Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

Bisphenol A; 4,4'-isopropylidenediphenol

EC number: 201-245-8
CAS number: 80-05-7

CLH-O-0000004110-93-03/F

Adopted

14 March 2014

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON BISPHENOL A;4,4'-ISOPROPYLIDENEDIPHENOL

Date	Country	Organisation	Type of Organisation	Comment number
11.10.2013	Netherlands		Member State	1
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26.09.2013	United Kingdom		Individual	3
11.10.2013	United States	North American Metal Packaging Alliance, Inc.	Industry or trade association	4
10.10.2013	Germany	Bayer Material Science AG	Company-Manufacturer	5
10.10.2013	Belgium	ReachCentrum BPA Consortium	Industry or trade association	6
10.10.2013	United Kingdom	Public Health England	National Authority	7
10.10.2013	Sweden	ChemSec	International NGO	8
09.10.2013	United Kingdom	Exponent	International NGO	9
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09.10.2013	Germany		Member State	26
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04.10.2013	Norway		Member State	28
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COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

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

ECHA accepts no responsibility or liability for the content of this table.

Substance name: bisphenol A;4,4'-isopropylidenediphenol
EC number: 201-245-8
CAS number: 80-05-7

Dossier submitter: France

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
11.10.2013	Netherlands		MemberState	1
Comment received				
[all comments are also submitted in a seperate document, for easier reading] • For many of the studies summarized in the proposal, it is not clear whether the studies are well performed and/or reported (how many animals were used, which analyses were performed, if GLP or guidelines were followed etc) and therefore how reliable these studies are. In addition, in most cases the quantity of effects and historical control range is not included, making it difficult to conclude whether observed effects are toxicologically relevant. • Some of the studies included in the CLH proposal have used very high dose levels (sometimes at 6% in diet corresponding with 68 mg/mouse according to Berger et al, 2007, but 180-240 mg/mouse according to our calculation corresponding with approximately 9000 mg/kg bw). It should be discussed whether effects observed only at these dose levels would warrant classification as described in paragraph 3.7.2.5.7 of Annex I of CLP. • Many studies were performed using subcutaneous injection or implanted time release pellets (bypassing the first pass effect). The relevance of these studies compared to humans and/or animal studies using relevant routes of exposure should be discussed based on the available toxicokinetic data in line with paragraph 3.7.2.5.6 of CLP. This should determine whether these studies can be used to support the proposed classification. The current chapter on toxicokinetic does not address this important question and in most studies using this exposure route, it is not or only very limited justified. Therefore, the relevance of these routes should be justified before using them for classification. The relevance could be dose				

dependent as sometimes a high dose is used subcutaneously which could result in very high exposure levels which may not be relevant as discussed under the previous bullet. The provided summaries focus mainly on the observed effects on development or fertility. However, information on maternal / other toxicity is often lacking. It is considered important to assess whether the observed reproductive effects are a direct effect of BPA or a secondary non-specific consequence of other toxicity. Further, it is unclear whether additional parameters have been determined in the studies which were not affected. This information is important for assessing the consistency between studies of effects observed in other studies.

• For all effects, it can be discussed whether these are effects on fertility or effects on development. Basically, effects on fertility (or the reproductive system) that are caused by developmental changes of the fertility system are primarily caused by an altered development and should therefore be seen as developmental effects. When fertility effects (or changes in the reproductive system) are observed in animals that are only exposed in utero, it can be concluded that these effects have arisen during development. If they are considered relevant for classification, they are relevant for classification for developmental effects, not fertility effects. Effects induced by post-natal exposure would not justify classification for development as described in Chapter 3.7.1.4 of the criteria. However, they could be considered as reprotoxic effects in general without differentiation as allowed in 3.7.1.1. It is suggested to first discuss which window of exposure would contribute to which differentiation of the classification for reproductive toxicity. Then the available studies could be split into those windows of exposure as a start before discussing the classification.

(ECHA note: The following attachment was provided - same content as in the comment above:
"Comments on the proposal for harmonized classification and labeling of Bisphenol A." [Attachment 9])

Dossier Submitter's Response

FR thanks NL for their comments and adds below few clarifications:

Concerning the reliability of the studies, in the summary tables presented in the CLH report, the number of animals and if the studies were GLP or OECD guidelines compliant is indicated only for the section on effects in females. It is clearly an oversight. Nevertheless this information is available in the IUCLID dossier and could therefore be consulted if needed. Moreover, FR indicates that the studies to be included on the report were chosen according to a protocol defined and validated by a working group of FR experts. Please see the response to COM no. 4 for the detailed methodology of reliability study assessment.

Concerning the doses used in the Berger *et al*. study (2007), the doses reported in the CLH report are those indicated in the publication. However, we agree with NL that while converting in mg/ kg bw/d, the dose used is very high. For the sake of comprehension, please find below the doses used in the Berger *et al.* studies:
2007: around 9000mg/kg bw/d
2007 subcutaneous and 2008: 0, 0.015, 0.045, 0.138, 0.429, 1.248, 3.75, 11.25, 33.75, 101.25, 303.75 mg/kg bw/d.
We recognise that the reporting of these studies is poor. However, we felt important to report these studies for sake of partiality. Indeed, these studies report negative results at doses (below 50 mg/kg bw/d) for which most of the other subcutaneous studies in mice are

positive for females. Indeed the 2 subcutaneous studies performed by Berger only show effects at doses> 100mg/kg bw/d:
-in number of pups born at 101 ($p < 0.05$) and 303 mg/kg bw/d ($p < 0.01$)
-in the percent of females that gave birth at 303 mg/kg bw/d ($p < 0.001$)
-in the number of implantation sites at 303 mg/kg bw/d

About the use of the studies quoted in the CLH report performed using a sub-cutaneous route of exposure: in a study by Taylor *et al*. (2008), a comparison was made in neonatal mice between the oral and the sub-cutaneous routes. It was concluded by the authors that there was no significant difference in plasma BPA following these 2 different routes of exposure for the neo-natal period. However, the CLH report details sorely the differences of BPA toxicokinetics of BPA, that seems to vary with the window of exposure, route of exposure and species. The data provided in the CLH report show that both routes of exposure may be acceptable, depending when they are used.
Moreover, if the oral route could be considered as the most relevant considering a dietary exposure only, the sub-cutaneous route could partially help to address the cutaneous exposure that occurs with BPA (via thermal paper used for receipts for instance).Finally, as NL mentioned, sub-cutaneous route bypass the first pass effect. It also allows a dosing at lower doses. Subcutaneous route can highlight effects at doses much lower than those administered orally. Therefore FR does not believe that studies performed subcutaneously should be disregarded.

In most cases the studies used in the report focused on reproduction only (except for the multi-generation studies). FR agrees that it has to be mentioned if others effects occurred; this was done in the report.

About the window of exposure, it is sometimes difficult to discriminate between the effects on fertility and a consequence of developmental effects. NL mentioned it in the COM 15 (see below) "the reduced number of litters/pups in the 3-generation study in rats without an effect on resorptions (also in F0) and the reduced number of litters/pups in the continuous breeding study in mice suggests an adverse effect on fertility. This effect is observed in two species and in multiple studies (i.e. the secondary effect of reduced litters/pups). However, summaries of the available developmental studies could further substantiate whether this is an effect on fertility or development. No reductions in offspring were observed in the developmental studies by Stump (2010) and Ryan (2010). This further justifies that the effects in the multigenerational studies are due to an effect on fertility instead of post-implantation loss." This is a possibility, another explanation, as noted in the CLH report could be that observed effects of BPA could rather be due to pre-implantation loss effects.
FR chose to address the fertility because our first analysis of the data prior the proposal writing and the conclusions of the ANSES report on hazards of BPA (ANSES, 2011 http://www.anses.fr/Documents/CHIM-Ra-BisphenolAEN.pdf) concluded to an impairment of fertility. Moreover due to limited resources available for the classification, FR was not able to address both fertility and development but would be very pleased if another MS could undertake this part of the classification. Several studies are available and a study conducted in DK showing effects on the development following an exposure during gestation and lactation will be published soon.

RAC's response
RAC consider that the studies using sub-cutaneous administration could be used as supporting studies in the weight of evidence analysis for the effects on sexual function and fertility following exposure to BPA. As regards the difficulties to discriminate between effects on fertility and effects on developmental, the CLP regulation in the description of effects includes no requirements regarding the time of exposure to the substance: *CLP 3.7.1.3 Adverse effects on sexual function and fertility: Any effect of substances that has the potential to interfere with sexual function and fertility. This includes, but is not limited to, alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems.*

Date	Country	Organisation	Type of Organisation	Comment number
04.10.2013	United States	Can Manufacturers Institute	Industry or trade association	2

Comment received
The Can Manufacturers Institute (CMI) is the US national trade association of the metal can manufacturing industry and its suppliers in the United States. The can industry accounts for the annual domestic production of approximately 124 billion food, beverage and other metal cans; which employs more than 28,000 people. A BPA warning label would convey a threat to human health that is unsupported by sound scientific evidence and is not found by conclusions drawn by FDA and most international public health regulatory bodies. Such an erroneous warning on packaged food could scare consumers away from these vital and affordable sources of nutrition. Our members are committed to providing safe, nutritious and refreshing canned food and beverages to consumers. CMI is opposed to the proposal to reclassify bisphenol A (BPA) as a reproductive toxicant Category 1B under Regulation (EC) No. 1272/2008. According to the Classification, Labeling and Packaging (CLP) regulations, substances to be classified as reproductive toxicant, Category 1B, must have clear evidence of an adverse effect in the absence of other toxic effects OR if other effects are seen, the reproductive toxicity effect must be the origin of the other toxic effects and not be a secondary, non-specific consequence. That is not the case with BPA. In studies where reproductive toxicity effects were observed, those effects occurred at dose levels above those where systemic toxicity effects. Thus, the criteria are not met and the reclassification proposal should not be accepted.
Dossier Submitter's Response
FR thanks CMI for their comments and invites them to refer to responses to comments of the ReachCentrum BPA Consortium (COM no. 21). We do not consider that reproductive effects seen following an exposure to BPA are secondary and a non-specific consequence of general toxicity. We rather think that BPA has several target organs, with lesions that are comparable (cysts) pointing to direct BPA effects of BPA on these organs.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment

				number
26.09.2013	United Kingdom		Individual	3
Comment received				
This substance must be better regulated and population better protected.				
Dossier Submitter's Response				
FR thanks you for this comment.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
11.10.2013	United States	North American Metal Packaging Alliance, Inc.	Industry or trade association	4
Comment received				

North American Metal Packaging Alliance, Inc.
Comments on French Government Dossier
Proposing Harmonised Classification and Labelling of BPA

October 11, 2013

The North American Metal Packaging Alliance, Inc. (NAMPA) submits these comments to the European Chemicals Agency (ECHA) in response to the July 2013 proposal from the French government for harmonized classification and labeling based on Regulation (EC) No. 1272/2008 for bisphenol A (BPA). NAMPA is a not-for-profit U.S. corporation committed to protecting health through the safety of metal packaging and metal packaged foods. NAMPA's membership includes companies and associations representing various sectors along the supply chain for the food and beverage packaging industry. Our members actively engage in worldwide trade and, as such, have a vested interest in the outcome of regulatory requirements within Europe. As discussed below, the French government proposal to reclassify BPA should not be adopted because it is not scientifically justified. NAMPA fully supports and incorporates by reference the comments submitted by the Bisphenol A REACH Consortium.

Background

BPA is currently classified as Category 2, defined as "substances which should be regarded as if they impair fertility in humans" under the Classification, Labeling and Packaging (CLP) regulation nomenclature. The French government has proposed that BPA be reclassified as Category 1B, which is defined as "presumed reproductive toxicant." According to the CLP regulations, a substance that is classified as Category 1B should have

☐ clear evidence of an adverse effect on sexual function and fertility or on development from exposure to the substance in the absence of other toxic effects or, alternatively,

☐ if toxic effects and the adverse effect on reproduction are both observed, the latter must not be a secondary, non-specific consequence of other toxic effects, but the origin of the effect.

BPA Data Do Not Support a "1B" Classification

The extensive available data on BPA do not support a "1B" classification. The generally recognized No Observed Adverse Effect Level (NOAEL) for general system toxicity for BPA is 5 mg/kg/day. The doses at which reproductive toxicity effects were observed were far higher than 5 mg/kg/day. A comprehensive review of applicable studies is included with the Bisphenol A REACH Consortium comments. As shown in that review, in those studies where animal fertility effects were observed, they occurred only at high dose levels at which other significant systemic toxicity effects were also observed. This clearly does not meet the Category 1B criteria and, as such, the proposal from the French government must be rejected.

The French Government Proposal Did Not Follow ECHA Procedures

As more fully articulated in the Bisphenol A REACH Consortium comments, the classification proposal from the French government did not follow specific procedures outlined in ECHA guidance. The proposal did not follow a weight of evidence approach, which is the specified approach for chemicals with large databases such as that supporting BPA. The BPA database is very large, and, as such, it is imperative that evaluations of available information be comprehensive and consider all pertinent data. It appears that the French government proposal included certain studies in its supporting documentation, deselected others for no apparent reason, and failed to integrate information on all studies, including numerous studies that showed no adverse effects. Likewise, several studies that the French government proposal cited as supportive to the reclassification were previously reviewed by the National Toxicology Program (NTP) and the European Food Safety Authority (EFSA) and found to be scientifically inadequate. This bias in a purported scientific evaluation is very concerning and raises credibility questions with the goal of the French government proposal.

