. The recovery of the total radiolabelled dosage was 99.92%. 95.9% remained at the treated skin site [30].

LLNA sensitisation study, which could draw attention to systemic toxicity, did not reveal effects leading to classification. The 20% sensitisation potential was determined within a Guinea pig maximization test [67].

Inhalation route

Inhalation absorption: default value 100%

The irritating effect of the substance occurred in a 11-d inhalation toxicity study with rats leading to a NOEL of 60 mg/m³ for 6 hours.

4-HBA metabolite from parabens non-human data

Oral route

In vitro test (human liver microsomes, UGT)

4- HBA was glucuronided as a result of paraben hydrolysis at very low rate within an *in vitro* assay with human liver microsomes in contrast to parabens. Incubation in maleate buffer, which increases the stability of acylglucuronides, did not enhance the production of 4-HBA glucuronides. The LC-MS experiment clearly identified the biosynthesis of an ether 4-hydroxyglucuronide after incubation with human liver microsomes.

Parabens are good substrates for UDP-glucuronosyltransferase in human liver microsomes and the rate of glucuronidation of parabens is much higher than that observed for 4-HBA, actually formation of 4-O-glucuronide could be attributed to parabens.

Investigation on parabens revealed, that 4-HHA does not arise, while unambiguously the formation of an ether glucuronide (which may also be formed by UGT without prior hydrolysis to the 4-HBA from parabens) was confirmed.

Study authors concluded, that parabens are extensively metabolized in human liver microsomes by the combined action of esterases and UGT of families 1A and 2B and it is likely to prevent their accumulation [46].

Regarding toxicokinetics of parabens there is a recent (2012) in vivo study investigating ADME on SD rats with clearly proven result by LC-MS, that 4-HBA is almost exclusively the main metabolite of parabens.

Toxicokinetic study on MeP, ProP a BuP: 100 mg/kg; oral, dermal or subcutaneous route, SD rats; presence of metabolites was evaluated with an HPLC/UV/radioactivity monitoring system in pools of plasma samples.

Examined parabens were orally well-absorbed, partially absorbed by dermal route, followed the ranking MeP > BuP > ProP and systemic exposure suggesting a higher skin penetration to short-chain parabens represented by MeP.

Excretion / Absorption	urine	faeces	tissue carcasses
oral (well absorbed)	> 70%	< 4%	2%
subcutaneous (well absorbed)	> 70%	< 4%	2%
dermal (> 50% unabsorbed)	14-27%	< 2%	

Route	$t_{max\ [h]}$
oral	0.5
subcutaneous	2-4
dermal	8

This study presented 4-HBA as the exclusive metabolite, as parabens had not been detected in significant amounts following exposure to all examined parabens (MeP, ProP, BuP used as

preservatives) by dermal and oral exposure route (plus BuP by sc route). This by authors basically means a systemic exposure to 4-HBA [47].

Dermal route

Parabens are able to penetrate human skin in intact form and are absorbed systemically (demonstrated *in vitro*, in vivo on humans) [45].

Uptake and metabolism of parabens in humans and animals was thoroughly reviewed recently [24]. Parabens after dermal absorption undergo conjugation and excretion through the bile and urine; a large part of the main metabolite 4-HBA is excreted as glycine conjugate (p-hydroxyhippuric acid, 4-HHA)

Inhalation route

Not relevant for this evaluation.

5.1.2 Human information

Oral route

No recent data available, however a few older assays/experiments are confirming the low toxicity of the substance and short-term tolerance of high doses up to 20 g/day. This amount is declared by study authors as maximum dosage, that human body is able to dispose of in the course of 24 hours at six hours intervals and 70% of dose was excreted unchanged with the urine (no information about metabolites, in the other provided human assays only about 50% were excreted with the urine, including identified metabolites).

Dermal route

The similar systemic exposures of rats and humans to free paraben at 100-fold different external doses is discussed in the SCCS report [26] and led to the assumption, that the difference is most probably based on markedly different toxicokinetics in rats and humans by dermal route. They discussed rat as inappropriate species for effects on humans by dermal route.