Finally, NAMPA notes that the proposal does not include more recent study data (post 2012), including an important research project from the U.S. Food and Drug Administration (FDA) National Center for Toxicological Research (NCTR). NAMPA urges that this study (Delclos 2013; "Relating Internal BPA Doses to Adverse Effects in Rodent Toxicity Studies"), which was specifically designed to address specific data gaps and lingering scientific questions on BPA, be carefully considered before any decision on classification is made.

For these reasons, the French government proposal to reclassify BPA should not be adopted because it is not scientifically justified, reflects an inappropriate bias, and does not consider all relevant and available scientific studies.

Dossier Submitter's Response

FR thanks North American Metal Packaging Alliance Inc. for their comments and invites them to please refer, for many points raised, to responses to the Reach Centrum BPA Consortium comments (COM no. 21).

Concerning the comment on the reliability of the studies included in the report, they were chosen according to a precise protocol defined and validated by a working group of FR experts and was the starting point for including studies in the CLH report. The methodology on the choice of the most reliable studies is explained as followed:
The CLH report is based on prior work undertaken by expert assessment authorities, and particularly the European Risk Assessment Report prepared in 2008 by the United Kingdom, the preliminary INSERM collective expert assessment report on BPA published in July 2010, and the expert appraisal work undertaken by AFSSA in 2010. The EFSA expert assessment report published in September 2010 and the report by the expert panel which met under the leadership of the FAO/WHO which was published in November 2010 were also taken into

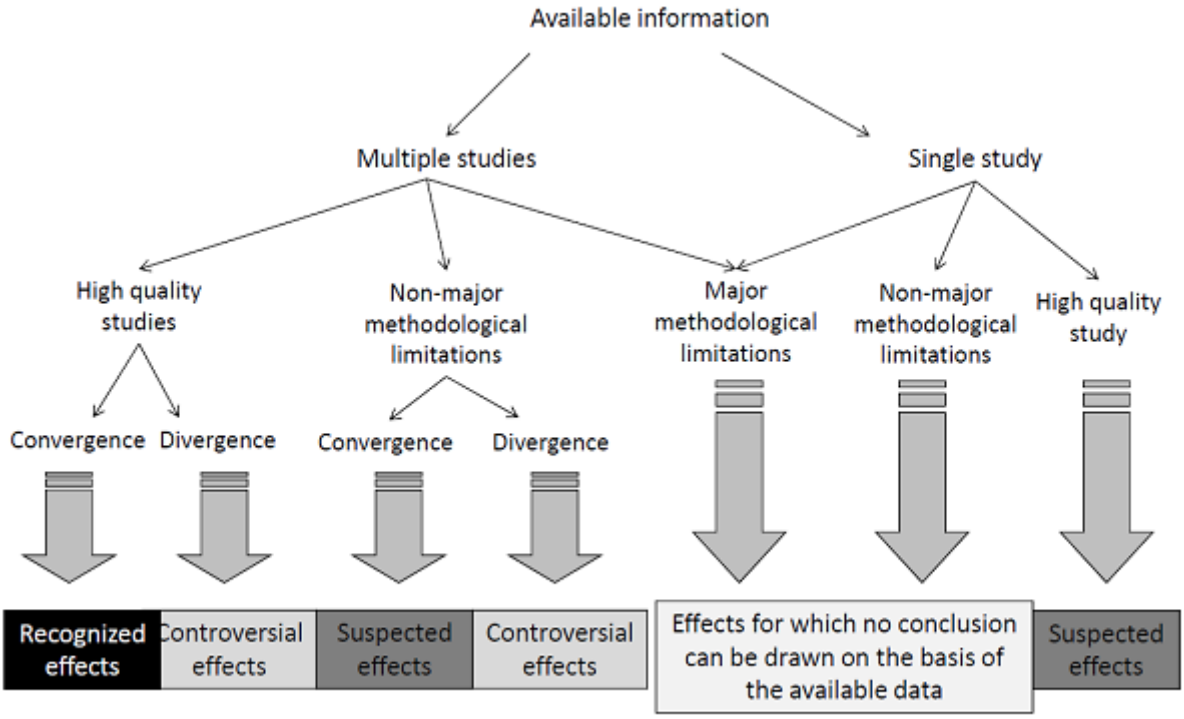
account.
In addition to these expert assessment reports on BPA which have recently been published, original papers of studies considered as key studies for certain types of effects linked to a BPA exposure were also analyzed. Furthermore, particular attention was paid to epidemiological studies likely to contain information that could be interpreted in terms of human effects and experimental studies using the subcutaneous route of exposure. In fact, the latter type of study has not undergone systematic analysis in past expert assessments, generally effects observed following oral route are considered as relevant, since they have been deemed more representative of dietary exposure. Nevertheless, given the fact that questions have recently been raised regarding non-dietary BPA exposure, including dermal exposure and that subcutaneous route can highlight effects at doses much lower than those administered orally, it was indeed considered as relevant to take these studies into account. So, epidemiological studies and studies using the subcutaneous route of exposure were included in the analysis.

New articles published from 2010 (when the preliminary INSERM report was published) to January 2011 (bibliography end date) were listed by ANSES and analyzed. In addition, some articles published after January 2011 were also included when they were relevant. Studies assessing the effects of BPA at doses lower than the NOAEL of 5 mg/kg bw/day, which was used to establish the current TDI, were assessed as a priority. It should be noted that considering the state of public resources, it is impossible to consider ALL the available studies on BPA. Nevertheless, no *a priori* was used to select studies. The only criterion was studies published after TC C&L previous discussions (2002) and considered as relevant new data. All the guideline studies published afterward and before TC C&L discussion were incorporated as milestones of the discussions on classification of BPA.

For each type of effect, the available data were presented by windows of exposure: gestational or *in utero*, prenatal, perinatal, neonatal, postnatal exposure or exposure during puberty or adulthood. The term 'exposure' does not provide information on the number of administrations (e.g. single or repeated).
For those articles considered as significant to provide information about the health effects of BPA, a publication analysis chart was used. The items on this chart list the important points to be specified when analysing articles, considering the limiting factors likely to interfere with the interpretation of results.
For each type of effect and on the basis of the expert *rapporteurs*' conclusions, the nature of the observed effects was determined and effects were characterised as:

- Recognised effects
- Suspected effects
- Controversial effects
- Effects for which no conclusion can be drawn on the basis of the available data.

In order to qualify the health effects of BPA, the following decision tree was used:



All the available information regarding a health effect was assessed using this decision tree, which can be interpreted as follows:

- When the available information was obtained from one or more studies, each study was analysed and considered either to be of 'good-quality', having 'non-major methodological limitations' or having 'major methodological limitations'.
  A 'good-quality' study was defined as containing an appropriate methodology (coherence of the exposure model, confounding factors taken into account, etc.) and a sufficient number of observations.
  A study was considered to have 'non-major methodological limitations' when one of the above aspects was not considered to be fully satisfactory. Nevertheless, the study could be taken into account in light of its contribution to the expert appraisal. Moreover, co-exposure had to be controlled (composition of feed for laboratory animals, type of cage, type of drinking container, etc.) or the way in which it was managed at least had to be mentioned. When a study had unacceptable shortcomings, it was considered as having 'major methodological limitations'.

- When the results of multiple 'good-quality' studies undertaken by different scientific teams:
  - converged: the effect was considered to be 'recognised',
  - diverged: the effect was considered to be 'controversial'.
- When studies having 'non-major methodological limitations':
  - converged: the effect was considered to be 'suspected',

- diverged: the effect was considered to be 'controversial'.

- Studies having 'major methodological limitations' were excluded as they could not be used to draw conclusions.

- Lastly, when information was reported in only one study, the methodology was assessed:

  - when it was 'good-quality', the effect was considered to be 'suspected',

  - when it had 'major or non-major methodological limitations', the study was considered to be excluded and could not be used to draw conclusions regarding the effect under consideration.

Lastly, once the various types of effects had been characterized according to their level of evidence, the significance of the observed biological effects was thus discussed in order to estimate their relevance for humans.

Then most of the studies included in the report were considered as good quality reliable studies. Others were included when considered as relevant for the WoE approach.

RAC's response

RAC considers that the CLH report would have benefitted from having included relevant information regarding the quality/reliability of the various studies in the CLH report since this exercise has been performed by the DS.

Date	Country	Organisation	Type of Organisation	Comment number
10.10.2013	Germany	Bayer MaterialScience AG	Company-Manufacturer	5

Comment received

Executive summary of
BPA Consortium comments to the CLH Proposal

These comments and attachments are the comments of Bayer MaterialSciene AG and the Bisphenol A REACH Consortium (BPA Consortium), which represents more than 30 of the main producers, importers and users of BPA in Europe. After careful review of the proposal in the CLH dossier, we have a number of concerns.
• The case has not been made that BPA merits classification as Category 1B (presumed reproductive toxicant) under the CLP Regulation. In fact, a review of the relevant studies shows that effects on animal fertility only occur at high doses of BPA and that, rather than being selective reproductive effects, they are merely related to systemic toxicity.
• The CLH proposal is not consistent with the procedure outlined in ECHA's "Guidance on the preparation of dossiers for harmonised classification and labeling" (ECHA 2010) [1] which directs the use of a weight-of-evidence approach for compounds with a large database, such as BPA. The CLH proposal
o does not consider "all available information;"
o does not follow the CLP Regulation standard regarding the request that "Both positive and negative results shall be assembled together in a single weight of evidence determination;" and
o fails to follow the CLP Regulation in that "The quality of the data shall be given

appropriate weight."
• The CLH proposal selectively relies only on studies, assessments, and the 1 out of 1.409 self-classifications that supports its proposal and, therefore, portrays an inaccurate and incomplete picture of the state of the science on BPA.
o Information is not comprehensive and inconsistent throughout the report.
o Statements related to the value of regulatory guideline studies compared to the value of exploratory studies are biased.
o Statements on multigeneration animal studies upon which regulators have relied (Tyl et al 2002 and 2008a) are inconsistent, incorrect and incomprehensible.
o Reference of one industry self-classification out of 1.409 is clear evidence of "cherry picking" information and ignoring contrary information.
• Recent (post December 31, 2012) and important scientific research from government agencies was not considered. These government studies do not support a classification of BPA as a Category 1B Reproductive Toxicant.

Given the above, the dossier should be rejected as not supporting a classification of BPA as Category 1B reproductive toxic. The CLH proposal does not fulfil the criteria outlined in ECHA's "Guidance on the preparation of dossiers for harmonised classification and labeling"

(ECHA 2010) because it fails to consider the quality of the data and it fails to consider all of the data in a weight of evidence analysis.
As can be seen from the BPA Consortium comments and from assessments of BPA conducted by other government regulators, when all high quality scientific studies on BPA have been considered and a weight of the scientific evidence evaluation is conducted, it will clearly demonstrate that BPA is not a selective reproductive toxicant. In conclusion, there is no basis to change the classification of BPA to Category 1B.

Dossier Submitter's Response

FR thanks Bayer Material Science AG for their comments and invites them to refer to the responses to North American Metal Packaging Alliance, Inc. (COM no. 4) and to the ReachCentrum BPA Consortium comments (COM no. 21).
We take the opportunity of the comment on Tyl studies to mention that there is a typing mistake in the CLH report on table 16, page 102/103. The table should be read as follows:

Mice Oral route 0 – 0.018 – 0.18 – 1.8 – 30 – 300 and 3500 ppm ((0, 0.003, 0.03, 0.3, 5, 50, or 600 mg BPA/kg/day) Exposure from 10 weeks before mating until adult age. N = 28 animals/dose	At 3500 ppm, ↘ body weight, increased kidney and liver weights, centrilobular hepatocyte hypertrophy, and renal nephropathy in males. At 3500 ppm, BPA also ↘ F1/F2 testes weights (with seminiferous tubule hypoplasia),slightly delayed preputial separation (PPS), and apparently increased the incidence of treatment-related, undescended testes only in weanlings ;Follow OECD guideline 416 (two generation reproduction toxicity study), TG 416 enhanced	Tyl *et al.*, 2008

	GLP compliant study	
Rat Oral route 0 – 0.015 – 0.3 – 4.5 – 75 – 750 – 7500 ppm corresponding to 0.0007-0.003, 0.015-0.062, 0.22-0.73, 4.1-15.4, 37.6-167.2 and 434-1823 mg/kg bw/day Exposure from 10 weeks before mating until adult age. 30 males/dose 30 females/dose	No effect on reproduction has been seen except at the highest dose: At 7500 ppm, preputial separation (PPS) were delayed in F1, F2, and F3 offspring,associated with reduced body weights. According to EPA OPPTS 837.38000, 1998 GLP compliant study	Tyl *et al.*, 2002
RAC's response		
Noted.		

Date	Country	Organisation	Type of Organisation	Comment number
10.10.2013	Belgium	ReachCentrum BPA Consortium	Industry or trade association	6
Comment received				

Executive summary of
BPA Consortium comments to the CLH Proposal

These comments and attachments are the comments of the Bisphenol A REACH Consortium (BPA Consortium), which represents more than 30 of the main producers, importers and users of BPA in Europe. After careful review of the proposal in the CLH dossier, we have a number of concerns.

• The case has not been made that BPA merits classification as Category 1B (presumed reproductive toxicant) under the CLP Regulation. In fact, a review of the relevant studies shows that effects on animal fertility only occur at high doses of BPA and that, rather than being selective reproductive effects, they are merely related to systemic toxicity.

• The CLH proposal is not consistent with the procedure outlined in ECHA's "Guidance on the preparation of dossiers for harmonised classification and labeling" (ECHA 2010) [1] which directs the use of a weight-of-evidence approach for compounds with a large database, such as BPA. The CLH proposal

o does not consider "all available information;"

o does not follow the CLP Regulation standard regarding the request that "Both positive and negative results shall be assembled together in a single weight of evidence determination;" and

o fails to follow the CLP Regulation in that "The quality of the data shall be given appropriate weight."

• The CLH proposal selectively relies only on studies, assessments, and the 1 out of 1.409 self-classifications that supports its proposal and, therefore, portrays an inaccurate and incomplete picture of the state of the science on BPA.

o Information is not comprehensive and inconsistent throughout the report.

o Statements related to the value of regulatory guideline studies compared to the value of exploratory studies are biased.

o Statements on multigeneration animal studies upon which regulators have relied (Tyl et al 2002 and 2008a) are inconsistent, incorrect and incomprehensible.

o Reference of one industry self-classification out of 1.409 is clear evidence of "cherry picking" information and ignoring contrary information.
• Recent (post December 31, 2012) and important scientific research from government agencies was not considered. These government studies do not support a classification of BPA as a Category 1B Reproductive Toxicant.

Given the above, the dossier should be rejected as not supporting a classification of BPA as Category 1B reproductive toxic. The CLH proposal does not fulfil the criteria outlined in ECHA's "Guidance on the preparation of dossiers for harmonised classification and labeling"

(ECHA 2010) because it fails to consider the quality of the data and it fails to consider all of the data in a weight of evidence analysis.
As can be seen from the BPA Consortium comments and from assessments of BPA conducted by other government regulators, when all high quality scientific studies on BPA have been considered and a weight of the scientific evidence evaluation is conducted, it will clearly demonstrate that BPA is not a selective reproductive toxicant. In conclusion, there is no basis to change the classification of BPA to Category 1B.

Dossier Submitter's Response

Same COM and same authors than comments no. 21, except that attachments were not mentioned here. Please refer to these RCOM (no. 21).

RAC's response

Noted.

Date	Country	Organisation	Type of Organisation	Comment number
10.10.2013	United Kingdom	Public Health England	National Authority	7

Comment received

This is a summary of the data collected on bisphenol A which attempts to explain the large inconsistencies in the outcomes of many of the studies undertaken on the potential toxicity of bisphenol A. Despite these inconsistencies, it is proposed that there is sufficient weight of evidence to classify as Repr. 1B-H360F or even Repr. 1A; H360F. The latter is inappropriate as there is no evidence for a direct effect of BPA in human reproductive toxicity. Data presented from human investigations shows correlation but not causation of effects. BPA is found in food contact material and there is no clear evidence that the human studies are not merely a representation of a poor diet having an effect upon fertility. Likewise there are a number of other inadequacies in the human data that make classification as Repr.1B suspect and Repr. 2 more appropriate (some evidence in humans but some deficiencies in the studies).
It should be noted that the document requires some editorial review.

Dossier Submitter's Response

FR thanks UK for their comments and notes the fact that the document requires editorial review, nevertheless the CLH procedure does not plan any update of the CHL report by the Dossier Submitter at that stage.

It should be noted that FR does not fully agree with the statement that there is no evidence that BPA is a reproductive toxicant for humans.
It is possible that the existing literature is not robust enough and requires further

investigation. Nevertheless showing causation of effect can be expected for pharmaceuticals only, but, with the techniques available so far, it seems hardly possible to raise causation between exposure to a chemical substance and an effect. Describing correlation is the best that can be provided by epidemiology so far taking into account all the existing bias.
A review of the most recent epidemiological data performed on BPA has recently been published (Rochester JR (2013), Bisphenol A and human health: A review of the literature. Reproductive Toxicology 42:132-155.). This review analyzes a lot of epidemiological studies and highlights effects of BPA on fertility (8 studies – the report indicated that there is some evidence that BPA contribute to infertility in humans), male sexual function (2 studies – reports that there is a strong link between BPA exposure and male sexual function which would be strengthened by replication of findings on workers in another cohort), sperm quality (3 studies – review concluded that there are high quality studies showing consistent results in different populations indicating clear relation of adult BPA exposure to (decreased) sperm quality in men), sex hormones concentrations (10 studies - the studies relating sex hormone concentration and BPA exposure are strong and fairly consistent effects across many types of populations and age groups confirming that BPA has antirational effects on circulation level of sex hormones), Polycystic Ovary Syndrome - PCOS (4 studies), endometrial disorders (3 studies) or pregnancy (3 studies). There is some evidence of a relationship between recurrent miscarriage and BPA exposure in women, which may be due to an increase in chromosomal abnormalities of the ovocytes due to meiotic disruption, shown in mice and There is a significant association between elevated total BPA and premature delivery). The studies included in this review were analyzed for quality. This study concludes that "While it is difficult to make causal links with epidemiological studies, the growing human literature correlating environmental BPA exposure to adverse effects in humans, along with laboratory studies in many species including primates, provides increasing support that environmental BPA exposure can be harmful to humans" and of the sub total of 34 most recent studies, 94% of them indicate a strong evidence on capacity of BPA to interfere with reproduction in humans. The causality is indeed still difficult to be demonstrated but effects of BPA in humans cannot totally be dismissed.

RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
10.10.2013	Sweden	ChemSec	International NGO	8

Comment received

ChemSec welcomes the French Proposal for a Harmonised Classification and Labelling (CLH) of Bisphenol A (BPA).

Overall the CLH report prepared is robust in order to justify an additional classification entry of BPA as Repro 1B H360F according to Annex VI of the CLP Regulation (Toxicity to reproduction – fertility Cat. 2; R60 according to the Dangerous Substances Directive).

However after our assessment of the available data, BPA should instead be classified as a Reproductive toxicant, Category 1A, as it is a known human reproductive toxicant based on evidence from humans.

BPA is associated with reproductive dysfunction, increased cancer risk, including breast and prostate, and a range of other chronic or irreversible health problems, often from very low levels of exposure. Both animal and human studies confirm these effects of very high concern. BPA is commonly detected in humans.

We therefore support this additional classification entry as Repro 1B (360F) but suggest that a classification entry as Repro 1A is more justified for this substance.
Dossier Submitter's Response
FR would like to thank ChemSec for its support to FR proposal.
RAC's response
RAC considers that the human epidemiological studies are regarded as weak supportive evidence for a classification for adverse effect on sexual function and fertility, but are not robust enough to justify a classification of BPA in Repr. 1A.

Date	Country	Organisation	Type of Organisation	Comment number
09.10.2013	United Kingdom	Exponent	International NGO	9
Comment received				
With respect to regulation EC 1272/2008 Table 3.7.1(a) " Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans" it is concluded based on the review of the human data that no such evidence is forthcoming and therefore the placing of BPA in Category 1 is inappropriate and unsupportable. *(ECHA note: The following attachment was provided:* *"Review of the epidemiology studies described in the ANSES 2013 report on harmonized classification and labeling of Bisphenol A" [Attachment 13])*				
Dossier Submitter's Response				
FR thanks Exponent for the document they submitted and their analysis. Nevertheless FR does not agree with the statement that there is no evidence that BPA is a reproductive toxicant for humans. Please refer to previous answer made to Public Health England (COM no. 7).				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
09.10.2013	Germany		MemberState	10
Comment received				
A classification of Bisphenol A as Repro 1B H360F is not supported by DE. In order to support Cat 1 B the criteria require that clear evidence on the adverse effect should be given. Instead, some evidence and inconsistencies across effects and discrepancies between studies confirms the present classification as Cat 2.				
Dossier Submitter's Response				
FR would like to thank DE for its comment and acknowledges the fact that DE does not support FR proposal.				

RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
11.10.2013	Switzerland	Dow Europe GmbH	Company-Manufacturer	11

Comment received

These comments and attachments are the comments of the Bisphenol A REACH Consortium (BPA Consortium), which represents more than 30 of the main producers, importers and users of BPA in Europe. The Dow Chemical Company endorses the positions taken in the BPA Consortium comments. The Dow Chemical Company possesses notable technical expertise in toxicology, especially in the area of reproductive toxicology. It also has considerable expertise in the areas of hazard and risk assessment. Finally, the Dow Chemical Company has been and is a significant stakeholder in the area of classification since it has promoted and contributed a great deal of the critical scientific information relevant to the classification issue at hand.

After a careful review of the proposal in the CLH dossier, we have a number of concerns.
• The case has not been made that BPA merits classification as Category 1B (presumed reproductive toxicant) under the CLP Regulation. In fact, a review of the relevant studies shows that effects on animal fertility only occur at high doses of BPA and that, rather than being selective reproductive effects, they are merely related to systemic toxicity.
• The CLH proposal is not consistent with the procedure outlined in ECHA's "Guidance on the preparation of dossiers for harmonised classification and labeling" (ECHA 2010) [1] which directs the use of a weight-of-evidence approach for compounds with a large database, such as BPA. The CLH proposal
o does not consider "all available information;"
o does not follow the CLP Regulation standard regarding the request that "Both positive and negative results shall be assembled together in a single weight of evidence determination;" and
o fails to follow the CLP Regulation in that "The quality of the data shall be given appropriate weight."
• The CLH proposal selectively relies only on studies, assessments, and the 1 out of 1.409 self-classifications that supports its proposal and, therefore, portrays an inaccurate and incomplete picture of the state of the science on BPA.
o Information is not comprehensive and inconsistent throughout the report.
o Statements related to the value of regulatory guideline studies compared to the value of exploratory studies are biased.
o Statements on multigeneration animal studies upon which regulators have relied (Tyl et al 2002 and 2008a) are inconsistent, incorrect and incomprehensible.
o Reference of one industry self-classification out of 1.409 is clear evidence of "cherry picking" information and ignoring contrary information.
• Recent (post December 31, 2012) and important scientific research from government agencies was not considered. These government studies do not support a classification of BPA as a Category 1B Reproductive Toxicant.
Given the above, the dossier should be rejected as not supporting a classification of BPA as Category 1B reproductive toxic. The CLH proposal does not fulfil the criteria outlined in ECHA's "Guidance on the preparation of dossiers for harmonised classification and labeling"

(ECHA 2010) because it fails to consider the quality of the data and it fails to consider all of the data in a weight of evidence analysis.

As can be seen from the BPA Consortium comments and from assessments of BPA conducted by other government regulators, when all high quality scientific studies on BPA have been considered and a weight of the scientific evidence evaluation is conducted, it will clearly demonstrate that BPA is not a selective reproductive toxicant. In conclusion, there is no basis to change the classification of BPA to Category 1B.
Dossier Submitter's Response
FR thanks Dow for their comments and invites to refer to responses to the Reach Centrum BPA Consortium comments (COM no. 21).
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
04.10.2013	Norway		MemberState	12
Comment received				
Norway would like to thank France for the proposal for harmonised classification and labeling of Bisphenol A (BPA), CAS- no. 80-05-7.				
Dossier Submitter's Response				
FR would like to thank NO for its support to FR proposal.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
11.10.2013	Sweden		MemberState	13
Comment received				
The SE CA supports classification of bisphenol A (Cas No 80-05-07) as specified in the proposal. SE agrees with the rationale for the classification into the proposed hazard class and differentiation.				
Dossier Submitter's Response				
FR would like to thank SE for its support to FR proposal.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
11.10.2013	Belgium	European Environmental Bureau (EEB)	International NGO	14
Comment received				
There is a review of ninety-one studies that links Bisphenol A (BPA) to health effects in humans that should be taken into account. A comprehensive review conducted by TEDX's Johanna Rochester, PhD, was recently accepted for publication in Reproductive Toxicology. Associations were revealed between Bisphenol A exposure and adverse perinatal, childhood and adult health outcomes in humans, including reproductive and developmental effects, metabolic disease and other				

health outcomes, particularly behavioral effects in children. These studies, over half of which were published in the last year, confirm that BPA can be harmful to humans at levels experienced by the general population, and well below levels considered safe by the EPA.

Abstract: There is growing evidence that bisphenol A (BPA) may adversely affect humans. BPA is an endocrine disruptor that has been shown to be harmful in laboratory animal studies. Until recently, there were relatively few epidemiological studies examining the relationship between BPA and health effects in humans. However, in the last year, the number of these studies has more than doubled. A comprehensive literature search found 91 studies linking BPA to human health; 53 published within the last year. This review outlines this body of literature, showing associations between BPA exposure and adverse perinatal, childhood, and adult health outcomes, including reproductive and developmental effects, metabolic disease, and other health effects. These studies encompass both prenatal and postnatal exposures, and include several study designs and population types. While it is difficult to make causal links with epidemiological studies, the growing human literature correlating environmental BPA exposure to adverse effects in humans, along with laboratory studies in many species including primates, provides increasing support that environmental BPA exposure can be harmful to humans, especially in regards to behavioral and other effects in children.

Link to the study: http://www.sciencedirect.com/science/article/pii/S0890623813003456

(ECHA note: The following confidential attachment was provided:
"Bisphenol A and Human Health: A review of the literature" [Attachment 14])

Dossier Submitter's Response

FR would like to thank EEB for this comment and this useful publication. It shows that the appreciation of the weight of evidence for BPA's effect on fertility is varying a lot depending on the comments received.

RAC's response

Noted.

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
11.10.2013	Netherlands		MemberState	15

Comment received

Effects on oocytes
• Effects on chromosome segregation could be considered induction of genetically based heritable effects on the offspring. According to chapter 3.7.1.1 it is considered more appropriate to address such effects under the separate hazard class of germ cell mutagenicity.