Inhalation route

No data available.

5.1.3 Summary and discussion on toxicokinetics

Oral route

4-HBA

An available study provided information on toxicokinetics after absorption of 4–HBA by oral and dermal route. Based on an inhalation toxicity study eventual absorption can occur by inhalation route but this is only relevant for industrial sites as there are no consumer or professional uses. Its dermal absorption is insignificant based on toxicokinetic data.

Parabens:

The recent toxikokinetic study on MeP, ProP, BuP with rats by oral, dermal (plus BuP subcutaneous) route based on HPLC detection concluded that insignificant amounts of intact parabens were identified due to rapid conversion to 4-HBA as almost exclusive metabolite.

Parabens can metabolize to 4-HBA at different rates. An inverse relationship between 4-HBA and 4-HHA was observed, because there are indications that parabens with longer alkyl chain form less 4-HBA metabolite after oral exposure in favour of 4-HHA.

It was found in a study on liver microsomes that the metabolism may take place in several conjugation ways - after hydrolysis of parabens but also by direct conjugation parabens without their previous hydrolysis, which reduces exposure to 4-HBA metabolite and increases the influence of direct metabolic detoxification products from parabens. This occurs more after intraduodenal administration then after intravenous as was presented in a comparative study with EtP. However, concomitant administration of salicylic acid with parabens can affect this process in the opposite direction.

Dermal route

4-HBA

The absorption of 4-HBA as such is insignificant after dermal exposure.

Parabens:

Parabens are able to penetrate human skin in intact form and are absorbed systemically (demonstrated *in vitro*, in vivo on humans).

Inhalation route

4-HBA

There are no data for inhalation route, although available data on inhalation toxicity indicate signs of upper airways irritation thus potential absorption. The substance is self-classified as STOT SE 3, H335 (May cause respiratory irritation).

5.2 Acute toxicity

5.2.1 Non-human information

5.2.1.1 Acute toxicity: oral

The studies on acute toxicity after oral administration result in LD50 ≥ 2000 mg/kg bw

based on 4-HBA.

5.2.1.2 Acute toxicity: inhalation

The available information from a provided 11 days inhalation toxicity study (SD rat, male/female, 4h inhalation/d) revealed irritation potential to respiratory tract.

5.2.1.3 Acute toxicity: dermal

LD50 ≥ 2000 mg/kg bw based on 4-HBA.

5.2.1.4 Acute toxicity: other routes

No data available.

5.2.2 Human information

Several older assays on humans are confirming the low toxicity of the substance and short-term tolerance with no signs of toxicity of high doses up to 20g/day, which is by authors declared as maximum dosage, that human body is able to dispose of in the course of 24 hours at six hours intervals.

5.2.3 Summary and discussion of acute toxicity

The information on acute toxicity indicated no hazard regarding the evaluated substance by oral and dermal route. The available information from the provided 11 days inhalation toxicity study revealed irritation potential to the respiratory tract. The substance has met classification criteria for STOT SE 3, H335 (May cause respiratory irritation) for exposure routes:

- oral (for extremely high dosages) and
- inhalation (depending on the physical state, the risk of inhalation can vary).

5.3 Irritation

5.3.1 Skin

Not relevant for this evaluation.

5.3.2 Eye

Not relevant for this evaluation. Self-classification: Eye Damage 1, H318 (Causes serious eye damage).

5.3.3 Respiratory tract

The substance has met classification criteria for STOT SE 3, H335 (May cause respiratory irritation) for exposure routes

- oral (for extremely high dosages) and
- inhalation (Depending on the physical state, the risk of inhalation can vary).

5.3.4 Summary and discussion of irritation

The substance has met classification criteria for respiratory irritation and also self-classification as Eye Damage 1, H318 (Causes serious eye damage) has been assigned.

5.4 Corrosivity

Not relevant for this evaluation.