Effects on the male reproductive tract
• When adult male rats were exposed to BPA, via gavage or subcutaneously, a decreased sperm count and an increased ventral prostate weight were observed. This might decrease reproductive performance and may be considered as an effect on sexual function. This effect was however not reported in the multigeneration studies. Because of the short study descriptions, it is not clear whether the studies that report these effects can be considered as reliable.

• The summary of the study by Sakaue et al, 2001 contains an error as fertility was not determined in this study.

Effects on reproductive performance
• The reduced number of litters/pups in the 3-generation study in rats without an effect on resorptions (also in F0) and the reduced number of litters/pups in the continuous breeding study in mice suggests an adverse effect on fertility. This effect is observed in two species and in multiple studies (i.e. the secondary effect of reduced litters/pups). However, summaries of the available developmental studies could further substantiate whether this is an effect on fertility or development. No reductions in offspring were observed in the developmental studies by Stump (2010) and Ryan (2010). This further justifies that the effects in the multigenerational studies are due to an effect on fertility instead of post-implantation loss.

Human data
• Two epidemiological studies suggest a relationship between BPA and endometriosis. However, both studies have a poor methodology and are therefore not reliable.
• Some epidemiological studies indicate that higher urinary or plasma BPA concentrations are related to reduced implantation, increased miscarriages, premature birth or ovarian response following ovary stimulation. Also these studies have serious methodological flaws with regard to confounders, population size etc.
Because human exposure to BPA largely comes via food intake and spot urine samples only reflect the last meal (Teeguarden et al, 2011), caloric intake should be included as confounder to exclude the possibility that the correlation is due to higher BPA intake due to higher food intake, in particular higher food intake via canned food (e.g. soft-drinks or beer). Only in some incidental epidemiological studies this possible confounding is mentioned but even in these few studies, the influence of this confounder was not taken into account.
• The methodology of the human data is insufficient, therefore these studies cannot be used for classification purposes. Not even as supportive evidence.

(ECHA note: The following attachment was provided - same content as in the comment above:
"Comments on the proposal for harmonized classification and labeling of Bisphenol A." [Attachment 9])

Dossier Submitter's Response

FR thanks NL for their comments.

1) Concerning the comment on the reliability of the studies including in the report, all the available information has been reported. It is known that scientific paper record of their protocol is often not detailed enough. For a detailed response on how studies were selected, please see response to NAMPA (COM no.4).

2) We acknowledge the fact that we do not report correctly Sakaue *et al.* report and that fertility was not assessed in this study.

3) Concerning the effects on reproductive performance, and your assertion on fertility, the CLH dossier although demonstrate that effects observed might be due to pre-implantation loss effects. Exhaustive review of developmental studies was not performed due to resources limits.

4) Concerning the epidemiological studies on endometrial disorders and their possible link with an exposure to BPA, as it was underlined in the CLH report and noted also in the recent review by Rochester (2013), FR agrees that these studies have a poor methodology and further research is needed. Nevertheless these studies have been included in order to have a complete picture of what has been done in humans on the BPA. FR does not agree with the statement that the methodology of all epidemiological studies included in the CLH report are poor and that the human data cannot be used for classification purpose. Please see response to COM no. 7 for a detailed response.

RAC's response

RAC consider that the human epidemiological studies are regarded as weak supportive evidence for a classification for adverse effect on sexual function and fertility, but are not robust enough to justify a classification of BPA in Repr. 1A.

Date	Country	Organisation	Type of Organisation	Comment number
11/10/2013	Italy		MemberState	16

Comment received

(*ECHA note: The comment below was submitted as a separate attachment with the name "Bisphenol classification" [Attachment 7])*

We support that classification of Bisphenol A for reproductive effects has to be reconsidered, since numerous studies have been produced in the recent years.
Under this respect, we consider that the studies performed by sub-cutaneous administration or other parenteral routes (intramuscular, i.p.) may serve as proof-of-principle, i.e., to show the potential of bishenol A to act as a reproductive toxicant and the main targets of its reproductive effects. However, for classification and labelling purposes, priority has to be given to studies using treatment routes relevant to consumer's exposure, such as oral, diet, drinking water, inhalation.
We support the classification proposal as 1b for reproductive toxicity (R60), based on the consistent observation of reproductive impairment at oral/dietary dose levels. Considering only studies performed after 2000 using oral (gavage, diet. drinking water) routes:

Female reproduction
- increased ovarian meiotic abnormalities upon prepubertal exposure in mice (Hunt et al., 2003)
- increased resorption rate in mice exposed since mating (Al-Hiyasat et al., 2004)
- dose-related delayed puberty (two-generation study in rats; Tyl et al., 2002)
- endometrial proliferation (increased thickness of uterine epithelia and stroma, less apoptotic cells) with reduced ER-α expression in rats exposed to 1.2 mg/kg bw in utero and during lactation (Mendoza-Rodriguez et al., 2011); at much higher in utero exposure (50 mg/kg) the thickness of the uterine epithelium is reduced with altered expression of estrogen receptors (Schönfelder et al., 2004)
- irregular estrous cycle upon developmental (pre- and neonatal) exposure (Rubin et al., 2001; Mendoza-Rodriguez et al., 2011)

Male reproduction
- Impaired spermatogenesis upon in utero exposure in rats (Tinwell et al., 2002; Iida et al., 2002) also with demonstrated impaired reproductive ability (Salian et al., 2009)
- Reduced testis and seminal vesicles weight, with reduced Leydig cells testosterone

production in rats exposed in utero and neonatally (Akingbemi et al., 2004)
- Reduced testosterone (Akingbemi et al., 2004; Della Seta et al., 2006) and LH (Akingbemi et al., 2004) serum levels in rat exposed during prepubertal phase (Akingbemi et al., 2004); reduced reproductive performance also observed (Della Seta et al., 2006)
- Delayed puberty in rats exposed in the prepubertal phase (Tan et al., 2003)
- Impaired spermatogenesisis with reduced testis and epidydimal weight and increased prostate weight in adult rats (Chitra et al., 2003)

Apparently the above effects were observed in the absence of other toxic effects that may cause reproductive impairment as a secondary consequence. Thus, the overall picture of BPA effects on fertility/reproductive function fulfills the criteria for classification as **1b**.
The data available hint to some further observations:
- The effects of BPA are more complex than being simply "estrogenic" and hint to modes of action modulated by dose, sex and lifestage; species- and strain-related susceptibility have been observed, mice appearing as less susceptible. This interplay among factors modulating BPA effects may partly explain why some studies contradict the majority of findings as no effects are shown; this aspect is surely worth investigating for a refined risk assessment. However, the overall the overall weight of evidence points to clearly point to an impaired reproductive function in both sexes, encompassing secveral endocrine, morphological and functional parameters and with enhanced sensitive of the prenatal and also prepubertal stages. Thus, for classification purposes BPA should be considered as a chemical capable to elicit clear-cut reproductive effects in both sexes through relevant exposure routes.
- Reproductive effects were apparently less evident in the two-generation studies, however these were not entirely absent as shown by the dose-related delayed puberty seen also at the intermediate dose in the rat two-generation study by (Tyl et al., 2002). This is another finding definitely worth investigating: data may suggest that the repeated pulse exposure related to gavage is much more effective than continuous low-level exposure elicited by the long term dietary administration.

One might speculate that the aggregate, multiple way exposure of humans is more similar to the repeated pulse scenario, whereas a continuous dietary exposure might be more relevant to cumulative chemicals.
Again, this issue is definitely worth investigating for risk assessment, but it bears little weight for classification purposes, that currently rely on rather stringent, hazard-based criteria

Moreover, consideration should be given to available human data:

- Biomonitoring data in humans indicate a continuous aggregate exposure, with a prolonged presence of detectable internal levels
- Humans may have more efficient detoxification mechanisms than rodents; however, many differences exist within the human population, related to genetics, sex and lifestage
- Epidemiological investigations suggest a relationship between BPA levels and increased risk of reproductive disorders in women (infertility, recurrent miscarriage, impaired IVF), while in males correlations with impaired spermatogenesis and altered endocrine balance (steroid hormones, FSH, Inhibin B) have been observed.

Overall human data are not robust enough to support a 1a classification; however, these studies support the evidence provided by the animal studies pointing out that bisphenol A should be classified as 1b for reproductive toxicity.

--- *End of attachment* ----

Dossier Submitter's Response
FR would like to thank IT for its support to the proposal and very interesting comments. FR invites IT to refer to response to COM no. 1 in which it is explained why we consider studies using sub-cutaneous route as relevant. FR appreciates the understanding of IT of the complexity arising from the available data on BPA's fertility effect. They, indeed, most probably show that these effects are not simply "estrogenic" and hint to modes of action modulated by dose, sex and lifestage; species- and strain-related susceptibility. We agree that some species/ strain appear as less susceptible than others. We agree that design is also probably playing a role on the outcome of the data. We are not sure, however, that the repeated pulse exposure related to gavage is more effective than continuous low-level exposure elicited by the long term dietary administration In particular, it seems that **in male, most of the negative results come from studies performed in SD rats by gavage. In female, studies performed in rats, particularly by gavage, give negative results.** We agree that the work performed in compiling the studies for this proposal did not manage to clearly indentify the interplay among those factors modulating BPA effects and therefore did not manage to explain why some studies contradict the majority of findings. We also agree that the overall weight of evidence points to an impaired reproductive function in both sexes, encompassing several endocrine, morphological and functional parameters and with enhanced sensitive of the prenatal and also prepubertal stages. Thus, for classification purposes BPA should be considered as a chemical capable to elicit clear-cut reproductive effects in both sexes through relevant exposure routes.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
26.09.2013	United Kingdom		Individual	17
Comment received				
It is known that effects exist on certain species of animals but this is not enough publicised, probably because the topic is very specific and not 'sexy' enough to attract a wide press coverage.				
Dossier Submitter's Response				
FR would like to thank you for your comment and your support. Nevertheless you should note that bisphenol A has pretty wide press coverage in recent years.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
04.10.2013	United States	Can Manufacturers Institute	Industry or trade association	18
Comment received				
CMI is opposed to the proposal to reclassify bisphenol A (BPA) as a reproductive toxicant Category 1B under Regulation (EC) No. 1272/2008. According to the Classification,				

Labeling and Packaging (CLP) regulations, substances to be classified as reproductive toxicant, Category 1B, must have clear evidence of an adverse effect in the absence of other toxic effects OR if other effects are seen, the reproductive toxicity effect must be the origin of the other toxic effects and not be a secondary, non-specific consequence. That is not the case with BPA. In studies where reproductive toxicity effects were observed, those effects occurred at dose levels above those where systemic toxicity effects. Thus, the criteria are not met and the reclassification proposal should not be accepted.

Dossier Submitter's Response

FR thanks CMI for its comment but does not agree with the statement that with BPA reproductive effects occurred only at doses where systemic toxicity is observed. Multigeneration and guideline studies show effects at doses >=50mg/kg bw/ d. However, even if there is subsequent signs of toxicity at the doses, there is nothing to prove that the fertility effects observed are secondary to these other signs of toxicity.

RAC's response

Noted

Date	Country	Organisation	Type of Organisation	Comment number
10.10.2013	Netherlands	Momentive Specialty Chemicals B.V.	Company-Manufacturer	19

Comment received

Executive summary of
BPA Consortium comments to the CLH Proposal

These comments and attachments are the comments of the Bisphenol A REACH Consortium (BPA Consortium), which represents more than 30 of the main producers, importers and users of BPA in Europe. After careful review of the proposal in the CLH dossier, we have a number of concerns.

• The case has not been made that BPA merits classification as Category 1B (presumed reproductive toxicant) under the CLP Regulation. In fact, a review of the relevant studies shows that effects on animal fertility only occur at high doses of BPA and that, rather than being selective reproductive effects, they are merely related to systemic toxicity.

• The CLH proposal is not consistent with the procedure outlined in ECHA's "Guidance on the preparation of dossiers for harmonised classification and labeling" (ECHA 2010) [1] which directs the use of a weight-of-evidence approach for compounds with a large database, such as BPA. The CLH proposal

o does not consider "all available information;"

o does not follow the CLP Regulation standard regarding the request that "Both positive and negative results shall be assembled together in a single weight of evidence determination;" and

o fails to follow the CLP Regulation in that "The quality of the data shall be given appropriate weight."

• The CLH proposal selectively relies only on studies, assessments, and the 1 out of 1.409 self-classifications that supports its proposal and, therefore, portrays an inaccurate and incomplete picture of the state of the science on BPA.

o Information is not comprehensive and inconsistent throughout the report.

o Statements related to the value of regulatory guideline studies compared to the value of exploratory studies are biased.

o Statements on multigeneration animal studies upon which regulators have relied (Tyl et al 2002 and 2008a) are inconsistent, incorrect and incomprehensible.
o Reference of one industry self-classification out of 1.409 is clear evidence of "cherry picking" information and ignoring contrary information.
• Recent (post December 31, 2012) and important scientific research from government agencies was not considered. These government studies do not support a classification of BPA as a Category 1B Reproductive Toxicant.

Given the above, the dossier should be rejected as not supporting a classification of BPA as Category 1B reproductive toxic. The CLH proposal does not fulfil the criteria outlined in ECHA's "Guidance on the preparation of dossiers for harmonised classification and labeling"

(ECHA 2010) because it fails to consider the quality of the data and it fails to consider all of the data in a weight of evidence analysis.
As can be seen from the BPA Consortium comments and from assessments of BPA conducted by other government regulators, when all high quality scientific studies on BPA have been considered and a weight of the scientific evidence evaluation is conducted, it will clearly demonstrate that BPA is not a selective reproductive toxicant. In conclusion, there is no basis to change the classification of BPA to Category 1B.
Please see for more details in the public attachment

(*ECHA note: The following attachments were provided:*
"Annex A - BPA REACH Consortium CLH male and female endpoints 10-10-2013"
"Annex B - BPA REACH Consortium CLH information on Tyl 10-10-2013"
"Annex C - BPA REACH Consortium CLH summary NCTR-2013 10-10-2013"
"Annex D - BPA REACH Consortium CLH overview relevant studies for BPA classification 10-10-2013"
"Annex E - BPA REACH Consortium CLH review epidemiology studies described in the CLH proposal 10-10-2013"
"BPA REACH Consortium comment on CLH proposal on BPA prepared by ANSES 10-10-2013_final"
[Attachments 1-6])

Dossier Submitter's Response

FR thanks Momentive Specialty Chemicals B.V. for their comments and invites them to see responses to the ReachCentrum BPA Consortium comments (COM no. 21).

RAC's response

Noted.

Date	Country	Organisation	Type of Organisation	Comment number
10.10.2013	Germany	Bayer MaterialScience AG	Company-Manufacturer	20

Comment received

Executive summary of
BPA Consortium comments to the CLH Proposal

These comments and attachments are the comments of Bayer MaterialScience AG and the Bisphenol A REACH Consortium (BPA Consortium), which represents more than 30 of the main producers, importers and users of BPA in Europe. After careful review of the proposal in the CLH dossier, we have a number of concerns.

• The case has not been made that BPA merits classification as Category 1B (presumed reproductive toxicant) under the CLP Regulation. In fact, a review of the relevant studies shows that effects on animal fertility only occur at high doses of BPA and that, rather than being selective reproductive effects, they are merely related to systemic toxicity.
• The CLH proposal is not consistent with the procedure outlined in ECHA's "Guidance on the preparation of dossiers for harmonised classification and labeling" (ECHA 2010) [1] which directs the use of a weight-of-evidence approach for compounds with a large database, such as BPA. The CLH proposal
o does not consider "all available information;"
o does not follow the CLP Regulation standard regarding the request that "Both positive and negative results shall be assembled together in a single weight of evidence determination;" and
o fails to follow the CLP Regulation in that "The quality of the data shall be given appropriate weight."
• The CLH proposal selectively relies only on studies, assessments, and the 1 out of 1.409 self-classifications that supports its proposal and, therefore, portrays an inaccurate and incomplete picture of the state of the science on BPA.
o Information is not comprehensive and inconsistent throughout the report.
o Statements related to the value of regulatory guideline studies compared to the value of exploratory studies are biased.
o Statements on multigeneration animal studies upon which regulators have relied (Tyl et al 2002 and 2008a) are inconsistent, incorrect and incomprehensible.
o Reference of one industry self-classification out of 1.409 is clear evidence of "cherry picking" information and ignoring contrary information.
• Recent (post December 31, 2012) and important scientific research from government agencies was not considered. These government studies do not support a classification of BPA as a Category 1B Reproductive Toxicant.

Given the above, the dossier should be rejected as not supporting a classification of BPA as Category 1B reproductive toxic. The CLH proposal does not fulfil the criteria outlined in ECHA's "Guidance on the preparation of dossiers for harmonised classification and labeling"

(ECHA 2010) because it fails to consider the quality of the data and it fails to consider all of the data in a weight of evidence analysis.
As can be seen from the BPA Consortium comments and from assessments of BPA conducted by other government regulators, when all high quality scientific studies on BPA have been considered and a weight of the scientific evidence evaluation is conducted, it will clearly demonstrate that BPA is not a selective reproductive toxicant. In conclusion, there is no basis to change the classification of BPA to Category 1B.

see public attachments

(*ECHA note: The following attachments were provided:*
"Annex A - BPA REACH Consortium CLH male and female endpoints 10-10-2013"
"Annex B - BPA REACH Consortium CLH information on Tyl 10-10-2013"
"Annex C - BPA REACH Consortium CLH summary NCTR-2013 10-10-2013"
"Annex D - BPA REACH Consortium CLH overview relevant studies for BPA classification 10-10-2013"
"Annex E - BPA REACH Consortium CLH review epidemiology studies described in the CLH proposal 10-10-2013"
"BPA REACH Consortium comment on CLH proposal on BPA prepared by ANSES 10-10-2013_final"
[Attachments 1-6])

Dossier Submitter's Response
FR thanks Bayer MaterialScience AG for their comments and invites them to see responses to the ReachCentrum BPA Consortium comments (COM no. 21).
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
10.10.2013	Belgium	ReachCentrum BPA Consortium	Industry or trade association	21

Comment received

Executive summary of
BPA Consortium comments to the CLH Proposal

These comments and attachments are the comments of the Bisphenol A REACH Consortium (BPA Consortium), which represents more than 30 of the main producers, importers and users of BPA in Europe. After careful review of the proposal in the CLH dossier, we have a number of concerns.

• The case has not been made that BPA merits classification as Category 1B (presumed reproductive toxicant) under the CLP Regulation. In fact, a review of the relevant studies shows that effects on animal fertility only occur at high doses of BPA and that, rather than being selective reproductive effects, they are merely related to systemic toxicity.

• The CLH proposal is not consistent with the procedure outlined in ECHA's "Guidance on the preparation of dossiers for harmonised classification and labeling" (ECHA 2010) [1] which directs the use of a weight-of-evidence approach for compounds with a large database, such as BPA. The CLH proposal

o does not consider "all available information;"

o does not follow the CLP Regulation standard regarding the request that "Both positive and negative results shall be assembled together in a single weight of evidence determination;" and

o fails to follow the CLP Regulation in that "The quality of the data shall be given appropriate weight."

• The CLH proposal selectively relies only on studies, assessments, and the 1 out of 1.409 self-classifications that supports its proposal and, therefore, portrays an inaccurate and incomplete picture of the state of the science on BPA.

o Information is not comprehensive and inconsistent throughout the report.

o Statements related to the value of regulatory guideline studies compared to the value of exploratory studies are biased.

o Statements on multigeneration animal studies upon which regulators have relied (Tyl et al 2002 and 2008a) are inconsistent, incorrect and incomprehensible.

o Reference of one industry self-classification out of 1.409 is clear evidence of "cherry picking" information and ignoring contrary information.

• Recent (post December 31, 2012) and important scientific research from government agencies was not considered. These government studies do not support a classification of BPA as a Category 1B Reproductive Toxicant.

Given the above, the dossier should be rejected as not supporting a classification of BPA as Category 1B reproductive toxic. The CLH proposal does not fulfil the criteria outlined in ECHA's "Guidance on the preparation of dossiers for harmonised classification and labeling"

(ECHA 2010) because it fails to consider the quality of the data and it fails to consider all of the data in a weight of evidence analysis.
As can be seen from the BPA Consortium comments and from assessments of BPA conducted by other government regulators, when all high quality scientific studies on BPA have been considered and a weight of the scientific evidence evaluation is conducted, it will clearly demonstrate that BPA is not a selective reproductive toxicant. In conclusion, there is no basis to change the classification of BPA to Category 1B.
see public attachments

(*ECHA note: The following attachments were provided:*
"Annex A - BPA REACH Consortium CLH male and female endpoints 10-10-2013"
"Annex B - BPA REACH Consortium CLH information on Tyl 10-10-2013"
"Annex C - BPA REACH Consortium CLH summary NCTR-2013 10-10-2013"
"Annex D - BPA REACH Consortium CLH overview relevant studies for BPA classification 10-10-2013"
"Annex E - BPA REACH Consortium CLH review epidemiology studies described in the CLH proposal 10-10-2013"
"BPA REACH Consortium comment on CLH proposal on BPA prepared by ANSES 10-10-2013_final"
[Attachments 1-6])

Dossier Submitter's Response

FR thanks ReachCentrum BPA Consortium for their comments and documents provided. Nevertheless you could find below few clarifications:

Concerning the choice of the studies included in the CLH report the studies analyzed for the ANSES's report on the hazards of BPA (ANSES, 2011) were included. Relevant recent studies and studies using doses over 5 mg BPA/kg bw/day were also added since they were not chosen for the ANSES report. As a proposal for modification of existing entry has to be based on new existing knowledge, FR chose to not cite explicitly studies performed before the publication of the EU-RAR used for the first proposal of a revision of the classification of the BPA (meaning before 2002) since they were already included in this report and were already a part of the argument to support in the previous the proposal. However, the summary of the outcome of the analysis of these studies was provided in annex and direct reference to previous evaluation was made. Finally, all the guideline studies published afterward and before TC C&L discussion were incorporated as milestones of the discussions on classification of BPA (and also to avoid FR being blamed of partiality as these studies show negative results at low doses).
Then, several studies qualified as "not included" and listed p.7 of the *"BPA REACH Consortium comment on CLH proposal on BPA prepared by ANSES 10-10-2013_final",* (for example Aikawa *et al*. (2004), Toyama *et al.* (2004), Ema *et al*. (2001), Cagen *et al*. (1999), vom Saal *et al*. (1998)…) are in fact in the report .They are cited either in the main document or in the Annex1 or Annex2 which report the UK EU-RAR on BPA from 2002. Our goal was obviously not to discriminate any studies and we did our best to have a complete, exhaustive review of the existing literature on fertility produced after UK Eu-RAR. But since new articles on BPA are published every week and considering the existing very large dataset it is possible that some were unintentionally omitted.
Moreover a very detailed methodology was used to choose the studies included in the ANSES report on hazards of the Bisphenol A. Studies were chosen according to a precise protocol defined and validated by a working group of FR experts and was the starting point

for including studies in the CLH report. The methodology on the choice of the most reliable studies is explained in the response to COM no. 4. Please refer to it.

Concerning the fact that non-guideline studies were included in the CLH report, most of the studies conducted in independent laboratories are not performed in a regulatory purpose and are then not following any guideline. This does not mean that the results found in these studies are less reliable especially when they are investigating very specific endpoints rarely addressed in more "classic" studies. It is sometimes difficult to precisely assess the details of the methodology used since it is not following a guideline. It is even more difficult to compare studies and effects observed when the same methodologies that have not been used However, it should be noted that this difficulty is also found when comparing guidelines of GLP studies in which strain/doses/route of exposure/window of exposure may differ. Indeed, if the doses at which effects are arising are coherent between the guideline studies, the effects described are not totally coherent.

Concerning the NCTR study, this study was not included in the CLH since it was not available at the time of the publication of the CLH report. We got access to the study during the RCOM period of time and analyzed it very closely. Our analysis of this recent preliminary study is attached in Annex 1.

Concerning the epidemiological studies, it is indeed possible that the existing literature is not robust enough and requires further investigation. Nevertheless a review of the most recent epidemiological data performed on BPA has recently been published (Rochester JR (2013), Bisphenol A and human health: A review of the literature. Reproductive Toxicology 42:132-155) which analyzed a lot of epidemiological studies and highlighted effects of BPA. Please refer to response to COM no. 7 for a detailed response.

About the history of the classification, it should be noted, as mentioned in the CLH report that, based on the 3 guidelines studies available in 2002 the proposal of UK to classify the Bisphenol A as reprotoxic Cat. 1B for fertility was first accepted. Their proposal was based partially on the fact that "the findings could not be attributed to general systemic toxicity". It is not pretty clear (contrary to what is indicated in your main document) why finally the proposal was not adopted. However, it is clear that the effects observed were not attributed to general systemic toxicity.
We believe that there is still no data to allow attributing fertility effects to secondary effects of general systemic toxicity. Moreover, we consider that the similarity of the lesions observed in various organs including ovary, render the plausibility of a specific effect of BPA on this type of lesion highly probable.

Regarding toxicokinetics: concerning the PBPK model mentioned in the ANSES report on hazards of BPA, it is still not available and under development. Concerning the inclusion of studies using the parenteral route of exposure, subcutaneous route can highlight effects at doses much lower than those administered orally and is then considered as relevant. Using a parenteral route allow you to better control the internal dose received by the animals. We agree that the relevance of each route of exposure has to be considered, although not specified as such in the guidance. These route are often use for testing low doses

(impossible to adequately assess by oral). Finally, in the specific case of BPA, subcutaneous studies are related to a major route of exposure to BPA via thermal paper. Please refer to RCOM no. 1 for a detailed response.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
10.10.2013	Denmark		MemberState	22
Comment received				
The Danish CA welcomes this thorough evaluation of the reproductive effects of BPA. Based on the total weight of evidence we agree that classification in Cat. 1B is warranted as adverse effects on fertility and the reproductive systems have been demonstrated in several studies in mice. These findings furthermore confirm the data obtained from various human studies, which i.e. indicate that exposure to BPA is associated with impact on sperm parameter and sexual dysfunction in males and effects on the reproductive organs in women. However, we are uncertain as to whether the human data are strong enough to justify a Cat. 1A classification.				
Dossier Submitter's Response				
FR would like to thank DK for their comments and support to FR proposal.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
10.10.2013	United Kingdom	Public Health England	National Authority	23
Comment received				
Part A1 A critical aspect of identifying an EDC especially at the high doses utilised in the majority of these studies is that the observed effects should be observed in the absence of systemic effects. The absence of any data on the acute oral toxicity or repeated dose toxicity for bisphenol A makes this data difficult to interpret. Part B2 Final section is incomplete 'Out of many sources, the general public might be exposed via thermal paper; in articles made of PVC….' The major source of BPA exposure is ingestion via use of BPA in food contact materials or in infants by mouthing behaviour of PVC-containing items. This is important information as the evidence presented later in the dossier shows conflicting data dependent upon whether BPA is dosed orally or injected subcutaneously. Part B4.1 BPA intake of 13ug/kg quoted. This is out of date and the current EFSA opinion under public consultation estimates daily exposure at <1ug/kg/day for all age categories. In metabolism section (4.1.3) it is stated that the enterohepatic circulation of BPA in rats has little consequence on clearance however, what is important is the reformation of the parent 'active' BPA from the inactive conjugates which makes the rat a more susceptible species than man. Also, the possibility of β-glucuronidase activity in placenta increasing exposure to the foetus is described despite the studies of Patterson et al 2010 showing that in primates, placenta contributes an increase in glucuronidation.				