5.5 Sensitisation

5.5.1 Skin

LLNA sensitisation study (OECD 429) did not reveal effects leading to classification.

5.5.2 Respiratory system

No data available.

5.5.3 Summary and discussion on sensitisation

Based on available data the criteria for classification for skin sensitisation have not been met.

5.6 Repeated dose toxicity

5.6.1 Non-human information

5.6.1.1 Repeated dose toxicity: oral

A study equivalent to OECD 422 is available for 4-HBA for the repeated dose toxicity endpoint by oral route.

The effects observed in the combined study were considered as unspecific changes caused by the acidic nature of 4-HBA, which can lead to a metabolic acidose in the blood and explains the

deviations in clinical chemistry and in haematology observed after high dosages of 4-HBA. Based on findings on the parental animals the registrants determined the NOAEL for systemic toxicity at 1000 mg/kg bw/d (highest dose).

Chronic toxicity studies by the oral route for substance methyl 4-hydroxybenzoate have been proposed for the purpose of read-across as a surrogate of results of a sub-chronic oral toxicity study.

- oral, chronic (96 weeks), rat, 1956 (pre-GLP);
- oral, chronic (422 days), dog, 1956 (pre-GLP)

However none of these studies (including OECD 422 study) provides individually the information equivalent to a 90-day repeated dose toxicity study, because they do not address (totally or partly) the following endpoints:

- detailed clinical battery (detailed clinical examination performed once a week)
- functional observation at the end of study: sensory reactivity, motoric activity, grip strength)
- oftalmological examination (at the beginning and at the end of study)
- consumption of water
- haematology (examination of coagulation parameters was not included in the combined study)
- urinalysis
- biometry of organs (weights of adrenal glands, spleen, brain and heart are missing)
- histopathology of organs (examination of the following organs is not included: spinal cord, pituitary gland, thyroid and parathyroid gland, oesophagus, salivary glands, stomach, small and large intestine, pancreas, adrenal glands, trachea, aorta, accessory sex organs, mammary gland, prostate gland, lymph nodes, ovaries, peripheral nerve, bone, skin, eyes)
- evaluation of reversibility of effects (recovery groups were not included in the combined study)

The justification of the adaptation for this information requirement is based on a toxicokinetic study on dogs conducted within the provided chronic toxicity studies for methyl 4-hydroxybenzoate in comparison to 4-HBA.

After oral administration 89% (21% as free acid) of methylparaben was eliminated in urine within this study. 94% (40% as free acid) of 4-hydroxybenzoic acid itself was eliminated. Similar elimination kinetics were found in plasma. Therefore the chronic study with rats and by oral route was deemed appropriate by registrants for evaluating the chronic toxicity of hydroxybenzoic acid as well.

Information for methylparaben was extended to ethylparaben (with subchronic feeding study on rat, NOAEL ≥ 1200 mg/kg bw/day) in the framework of read-across document data matrix.

As more information about uses and relevant amounts used in EU confirmed that there is no consumer or professional use for concerned registrants or high tonnage of 4-HBA used as substance itself (see section Exposure assessment), an overall assessment led to conclusion not to address the additional concern in relation to repeated dose toxicity with combination high tonnage and use.

5.6.1.2 Repeated dose toxicity: inhalation

There is an experimental 11 days inhalation toxicity study (SD rat, male/female, dust, whole body, no guideline, non- GLP, well documented) with a NOEL of 60 mg/m³ air (male/female) [3]

5.6.1.3 Repeated dose toxicity: dermal

Data waiving based on insignificant dermal absorption (2% + ca 2% remained into treated skin) supported by toxicokinetic study with radiolabelled 4-HBA substance. [30]

5.6.1.4 Repeated dose toxicity: other routes

There is a dose-setting study provided on mice CD-1, female (3 days, subcutaneous administration) for an uterotrophic assay in Reproductive toxicity section. Study conclusion: NOEL: >= 100 mg/kg bw.[4]

5.6.2 Human information

No data with 4-HBA are available. As support information a short-term assay on humans was provided. The total dose of 3g/d was administered in several doses within day in water for 5 consecutive days. Samples were taken 2 days after end of administration.

Total recovery after administration of 4-HBA itself was 46.2% in urine, of which 28% as 4-HBA, 11.0% as hippuric acid (HA), 7.2% 4-hydroxyhippuric acid (4-HHA). *"As metabolites "Hippuronsäure" 1.7 g (ca. 11%) and "p-Oxybenzursäure": 1.42 g (7.2%) were found."* [12]

With respect to parabens there is a placebo-controlled study (conducted from 1.1.90 to 31.12.94) with weekly orally administered mixture of 100mg methylparaben (MeP) + 100 mg propylparaben (ProP) involving 14 paraben-sensitive patients. Two patients had flares of their usual recurrent vesicular hand eczema within 24 hours after administration but not after the placebo [48].

5.6.3 Summary and discussion of repeated dose toxicity

A study equivalent to OECD 422 Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test on rat was provided for repeated dose toxicity endpoint.

Based on findings on the parental animals the registrants determined the NOAEL for systemic toxicity at 1000 mg/kg bw/d (highest dose).

More information about uses and relevant amounts used in EU confirmed that there is no consumer or professional use for concerned registrants or high tonnage of 4-HBA used as substance itself. Therefore an overall assessment led to conclusion not to address the additional concern in relation to repeated dose toxicity with combination high tonnage and use.

5.7 Mutagenicity

5.7.1 Non-human information

No adverse effect was observed with respect to genetic toxicity in all performed *in vitro* tests. Based on the available data no classification criteria have been met.

5.7.2 Human information

No data available.

5.7.3 Summary and discussion of mutagenicity

No adverse effect was observed with respect to genetic toxicity in all *in vitro* performed tests. Based on the available data no classification criteria have been met.

5.8 Carcinogenicity

Not evaluated.

5.9 Toxicity for reproduction

5.9.1 Effects on fertility

5.9.1.1 Non-human information

A study equivalent to OECD 422 (Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test) was provided for 4-HBA for the reproduction toxicity endpoint. The chronic studies on MeP were proposed as surrogate (see section 5.6 Repeated dose toxicity**)**. Information for methylparaben was extended to ethylparaben in the framework of read-across document data matrix.

The screening study has short duration, small number of animals, small number of reproductive/developmental endpoints.

The studies submitted for developmental toxicity on different species with MeP (including screening study), do not address (totally or partly) the following reproduction endpoints:

- biometry of organs (weights of uterus, ovaries, pituitary gland, prostate gland are missing)
- histopathology of organs (examination of the following organs is not included: pituitary gland, prostate gland, seminal vesicles, mammary gland, ovaries, vagina)
- sperm analysis (motility and morphology)

The results from available UT assays (see section 5.10 Endocrine properties) indicate that the substance exerts equivocal effects and any positive result could be an alert for testing. However, the positive result from the first UT assay [55] is isolated with regard to other later results linked to 4-HBA. In addition, this result was also subsequently connected with a negative result for 4-HBA and MeP in a binding assay conducted by the same authors [59].

The endocrine activity for parabens with decreasing potential with shorter alkyl chain was identified in general, however having regard to the available data it seems that 4-HBA itself is not part of this line as it has been shown in several studies where 4-HBA was evaluated as next substance together with parabens as it is the major metabolite.

Studies provided with MeP and EtP on fertility in rats did not show spermatotoxic effects [24].

As more information about uses and relevant amounts used in EU confirmed that there is no consumer or professional use for concerned registrants or high tonnage of 4-HBA used as substance itself (see section Exposure assessment), an overall assessment led to conclusion not to address the additional concern in relation to reproductive toxicity with combination high tonnage and use.

5.9.1.2 Human information

No data available.