The very low proportion of aglycone in body fluids including breast milk highlight the facts that high dose levels by routes other than oral that avoid first pass effect are not representative of the risks to humans and wildlife.
Section 4.11.
Several different animal models have been utilised to investigate the effects of BPA. Many of the effects observed in these studies occur spontaneously in different animal strains eg ovarian cysts. Where these have been presented data should referenced to the range of expected spontaneous incidence in that species.
If a weight of evidence approach is being used, less weighting should be given to studies using inappropriate routes of dosing.
4.11.1.1.5 Multigenerational exposure
In the continuous breeding study weight loss is observed in the female mice in the treated groups suggestive of systemic toxicity which may affect the small declines in fertility of these animals.
Tyl et al 2008 saw no reprotoxicity except at the highest dose (where the authors report systemic toxicity) in CD-1 mice. Organ specific toxicities were observed for kidney and liver at 300 ppm (50 mg/Kg, Fo) and 0.018 ppm (3ug/kg, F1, F2) suggest the Repro. Tox is a secondary effect and that classification based upon repeated dose toxicity is more appropriate.
Likewise the same authors reported renal tubular degeneration and chronic hepatic inflammation in SD rats exposed to 750 and 7500 ppm but only observed reprotoxic effects at the highest dose (7500 ppm)
4.11.1.1.6 Transgenerational exposure
Hiyama study, all doses are in excess of the doses inducing organ toxicity (Tyl et al)

4.11.1.1.7
No effects on meiosis and oocyte development were observed in the 4 guideline studies.
The spontaneous incidence of ovarian cysts is well documented in CD-1 mouse strain. It should be made clear whether the test groups described here fall within the range of spontaneous incidence or not especially as this was the only significant finding in these studies.
Hoxa10 and Hoxa 11 expression data are contradictory in two studies therefore little can be inferred from the data at this time.
Several of the mechanistic studies take place in ovariectomised animals and therefore the studies should be given less weight as they are not in intact animals.

Effects on the female reproductive capacities
Effects are only observed when BPA is dosed by subcutaneous injection. Oral dosing eg cabaton et al, Tyl et al induced no effects
The authors make reference to publications for the lack of sensitivity of SD rats to estrogens. This evidence needs to be made clearer as the research referenced as Nagao et al and Kwon et al 2000 demonstrate that SD rats respond to DES or estradiol benzoate but not BPA. Without this the intent of the authors to allow less emphasis on the negative studies conducted in SD rats is unfounded.
Is it correct that the doses in the Berger study are 3375 mg/day and 10,125 mg/day? Is this even possible? If so, was a full toxicological evaluation conducted? Were any other organ toxicities observed?
Conclusion on female reproductive system in animals
The increased incidence of ovarian cysts in CD-1 mice needs to be confirmed as significantly greater than the well documented spontaneous occurrence rate if this effect is to be proven to be attributed to BPA.
There is significant inconsistency between studies with uncertainties associated with strain and species differences, studies showing no toxicity (particularly those using OECD guideline

tests), use of ovariectomised animals, routes of exposure and hepatic and renal toxicity at concentrations lower than those inducing reproductive effects. As such, a categorisation of BPA as Repr. 2 is appropriate.
4.11.1.2 Human information
4.11.1.2.2 Effects during pregnancy
No significant well conducted studies are presented here.
In addition to the criticisms of these studies by the authors; the case-control study of Sugiura-Ogasawara is also suspect as non-pregnant women are used as controls for women with recurrent miscarriage. The correct control would have been first trimester women with no history of miscarriage. Inoue et al (2005 Drug Metab Dispos) demonstrated that in rats biliary excretion of BPA glururonide is decreased in pregnant rats and circulating BPA glucuronide is increase. ELISA will not differentiate between parent and glucuronide and so in this study an increased BPA level in the pregnant women with a history of miscarriage is to be expected.

4.11.1.2.3 Effects on Ovary
Data are contradictory between studies for the effects of obesity for example BPA is increased in obese women in Tekeuchi et al but not Kandaraki et al. and data is questionable because of the use of ELISA and urinary BPA levels negatively correlate with oocytes collected (Mok-Lin et al) but not between serum BPA and oocytes collected (Bloom et al).

4.11.2 Effects on male reproductive tract
4.11.2.1.1 in utero and lactation exposure
Strain differences
Some studies show effects on prostate others don't. Some studies show effects on male reproductive tissues others no not. Majority of the more recent studies were negative

4.11.2.1.2 neonatal exposure
The study of Salian et al shows no dose response but a consistent post-implantation loss of around 25% for doses 400, 800 and 1600ug/kg bw/d. How does this vary compared with the natural variability in Holzman rats? There is a dose dependent effect on sperm count and motility however these effects wouldn't cause infertility as counts are still within the normal range of a fertile animal.
In the description of the study of Aikawa et al there is inconsistency in reporting the dose ug or mg?
No effects on sperm count seen in this study despite comparable doses to Salian et al study?
Toyama and Yuasa (2004b) and Kato et al (2006) suggest that BPA dosing of neonates has no effect on fertility.

4.11.2.1.3 Prepubertal exposure
There is no consistency across these studies with respect to effects on oestradiol or testosterone levels. Some studies show an alteration of LH but there are no signs of adversity in any of the studies with respect to reproduction.
Akingbemi et al , no dose response with significant effects only observed at one dose and no indications of adversity.
Nakamura et al 2010 only demonstrate effects on testis weight and reduced epididymis and seminal vesicles at doses responsible for weight loss and therefore potential systemic toxicity.
Tan et al showed little significant effect whereas Takahashi and Oishi did show toxicity in Wistar rats but only when dosed ip or sc and no toxicity when dosed via the oral route.

4.11.2.1.4 Adult exposure
Consistent transitory effects of adult exposure on sperm parameters following prolonged, high dose exposures, using an inappropriate route of exposure in rat only suggesting a Repr. Tox 2 categorisation.

4.11.2.1.5 Multiple Exposure
No consistent significant effects

4.11.2.1.6 Multigenerational exposure
No evidence of any multigenerational effects even at very high doses.
No clear reproductive toxicity is seen in these mouse studies. Effect on reproductive organ weight in the continuous study is accompanied by a marked increase in liver and kidney weights. It is not clear from the report which characteristic toxicity response (liver, kidney, reproductive organs) happens at the lowest dose and therefore is the key/primary target organ in this study and consequently whether BPA should be classed as a repro, hepatic or renal toxicant.
In rat (Emma et al), no statistically significant changes to AGD or sperm statistics nor any dose-response for seminal vesicle weight and no morphological changes and therefore no adversity. Likewise the study of Tyl et al 2002 only demonstrated effects at the highest dose which was accompanied by body weight loss suggesting toxicity at other sites
4.11.2.1.7 Transgenerational exposure
The study of Salian et al demonstrates a transgenerational effect of BPA with some dose response and indication of adversity (post-implantation loss). This is inconsistent with the multigenerational studies described above and is the only study of its type further highlighting the large unexplained discrepancies between studies.

Conclusions on male reproduction
The authors state that oral studies may elicit more effects that sub-cutaneous studies which isn't supported by the data. For example Tan et al showed limited toxicity by sc route and non by the oral route.
It is apparent that many studies are conducted in SD rats which require 100- to 400-fold higher doses of estrogens to observe an effect which is a shortfall. However, doses used in many of these studies are 1000-fold or higher than expected human exposures and so some effects would still be expected to be observed.
Additional information
Although this section is of interest, the effects observed on various proteins do not demonstrate adversity
4.11.2.2 Human information
There is marked inconsistency between studies. No consistent association between urinary BPA and adverse semen parameters in Mendiola et al (2010). Where correlations exist this is not evidence of a causal relationship. BPA exposure will be higher in people consuming a processed food diet rich in sugary, carbonated drinks. This group is more likely to have dietary insufficiencies, to be overweight and have high blood glucose levels which are all associated with lowered fertility/sperm counts. It is very difficult to prove causality and the concentrations of BPA found in biofluids of humans are orders of magnitude lower than those expected to occur in the animal models at the effective doses of BPA in vivo.
The marked significant decrease in AGD in boys whose mothers (P=0.003) but not fathers (p=0.15) were exposed to BPA is an important finding but it shows no adversity and BPA levels in cord blood were not significantly different between cryptorchid boys and controls
Conclusions in humans (men)
It is wrong for the authors to state that 'all point out a correlation between higher BPA levels and different sexual parameters' as not all studies in men do and the observations of Meeker et al (2010a) and Mendiola et al (2010) report inconsistent relations between

urinary BPA and FSH. What these studies do consistently show is the very low (single digit ng/ml or lower) plasma levels of BPA in the human population which are inconsistent doses employed inducing any detrimental effects observed in animal models.
4.11.5. Summary…
The authors state 'the significance of the positive trend between BPA and testosterone was not reached' for three studies in humans. This suggests that BPA causes an increase in testosterone which would be expected to cause an increase in sperm count. This is inconsistent with proposed effects on sperm populations and AGD in rodents in vivo.
Most consistent effect is ovarian cyst occurrence but this is observed in CD1 mice which have a predisposition for ovarian cysts.
'…to show that the hazard demonstrated in animals can be observed as a risk in humans.'
This is not a truly accurate statement. Although more work on measuring tissue and biofluid levels in animals during these experiments should be conducted to verify concentrations of BPA in circulation it is very likely that the doses of BPA employed to achieve the low ng/ml levels seen in patients consistently show no detrimental effects in vivo. This is emphasised more when it is considered that rats demonstrate significant enterohepatic circulation of BPA that humans do not.
4.11.6 Comparison with criteria
Category 2. The data provided in this dossier provides some evidence from humans but this is limited to correlations and has a lack of consistency and lots of inconsistent studies in experimental animals most of which do not demonstrate an adverse effect on sexual function and fertility, or on development. Furthermore, these effects are not always observed in the absence of other toxic effects (weight loss, increases in liver and kidney size) suggesting that the evidence is not sufficiently convincing to place the substance in Category 1. For a classification of Category 1B this requires that it should be known that BPA produces adverse effects on sexual function and fertility in humans which the evidence to date does not support. Alternatively, a strong presumption that interference with reproduction in humans based upon clear evidence from animal experiments may also determine a Category 1B classification. There is no clear data from animal experiments that BPA by the oral route (the route of exposure in humans) is responsible for reproductive toxicity with the majority of the oral or dietary doses showing no toxicity or, at very high doses, showing toxicity to reproductive tissues, liver and kidney and inducing weight loss indicative of systemic toxicity. This together with the lack of data on acute toxicity suggest that secondary non-specific reproductive toxicity may be the cause of at least some of the observed toxicities. A classification of Category 1A is not appropriate

Dossier Submitter's Response

FR would like to thank UK for their comments and acknowledges the remarks made. Please find below some clarifications.

Part A1: First, our goal in this CLH report was not to demonstrate the EDC properties of BPA. However, our classification proposal covers the fertility endpoint.
Secondly, the criteria[1] for identifying EDs specify that the [animal studies/ experimental animal studies] shall provide clear evidence of ED-mediated adverse effects in the absence of other toxic effects, or if occurring together with other toxic effects, the ED-mediated adverse effects should be considered not to be a secondary non-specific consequence of other toxic effects. Therefore, you will find below the data required (the DNEL proposed for the general population in the registration dossier) although we do not believe they are of any added value for our dossier:

[1] ED-AD-HOC-5/2012/04; THE COMMUNITY STRATEGY FOR ENDOCRINE DISRUPTORS 5TH AD HOC MEETING OF COMMISSION SERVICES, EU AGENCIES AND MEMBER STATES: Initial thoughts on the criteria for endocrine disruptors.

		Acute/short-term	Long-term
Inhalation route	Systemic effects	5 mg/m^3	0.25 mg/m^3
	Local effects	5 mg/m^3	5 mg/m^3
Dermal route	Systemic effects	0.7 mg/kg bw/day	0.7 mg/kg bw/day
Oral route	Systemic effects	0.05 mg/kg bw/day	0.05 mg/kg bw/day

These data, also do not apply for our fertility proposal. Indeed, guidance on the Application of the CLP Criteria (ECHA), in paragraph 3.7.2.2.1 Classification in the presence of parental toxicity, 3.7.2.2.1.1 Effects to be considered in the presence of marked systemic effects that: "In general all findings on reproductive toxicity should be considered for classification purposes irrespective of the level of parental toxicity. A comparison between the severity of the effects on fertility/development and the severity of other toxicological findings must be performed.