5.9.2 Developmental toxicity

5.9.2.1 Non-human information

A screening study equivalent to OECD 422 is provided. In addition, several supporting studies on different species (mouse, rat, hamster, and rabbit) with MeP for the developmental toxicity endpoint are available together with read-across document data matrix for methylparaben extended to ethylparaben.

Data from a study on ethylparaben were used as demonstration, that although parameters in that study are satisfactory, maternal and fetal NOAELs are high (470 and 517 mg/kg bw for 4-HBA equivalents) and observed effects are not specific for EtP, but attributed to mothers' malnutrition and a decrease in maternal food consumption due to high amounts of test substance.

As more information about uses and relevant amounts used in EU confirmed that there is no consumer or professional use for concerned registrants or high tonnage of 4-HBA used as substance itself (see section Exposure assessment), an overall assessment led to conclusion not to address the additional concern in relation to developmental toxicity with combination high tonnage and use.

5.9.2.2 Human information

No data available.

5.9.3 Summary and discussion of reproductive toxicity

A study equivalent to OECD 422 (Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test) for reproduction toxicity endpoint is available with results

- NOEL for Reproductive toxicity: 1000 mg/kg bw/day

- NOEL for Developmental toxicity: 1000 mg/kg bw/day

No multi-generation reproduction toxicity study is available for 4-HBA.

A read-accros approach was supported for developmental toxicity by several studies on different species on methylparaben as well as the fertility endpoint with studies on methylparaben and ethylparaben.

Data from read-across substances has been evaluated by registrants with regard to toxicity for reproduction in a weight of evidence approach.

Although the information provided has shortcomings, due to extended read-across document data matrix and information about uses and relevant amounts used in EU (no consumer/professional use or high tonnage of 4-HBA used as such), an overall assessment led to conclusion not to address the additional concern in relation to toxicity for reproduction with combination high tonnage and use.

5.10 Endocrine disrupting properties

This evaluation addresses the potential of 4-HBA to cause endocrine effects as the substance is the main metabolite after exposure to wide dispersively used alkyl esters, parabens.

The endocrine effects for parabens, even though weak, were demonstrated in various studies for human health and environment. If 4-HBA was assessed as part of such studies under the same condition and such activity had been observed for 4-HBA then it was by orders of magnitude below or with the conclusion inactive.

Several consecutive projects of European Commission have been carried out to determine the chemical substances with endocrine potential. In the most recent of them ("Study on enhancing the Endocrine Disrupter Priority list with a focus on low production volume chemicals" 2007 by DHI [66]), within the additional assessment of low production volume chemicals, an *in vivo* uterotrophic study [55] on 4-HBA was regarded as key study since it provided *in vivo* evidence about endocrine response in intact organism.

A categorisation was set within the database: one study *in vivo* with evidence ED response means Category 1. This led to the inclusion of 4-HBA into a Priority list database which addresses substances with potential of endocrine disruption properties based on available literature data at the time of processing the project.

It was followed by subjective assignment to exposure concern. In this case the substance was assigned with Medium due to the fact, that 4-HBA as main metabolite of paraben preservatives in food and cosmetics is connected to implicit human exposure taking into account that the substance is readily biodegradable with low potential pro bioaccumulation (logKow of 1.58; DHI project value). Low concentrations in environment and insignificant contents in fish, predators and human food are expected based on project EUSES calculations (calculations for <1000 t/y as LPV substance).

However it is noted by the project authors, that inclusion of any substance into the database does not mean accomplishment of a full risk assessment but only brings together the identified candidates for evaluation of their role in endocrine disruption and after assessment with negative result it can be deleted.

1997 Lemini in vivo UT assay – key study for inclusion to Priority list

The key study referred in the database [55] was conducted on immature and adult ovariectomized female CD1 mice and after subcutaneous administrations. It led to vaginal cornification and a dose dependent increase in the uterine weight.

Although the UT assay is specific to estrogenic effects and oral administration with ADME can detect chemicals that need activation, the uterotrophic response does not occur only due to estrogenic chemicals and results should be supported by information about ER binding or transcriptional activation [27].

In contrast to the 1997 Lemini study another uterotrophic study with 4-HBA on Alpk:APf CD-1 mice by subcutaneous administration [5] led to a negative uterotrophic response of 4-HBA. Although it was carried out in 2000 this study was not available to DHI project authors as it is an unpublished report.

Two additional supporting studies for Priority list

Two additional supporting studies were also reported. The first one [57] by Hossaini et al. reports a negative result for 4-HBA by subcutaneous route on mice at 5 and 100 mg/kg/d and on rats at 5 mg/kg/d as well. And generally conclude no uterotrophic effect for individual parabens or their combination except BuP on rats 600 mg/kg/d (an increase in wet and dry uterine weight). Thus this study at the very least does not support the project key study outcome in relation to 4-HBA.

The second study [61] by Pugazhendhi et al. reports a similar relative binding of 4-HBA and MeP to the estrogen receptor (intrinsic estrogenic activity) and that 4-HBA can give estrogenic responses in human breast cancer cells. However its estrogenic activity on gene expression and cell proliferation was lower than with MeP. Proliferation of two human breast cancer cell lines (MCF7, ZR-75-1) could be increased by 10^{-5} M 4-HBA.

But one of the authors had noted in a following study, that estrogen action can influence more than proliferation (it could only enhance the effects of random replication errors) via actions on apoptosis, differentiation, cell adhesion and cell motility [62].

2003 Lemini UT and ERRγ binding study

The mentioned negative study [57] by Hossaini et al. from the DHI project as well as another study with negative response of 4-HBA [58] by Routledge et al. are referenced in the subsequent study by Lemini et al. [59], which was carried out to clarify different results from those studies and their own older study from 1997. Within re-evaluation of 4-HBA on immature CD1 mice the estrogenic effects of 4-HBA has been found again and NOELs for uterotrophic activity in immature mice were from 0.6 to 6.5 mg/kg per day. Based on values in immature Wistar rats 4-HBA was deemed inactive. Values for parabens were 1,000-100,000 times less potent than E_2.

A study section dedicated to a receptor binding analysis led to the conclusion that 4-HBA and MeP were not able to displace [^{3}H]-E_2 thus do not elicit estrogenic binding activity in contrast with other parabens. This confirmation study finally presented the conclusion that an influence of species and strain could be more significant than was assumed and the extrapolation to humans cannot be done directly.

All data above suggest a consideration of influence of species and strain of animals tested although according to OECD Conceptual Framework qualitatively comparable uterus responses in rats and mice are assumed with regard to the OECD TG 440 UT assay, however the rat is still the preferred species for testing effects relevant for humans due to metabolism similarity.

A following study by the same group of authors [60] builds on this work and the effects of parabens on the uterus are further investigated by a morphometric method. The influence of 4-HBA on uterus in the UT assay from 1997 was mentioned again, but probably with respect to the receptor binding analysis negative results from previous re-evaluating study [59], 4-HBA was not part of results.

The uterotrophic UT assay is set up as sensitive and for weak phytoestrogens may lead to exaggerated results. Despite the inclusion of 4-HBA into the Priority list it should be noted, that overall consideration of effects of 4-HBA as such suggests only negligible links to endocrine activity based on different assays and studies.

Other data sources

Recent review from 2013 [24] sums up the information in relation to parabens. The need for additional reproduction studies on both long- and short-chain parabens was identified among other issues because of the risk of estrogenic burden of free parabens and 4-HBA in blood, which may exceed estradiol levels for a sensitive group of population – children. But in conclusion is also mentioned that estimates on estrogenic potencies and blood concentrations of parabens and 4-HBA are rather uncertain.

Following SCCS (The Scientific Committee on Consumer Safety) report did not rule out this concern of exposure by the dermal route through damaged skin in nappy area. A margin of safety and a proposal for decreasing allowed concentration of butylparaben (BuP) in cosmetics was presented.