Fertility effects: Adverse effects on fertility and reproductive performance seen only at dose levels causing marked systemic toxicity (e.g. lethality, dramatic reduction in absolute body weight, coma) are not relevant for classification purposes. There is no established relationship between fertility effects and less marked systemic toxicity. Therefore it should be assumed that effects on fertility seen at dose levels causing less marked systemic toxicity are not a secondary consequence of this toxicity. However, mating behaviour can be influenced by parental effects not directly related to reproduction (e.g. sedation, paralysis), and such effects on mating behaviour may not warrant classification."

None of the cases described above applies to the studies on BPA, neither at low doses, nor at higher doses for which mild systemic effects are described (liver and kidney cysts, low decrease in mother BW gain°

Part B2: FR acknowledges your remark; unfortunately, the CLH report is not expected to be updated by the Dossier Submitter at this stage. Concerning the oral route and the other routes of exposure, as indicated in the FR report on assessment of the hazards of BPA (Anses, 2011), subcutaneous route can highlight effects at doses much lower than those administered orally and is then considered as relevant (see response to COM no. 1 for justification). Moreover, this recent French risk assessment of BPA shows that dermal exposure via thermal paper is also a major source of contamination due to high quantity of BPA on the paper and the fact that it is not embedded to matrix. We agree that the relevance of each route of exposure has to be considered, although not specified as such in the guidance. These route are often use for testing low doses (impossible to adequately assess by oral).

Part B4.1: FR acknowledges UK comment on the daily exposure.
Regarding metabolism, glucuronidation and placenta metabolism, there are a lot of contradictory studies. Due to these inconsistent results, French experts decided that

bioavailability had to be set up at 100% for the inhalation and dermal routes (based on the fact that for the dermal route, the absorption factor is unknown but 100% of the internal BPA following a dermal exposure is considered as bioavailable) and 3% for the oral route.

Section 4.11: As indicated previously FR does not consider any routes of exposure as inappropriate.

Section 4.11.1.1.5:
In the continuous breeding study weight loss is observed in the female mice in the treated groups suggestive of systemic toxicity. Nevertheless, we do not agree with the assertion that in the continuous breeding study, the decline in fertility is explained by a decrease in maternal bw. A stated in the CLH report, the bw decrease was pretty small (between 6-9%) and could not explain totally the effect observed on fertility (decrease in number of litters and their size) It was stated in the UK RAR about this continuous breeding "In the F_0 generation, no effects on fertility were seen at 300 mg/kg/day, but at dose levels of approximately 600 mg/kg/day and above, reductions in the numbers of litters produced, litter size and numbers of live pups per litter were observed in each of the 4-5 litters produced. These effects were observed in the absence of significant parental toxicity."

As indicated in the CLH we do not consider the effects observed (in liver and kidney) in the Tyl *et al*. study as a general toxicity but rather a direct systemic effect on these organs. Indeed, most of the studies using doses >=100 mg/ kg bw/d report effects on liver and kidney. It appears that these organs are target organs for effects of BPA: they develop cysts having an effect on creatinine only at high dose. This is not surprising as these organs are highly sensitive to hormones. However, there is no sign of general over toxicity at this dose of BPA in the recent NCTR study (as a part of the CLARITY-BPA, see Annex 1 of this RCOM)and there is ABSOLUTLY NO DATA showing that reprotoxicity effects observed would be secondary to the effects on kidney and liver. In this study some reproductive effects, especially in females, have been observed at doses for which the only systemic toxicity in a decrease of the bodyweight.

Section 4.11.1.1.7: To our knowledge the meiosis and oocyte development have not been investigated in the 4 guidelines studies. Concerning ovarian cysts, this effect has also been observed in rats like in the recent NCTR study performed in SD rats. These kinds of effects occur more spontaneously in aging rats than in juveniles. One could also argue that these ovarian cysts are in line with the cysts observed in other organs such as kidney and liver, rendering their appearance in ovary linked to BPA exposure rather than chance!

The recent NCTR study shows effects of EE2 in Sprague Dawley rats contradicting previous publications (Kwon et al., 2000 and Nikaido *et al*., 2005). However, it should be noted that the serum levels of estradiol measured in females' pups at PND80 after treatment of 0.5 and 5 µg/ of EE2 are both equivalent and smaller in these 2 EE2groups than in animals treated with the 2 highest doses of BPA. Therefore, the responsiveness (dose-response relationship)of SD to EE2 still needs to be clarified.

Concerning the Berger study, a mistake occurred when reporting the doses and doses 1000 times lower should be understood. Please note that in response to COM no.1 we indicated the doses expressed in mg/kg bw/day

Human information:
Concerning the epidemiological studies and their reliability, please refer to response to COM no. 7.
More specifically on 4.11.1.2.2 Effects during pregnancy/on ovaries: We agree that in epidemiological studies using an ELISA assay to measure BPA levels, this method does not allow discriminating the various forms. Nevertheless, a link between BPA exposure and effects on reproductive parameters has been reported in studies using other types of measurement like HPLC. As stated in the publication by J. Rochester (2013) "Total BPA contains both the unconjugated and the biologically inactive conjugated fractions. Thus, measurement solely of unconjugated BPA may more accurately reflect the biological activity but only a few of the human studies measured unconjugated serum BPA as the sole biomarker for BPA exposure. This measurement may be less relevant for urinary BPA, as most of the BPA excreted in the urine is the conjugated form. In fact, a few of the studies used only urinary conjugated BPA as a biomarker of exposure. An important area of future research would be to develop a scientifically sound, consistent, established protocol for measuring BPA exposure in humans in order to better carry out inter-study comparisons."

4.11.2
In your comments you underlined one of the difficulty of having such an important dataset, it is pretty difficult to compare the studies since generally the strain/way of exposure/window of exposure/doses used are different.

Concerning the Holzman rats (used in Salian *et al*. study), they are historically used in physiologically based pregnancy studies. And the variability in post-implantation loss is not mentioned in the study. Nevertheless, according to a study by Cummings *et al.* (2000) on the effect of atrazine on 4 different strains of rats, Holzman rats seemed to be more susceptible to post-implantations loss than the other strains at 100 and 200 mg/kg especially compared to Sprague-Dawley rats. But a significant effect on pre-implantation loss was also observed in Salian *et al.* study. We may agree with the fact that the sperm counts in treated animals are within the normal range. But, as you mentioned, the BPA induced a dose-dependent decrease on motility also and you cannot ensure that fertility won't be affected until it is assessed since several parameters are involved in fertility and not only the sperm count, especially in rodents.
Concerning the Aikawa study, the doses used are 0.5 and 50 µg and the sperm count is not indicated.

4.11.2.2.1.6: . Effect on reproductive organ weight in the continuous study is accompanied by a increase in liver and kidney weights. However, there is no reason for identifying whichtoxicity response (liver, kidney, reproductive organs) happens at the lowest dose as the reprotoxicity described in the report is not a consequence of hepatic or renal toxicity.

See above on the relevance of the epidemiological data.

4.11.5 Summary: We agree that BPA seemed to cause an increase in testosterone levels and that animals studies seemed to show an opposite trend. Nevertheless the effect may be different according to the dose used according to what is observed in the recent NCTR study. In this study the serum testosterone level were measured at PND80 following an exposure from GD6 to PND90 and there was first a trend to increase of the level from 2.5 to 25 µg/kg bw/day compared to the vehicle control and then a decreased from 80 µg/kg

bw/day onward to 300 mg/kg. The serum level observed in both doses of EE_2 (reference estrogen used) were between the level observed the 100 and 300 mg BPA groups. This study therefore suggest that effect of BPA exposure on the serum level of testosterone follow a non-monotonic distribution. In most of the studies, regardless of the dose or the period of exposure, an exposure to BPA leads to a decrease of the serum testosterone level except in Watanabe *et al*. (2003) in which in rats exposed by gavage to 4, 40 or 400 mg BPA/kg an increase of the Testosterone level was reported at 4 mg only.

About the ovarian cysts some have also been observed in others species than CD1 mice,. On NCTR study for example (see Annex1) ovarian cysts were observed in SD rats. As discussed previously liver and kidney develop also cysts

Finally concerning your analysis of the relevance of effects observed in animals for humans: we would like, one more time to emphasise that when comparing effects *in vivo* and epidemiological data available, some effects are consistent. For example, it has been shown that approximately three-quarters of patients with PCOS (by the diagnostic criteria of NIH/NICHD 1990) have evidence of hyperandrogenemia. Bisphenol A has been shown by Wetherill YB *et al*. (2002) to induce inappropriate androgen receptor activation. This might explain the effects of BPA on polycystic ovaries in animals and humans.

RAC's response

Noted.

Date	Country	Organisation	Type of Organisation	Comment number
10.10.2013	Sweden	ChemSec	International NGO	24

Comment received

BPA is a largely studied chemical and its health effects have been officially recognized through its 29th ATP entry (Directive 2004/73) It is also classified as specific target organ toxicity Cat. 3.

We would like to remind that the proposed classification entry of BPA follows also the initial findings of the UK CA submission in 2002 to classify it as Repr. Cat. 1B.
ChemSec supports the findings justifying a classification as at least Repr. Cat. 1B on adverse developmental effects indentified in the 2011 ANSES report. The findings conclude that animal studies show effects that could be confirmed on male sperm production, induction of ovarian cysts, endometriosis and advanced puberty in females. The ANSES 2011 study also confirms adverse behaviour effects, effects on lipogenesis and breast development but also cardiovascular diseases. There is a strong body of evidence available allowing stakeholders to presume -without doubt- that the substance has the capacity interfere with reproduction in humans.

A large amount of studies show that is acts as an endocrine disruptor on fertility at low doses. About 800 peer-reviewed studies make the case that BPA is toxic at low human exposure levels.

As with other hormones, effects are often not observed until later in the lifecycle (making the causal link to BPA exposure difficult). However in this regard, we would wish to remind that the purpose of the CLP Regulation is to ensure a high level of protection of human health and the environment (Article 1 of CLP Regulation). The assessment of the weight of

evidence and the application of the classification criteria set within the CLP Regulation need to bear in mind its underlying purpose.

A further but important issue relates to underlying test protocols used and the non-monotonic dose-response curves for BPA. It has been found since 1997 (Colerangle and Roy) that BPA is more potent at low dose effects and that the window of exposure also matters (confirmed by numerous studies such as Vandenberg et al. 2012). Several studies make the case that irreversible developmental effects are caused depending on time windows of exposure during foetal, neonatal or juvenile periods of exposed organisms (Richter et al. 2007; Palanza et al. 2008; and Soriano et al 2012). Studies indicate that BPA does affect females: alteration of ovarian cyclicity / induction of early cessation of oestrus cycles, impairs reproduction, alters mammary gland development (Rudel et al. 2011b) and induces gland neoplasia, interferes with sexual differentiation of brain, alteration of behaviour (Soto and Sonnenschein 2010). Studies also indicate BPA can interfere with spermatogenesis (vom Saal et al. 1998; Okada et al. 2008a).
Based on results from resent in vivo and in vitro studies the past assumption that BPA would be a weak oestrogen could be reversed: BPA can act through non-classical membrane-bound oestrogen receptor (Nadal et al 2000,2004 and Alonso-Magdalena et al 2005), or can bind with high affinity to estrogen related receptor (ERR-γ) (Okada et al. 2008b) or various receptors: GPR30 (Thomas and Dong 2006) and AhR (Kruger et al. 2008).

The EEA has found in its second version "Late lessons for early warnings" that the case of BPA is very revealing on where independent research deviates strongly from industry-sponsored studies (please refer to tables 10.1 and 10.2 with summaries of mammalian studies on BPA with effect level at or below 50µ/kg bw d (oral)) and the list of references pages 230-239).
It appears that 2 studies -sponsored by the society of the Plastic Industry Inc- (Tyl et al 2002 and Ryan 2010) have not identified major concerns of BPA, and have been used as the main studies for regulatory purposes because these have been done according to GLP testing protocols.
The issue of potential conflict of interests needs to be carefully considered by ECHA when ranking the various studies undertaken on BPA and put forward by various stakeholders under this public consultation.

Justification for classification as reproductive toxicant Cat 1 A:
According to the CLP Regulation, substances are to be classified in Category 1A if it is "largely based on evidence from humans". What matters for the Category 1 classification is on whether there is "a strong presumption that the substance has the capacity to interfere with reproduction in humans".

Based on the evidence available and the further attached submissions, ChemSec deems these criteria to be fulfilled.

A recent review of literature research on human health effects of BPA has been conducted by Johanna R. Rochester from The Endocrine Disruption Exchange (TEDX) [Rochester JR, Bisphenol A and Human Health: A review of the literature., Reproductive Toxicology (2013), http://dx.doi.org/10.1016/j.reprotox.2013.08.008].

A comprehensive literature search found 91 epidemiological studies linking BPA to human health effects in humans; of which 53 published were within the last year.
The review outlines this body of literature, showing clear associations between BPA exposure and adverse prenatal, childhood, and adult health outcomes, including

reproductive and developmental effects, metabolic disease, and other health effects. These studies encompass both prenatal and postnatal exposures, and include several study designs and population types.

The growing human epidemiologic studies correlating environmental BPA exposure to adverse effects in humans, along with laboratory studies in many species including primates, provides increasing support that environmental BPA exposure can be harmful to humans, especially in regards to behavioural and other effects in children.

In respect to the literature review (attached) ChemSec would wish to highlight:
- 22 studies in humans focusing on BPA and children's health outcomes that were published between 2002 and April 2011;
- 53 epidemiologic cross-sectional, prospective cohort, case report, case-control and randomised clinical trial studies have been examined;
- a further reference to 16 earlier studies is included in this literature review.

Most of the studies are also references in the HCL report submitted by France.