The SCCS report considers parabens conjugates (glycine-, sulfate- and glucuronide) as non estrogenic as the steroid conjugates cause no effect at the receptor, but the concern has been linked to the presence of intact parabens after dermal exposure to cosmetics.

However, oral route of exposure to parabens is the main area of interest as it consequently means exposure to 4-HBA. The large differences in estrogenic activity (*in vitro, in vivo*) and toxicity of individual parabens suggest that if the common metabolite 4-HBA would also be able to exert endocrine activity such differences could not be plausible. [25]

In addition, 4-HBA did not elicit endocrine activity in environmental tests (see section 7).

Therefore the available data does not support the endocrine activity of 4-HBA thus leading to conclusion of very low concern.

Conclusion

The estrogenic activity of 4-HBA is deemed to be insignificant, thus leading to the conclusion of low concern considering these circumstances

- even though there are equivocal results in UT assays, the positive result from first UT assay is isolated with regard to later results,
- negative in binding assay(s) on ERRγ unlike parabens in the same assay,
- very low activity in study with MCF-7 cells comparing to MeP
- no activity comparing to parabens in toxicity study by yolk protein induction addressing hazard towards environment.

All available data suggest that 4-HBA as such is inactive in connection with the endocrine activity and there is no concern for endocrine disruption.

5.11 Other effects

No data available.

5.12 Combined effects

No data available.

5.13 Derivation of DNEL(s) / DMEL(s)

There is agreement with the DNELs provided by the registrants for relevant exposure routes.

5.13.1 Quantitative descriptor for critical health effects

The NOAEL value of 40 mg/kg bw/day obtained from the screening study OECD 422 was used as the dose descriptors for the exposure assessment via oral route and the NOEC of 60 mg/m^3 via inhalation route.

6 HUMAN HEALTH HAZARD ASSESSMENT OF PHYSICO CHEMICAL PROPERTIES

Not relevant for this evaluation.

7 ENVIRONMENTAL HAZARD ASSESSMENT

Data for short-term and long-term aquatic toxicity on 4-HBA was provided in the dossier with results obtained with pH-adjustments to neutral values. Those values were used as key values as the pH-values in tests without pH neutralisation were outside the acceptable range in the guidelines. The value of 100 mg/L was determined as a NOEC as a worst case for CSA.

Other compartments are of minor importance for this evaluation as the substance is readily biodegradable with very low potential for adsorption and bioaccumulation and naturally occurs in the environment. In case of industrial release to water practically all amount of substance is detained in water. An eventual release to other compartments is subsequently distributed as ca one fourth to water and rest to soil. However, all such releases from production site are prevented by technical measures.

There is a published study related to estrogenic action, which directly examined an action of 4-HBA in water compartment. Within an in vivo fish assay the EtP, ProP and BuP and 4-HBA were tested for the estrogenic activity in vivo (injection of the compounds; yolk protein induction; sexually immature rainbow trout; doses between 100 and 300 mg/kg) with the conclusion that tested parabens were estrogenic (ProP, BuP comparable to bisphenol A, EtP approximately sixty times weaker) but 4-HBA did not exert such activity [63] although for the proximate ester MeP an estrogenic activity including bioaccumulation in a dose dependent response was approved as well [64]. So there is indication that 4-HBA as such does not elicit estrogenic response in wildlife animals.

8 PBT AND vPvB ASSESSMENT

Not relevant for this evaluation.

9 EXPOSURE ASSESSMENT

It is stated in “Justification for the selection of a candidate CoRAP substance” that potential uses as preservative in cosmetics were identified from publicly available information and Danish SPIN DB also indicates narrow uses.

However, the information in the SPIN database revealed that there are very few applications (1-3). Moreover a direct use of 4-HBA in cosmetics is not problematic from the view of systemic availability as dermal absorption 4-HBA itself is insignificant based on results from toxicokinetic studies and endocrine activity has not been confirmed.

Thus the concern of use of 4-HBA itself in consumer cosmetic products has a negligible significance. As preservatives in food or pharmaceuticals are predominantly combinations of short-alkyl parabens, this use of 4-HBA by itself has insignificant importance as well.

Some considerations about exposure amounts of parabens are presented within the section “5 Human health hazard assessment, Conclusions related to the concern, Introduction”, as 4-HBA was connected with consumer use concern in the CoRAP Justification document as well as in the Priority list (database of substances with ED potential).

The substance exhibits low acute toxicological activity. Human exposure by oral route can possibly take place as 4-HBA occurs as a metabolite from parabens, which are used as preservatives in foods and pharmaceuticals, next to natural food resources of 4-HBA. The contents in these resources had

been examined with study authors statement, that there is unknown contribution of other classes of phenols to 4-HBA burden. As these known levels are lower than levels used in studies, the biological effects associated with dietary sources are considered to be weak. However, current studies show increasing of long-term daily intake of parabens from various sources at several milligrams (in some examined groups with dozens of milligrams). In any case, this intake is not intake from natural sources, even though 4-HBA or even parabens themselves are also part of the flora and fauna.

Although the concern about the estrogenic activity of 4-HBA has not been confirmed, regular uptake, which possibly can become excessive, may cause adverse effects like those described for benzoic acid, when its conversion to hippuric acid is a saturable process relating to availability of glycine and sufficiently high doses may have consequences for metabolism even with rapid excretion of the acid itself (benzoic acid ADI of up to 5mg/kg bw).

Therefore there was need to take into account the possibility of long term and reproductive toxicity effects of 4-HBA due to the nature of concern and as metabolite due to wide dispersive use of a mixture of parabens in a variety of products.

It should be noted, that the identified use presented in the registration dossiers is only intermediate (transported, isolated) for industry sectors.

Information about uses and relevant amounts used in EU confirm that there is no consumer or professional use. It became clear that uses, which might lead to additional concerns on the basis of tonnage and exposure to general population, are not relevant for the evaluated substance. The information from concerned registrants of the evaluated substance indicates that the produced and used amount of 4-HBA, which might be used in the above mentioned context, is of minor importance.

Registrants claimed: “There is no direct nor indirect exposure (via the environment) to consumers and humans from the manufactured 4-HBA through its uses at the manufacturing site and any downstream uses as intermediate for production of polymers and chemicals.”

The exposure scenarios for manufacture and intermediate use result in no significant exposure to workers at the manufacturing site and a few industrial use sites (RCRs well below 1).

Almost all production is used in EU as the polymer. The concern for workers is low as the amount of 4-HBA contained as monomer is below the detection limit of 50 ppb, in connection with the fact that concern about endocrine disruption has not been confirmed.

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ABBREVIATIONS

2-HBA	2-hydroxybenzoic acid, salicylic acid
4-HBA	4-hydroxybenzoic acid
4-HHA	4-hydroxyhippuric acid
BuP	butylparaben
BenzP	benzylparaben
CLP	Classification, labelling and packaging
CoRAP	Community Rolling Action Plan
DNEL	Derived No Effect Level
E_2	estradiol
EA	Exposure Assessment
ECHA	European Chemicals Agency
EtP	ethylparaben, ethyl 4-hydroxybenzoate
ES	Exposure Scenario
HA	hippuric acid
HSDB	Hazardous Substances Data Bank
IUCLID	International Uniform Chemical Information Database
LOQ	Limit of quantification
LPVC	Low production volume chemicals
MeP	methylparaben, methyl 4-hydroxybenzoate
NOAEL	No Observable Adverse Effect Level
OECD	Organisation for Economic Co-operation and Development
ProP	propylparaben, propyl 4-hydroxybenzoate
RAAF	The Read-Across Assessment Framework
RCR	Risk Characterisation
RDT	Repeated Dose Toxicity
RMM	Risk Management Measure
RMO	Risk Management Option
SEV	Substance Evaluation
SIDS	Screening Information Data Set