The shortlisted studies were analysed for quality based on the National Toxicology Program Office of Health Assessment and Translation (OHAT) approach and for rigorous assessment on strength of evidence. The review includes a sub total of 34 recent human studies (2010-2012), of which 94% indicate strong evidence on capacity to interfere with reproduction in humans .

The overview tables per category of effects on human health are provided in a separate document.

The conclusions of these findings are summed up as follows:

Fertility:
- the report indicated that there is some evidence that BPA may contribute to infertility in humans

Male Sexual function:
- the report concludes that there is a strong link between BPA exposure and male sexual function which would be strengthened by replication of findings on workers in another cohort

Reduced Sperm Quality:
- there are high quality studies showing consistent results in different populations indicating clear relation of adult BPA exposure to (decreased) sperm quality in men.

Sex Hormone Concentrations:
- the studies relating sex hormone concentration and BPA exposure are strong and fairly consistent effects across many types of populations and age groups confirming that BPA has antirational effects on circulation level of sex hormones.

Miscarriage:
There is some evidence of a relationship between recurrent miscarriage and BPA exposure in women, which may be due to an increase in chromosomal abnormalities of the oocovytes due to meiotic disruption, shown in mice.

Premature Deliveries:

There is a significant association between elevated total BPA and premature delivery.

Childhood Behaviour / Neurodevelopment
The report concludes that there is strong evidence that BPA is associated with neurobehavioral problems in children. (However it is unclear if BPA alone is responsible for the behavioural effects)

Childhood Asthma / Wheeze
The report suggests that there may be prenatal and postnatal windows of susceptibility, which may change the magnitude/direction of the health effect. Prenatal BPA exposure has been confirmed to induce asthma in mouse pups, additional longitudinal studies with different populations would be needed to verify the link.

Metabolic Disease:
The report concludes that there is strong evidence in human studies that Type-2 Diabetes is associated with BPA.
Further there is strong evidence that adult exposure to BPA is associated with cardiovascular diseases, in many populations.

Overall conclusion:
Strong evidence available today and recent human studies corroborating the animal findings confirm a strong presumption that BPA has the capacity to interfere with reproduction in humans, suggesting a HCL entry as Repr. Cat 1A.

There is strong evidence that BPA has the capacity to alter human reproduction, i.e. reduced ovarian response and IVF success, reduced fertilization success and embryo quality, implantation failure, miscarriage, premature delivery, reduced mal sexual function, reduced sperm quality, altered sex hormone concentrations, PCOS, altered hormone concentrations, blunted immune function, type-2 diabetes, cardiovascular disease, altered liver function, obesity, albuminaria, oxidative stress and inflammation, and altered epigenetic markers and gene expression.

Exposure to BPA at certain exposure windows can also cause increased spontaneous abortions / male genital abnormalities / abnormal gestation time / reduced birth weight and childhood obesity. There is particularly strong evidence of BPA effects on altered behaviour and disrupted neurodevelopment in children. There is also a strong presumption that BPA is linked with infertility.

The report submitted by France also confirms that all studies in humans assessed point out to "a correlation between higher BPA levels and different sexual parameters (quality of sperm, sex hormones, and sexual function and quality) and then strengthen the plausibility of causality." [see page 109 in the HCL report]
The epidemiological studies performed by Meerker et al. 2010, Mendiola et al. (2010) and Galloway et al. (2010) , Li et al (2010) have been addressed. Further it states that little boys among whom one or both parent(s) had an occupational exposure to BPA exhibit shorter AGD when compared to control boys, with irrevocable results. A dose-response relationship has also been established between increased BPA exposure levels during pregnancy and shortened AGD in male offspring. Effects are also recognised on the issue of IVF (Bloom et al. 2011). The report concludes that "the effects seen in men are consistent with the one observed in animals like effects on the sexual hormones and on male sexual function including sperm parameters" [See page 113 in the HCL report].

A classification to Repr. Cat 1A will lead to immediate positive effects for human health effects and environmental protection because of immediate regulatory response in terms of labelling and further restrictions for certain uses of BPA containing products. We like to remind that the effects identified by BPA are irreversible.

The full review paper is attached for your consideration.

(*ECHA note: The following attachments were provided:*
"Background information on new literature research of BPA by TEDX"
"Bisphenol A and Human Health: A review of the literature, Accepted Manuscript"
"Table 1. Studies on Bisphenol A (BPA) and Health Effects. From Rochester JR, Bisphenol A and Human Health: A review of the literature, Reproductive Toxicology (2013 in press)."
[Attachments 10-12])

Dossier Submitter's Response

FR would like to thank ChemSec for its support and the additional data provided. They should be valuable in the following of the CLH process

RAC's response

RAC considers that the human epidemiological studies are regarded as weak supportive evidence for a classification for adverse effect on sexual function and fertility, but are not robust enough to justify a classification of BPA in Repr. 1A.

Date	Country	Organisation	Type of Organisation	Comment number
09.10.2013	United Kingdom	Exponent	International NGO	25

Comment received

This document reviews the epidemiologic literature in the ANSES CLH report on Bisphenol A and is a response to the invitation from ECHA for public comments. It is divided into two sections, parallel to the ANSES report, on 1: the female reproductive system and 2: the male reproductive tract. The concern about the poor quality of many studies expressed in the report on page 10 is shared. Most importantly, nearly all the studies rely upon a spot serum or urine sample with which to determine exposure. Given the short half-life of BPA, a single sample is not representative for general exposure, a concern also raised by a joint committee of FAO/WHO (2010) and by Teeguarden et al (1). Further with few exceptions, none of the samples was collected during the etiologic relevant period for disease. The population-based studies did not collect any information from the subjects to distinguish any dietary or other behaviors and to confirm the observed BPA concentrations. Most of the studies employ a cross sectional design which can only demonstrate a mathematical correlation of the observed data and not temporal association or causal connection. After a thorough review of the epidemiology studies included in the ANSES report, we conclude that the inconsistent results, in combination with the poor quality, do not provide additional evidence for BPA toxicity. The inconsistency, null findings, doubtful results and contradictory findings in the 20 epidemiology studies are best compatible with a situation where there is no biologically plausible relationship that can be established. Consequently, the presence of any robust adverse health effects caused by BPA can be excluded.

(ECHA note: The following attachment was provided:
"Review of the epidemiology studies described in the ANSES 2013 report on harmonized classification and labeling of Bisphenol A"

[Attachment 13])
Dossier Submitter's Response
FR thank you for the document you submitted and your analysis. FR does not agree with the statement that there is no evidence that BPA is a reproductive toxicant for humans. Please see the detailed answer in response to COM no. 7.
RAC's response
RAC consider that the human epidemiological studies are regarded as weak supportive evidence for a classification for adverse effect on sexual function and fertility, but are not robust enough to justify a classification of BPA in Repr. 1A.

Date	Country	Organisation	Type of Organisation	Comment number
09.10.2013	Germany		MemberState	26
Comment received				
In the present CLH proposal on BPA predominantly „low-dose effects" are proposed as leading effects for an upgrade of classification for reproductive toxicity from Repr. 2H361 to Repr. 1BH360F. The hypothesis of low dose effects in terms of adverse effects relevant for human health, however, is far from being consented by the scientific community and it is still a matter of scientific debate. The observation of „low-dose effects" of any substance including BPA is currently considered insufficient (and not appropriate) to serve for the scientific justification of regulatory measures such as classification and labeling of chemical substances. With regard to low-dose effects, it is to note that the dose-relationship of the observed effects gives in general the strongest evidence on the causal proof that the effect of concern is related to the substance administered. Despite the absence of monotonic dose-related effects, as seen in many study using very low doses, the observed effects at singular doses (independent of the dose level) are explained as evidence of non-monotonic effects caused by BPA without any plausible mode of action for the non-monotonic action. The DS interpretation of monocausality of low-dose, non-monotonic effects that often showed diverse directions of their responses and contradictory effects is seen as rather uncertain and from a scientific aspect considering the strength of evidence as non-justified. In addition consistency on each of the observed 'low-dose' effects across studies is rather limited. The CLH report appears to weigh the evidence from studies that are in compliance with OECD test guidelines less than the indications from low dose studies using artificial applications. In order to support Cat 1 B the criteria require that clear evidence on the adverse effect should be given. Instead, some evidence and inconsistencies across effects and discrepancies between studies confirms the present classification Cat 2. Preference should be given to oral and dermal studies following the OECD testing regimen, in particular studies testing multiple doses should be considered as key studies. Those studies using invasive administration routes (e.g. subcutaneous injection or implantation of pumps) were not in compliance with standard requirements of OECD test guidelines. The relevance of these studies using other administration routes than the oral, dermal or inhalation route for classification purposes is questioned. Evidence from such studies can only be used as giving supportive evidence, if they are (as regards the observed effects) coherent to the outcome of those studies with relevant routes. To assess the dose-dependency of effects, their outcome should be assessed taking into account the uncertainties as regards the dermal absorption rate and differences in metabolism due to				

different sites of administration (dermal vs. subcutis).

With regard to effects on the female reproductive system, it has been stated that the guideline (key) studies contradict the findings of other studies. However, a critical appraisal as regards the robustness of low dose studies using artificial dosing and their contribution to the overall evidence was not conducted. The limited evidence for fertility effects as seen in the multigeneration study (Tyl et al., 2002, 2008, Ema et al., 2001, NTP, 1985a) that are key for the decision on Cat 2 or Cat 1B taking into account the effects seen in the other studies with oral administration (Mendoza-Rodriguez et al., 2011, Ryan et al., 2010, Rubin et al., 2001, Hunt et al., 2003) is limited. E.g. the effects on the oestrus cycle were rather inconsistent across studies and the observation of meiotic abnormalities at low doses in the Hunt study was not confirmed by any other (multigeneration) study. Similar shortcomings may also apply to the section on effects on the male reproductive tract. E.g., assumed key effects on sperm production (reduction) as obtained from the studies of Chitra et al., 2003 or Herath et al., 2004 were not seen in the multigeneration studies (Tyl et al., 2002, 2008) and are of questionable significance.

Dossier Submitter's Response

FR would like to thank DE for their comments and notes its reluctance to change Bisphenol A classification.

RAC's response

RAC considers that the adverse effects on fertility and on male and female reproductive organs reported in the guideline studies and supported by the non-guideline studies are considered not to be a secondary non-specific consequence of other toxic effects. The MoA is considered to be relevant to humans. Human data also provides some evidence of the BPA effects on fertility. RAC considers that the criteria for Repr. 1B for adverse effects on sexual function and fertility is fulfilled.

Date	Country	Organisation	Type of Organisation	Comment number
11.10.2013	Switzerland	Dow Europe GmbH	Company-Manufacturer	27

Comment received

See public attachments.

(*ECHA note: The following attachments were provided:*
"Annex A - BPA REACH Consortium CLH male and female endpoints 10-10-2013"
"Annex B - BPA REACH Consortium CLH information on Tyl 10-10-2013"
"Annex C - BPA REACH Consortium CLH summary NCTR-2013 10-10-2013"
"Annex D - BPA REACH Consortium CLH overview relevant studies for BPA classification 10-10-2013"
"Annex E - BPA REACH Consortium CLH review epidemiology studies described in the CLH proposal 10-10-2013"
"BPA REACH Consortium comment on CLH proposal on BPA prepared by ANSES 10-10-2013_final"
[Attachments 1-6])

Dossier Submitter's Response

FR acknowledges your comment and invites Dow to see FR responses to the Reach Centrum BPA Consortium (COM no. 21).

RAC's response

Noted.

Date	Country	Organisation	Type of Organisation	Comment number
04.10.2013	Norway		MemberState	28
Comment received				
The last time the classification of BPA for human health was discussed and decided, in TC C&L in 2002, the proposal from UK was to classify the BPA as Repr. Cat 2; R60. Nevertheless some members stressed the fact that classifying the BPA as Repr. Cat. 2 was a borderline it was agreed to rather classify as Repr. Cat. 3. Since 2002, several new studies with more mechanistic data, supporting a Repr. 1B classification, have been published We support the proposal to classify BPA for reproductive toxicity with Repr. 1B - H360F based on the weight of evidence of numerous animal studies where it appears that BPA impacts the male reproductive system with effects on the seminiferous tubules, the reproductive hormones levels and the quantity and quality of sperm. In female animals an increased occurrence of ovarian cysts or disturbance of estrous cycle are observed in all studies. Decrease in the number of pregnancies and implantations was systematically reported and pre-implantation loss seems to be responsible of the effect of BPA on fecundity in rodents. These observations corroborate risks identified in humans through epidemiological studies. There are methodological limitations in the epidemiological studies. Human data should therefore be considered as additional/supportive evidence in the weight of evidence for classification with Repr 1B, but not sufficient for a classification with Repr. 1A.				
Dossier Submitter's Response				
FR would like to thank NO for its support to the proposal of upgrading BPA classification for reproduction. FR also notes the NO point of view on the weight of the epidemiological studies.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
11.10.2013	Belgium		MemberState	29
Comment received				
We welcome the FRENCH proposal on the BPA classification. Please find our comments in attachment. (*ECHA note: The following attachment was provided:* *"Proposal for harmonised classification and labelling : Bisphenol A"* *[Attachment 8])*				
Dossier Submitter's Response				
FR would like to thank BE for their comments. Concerning the Sakaue *et al.* study, FR agrees that a mistake occurred in the summary table concerning this study on the length of exposure and the effects observed. FR agrees and welcomes the observation (As stated in Annex 1 About the recent NCTR study) that it is difficult to compare the various studies available since the way of exposure, the window of exposure, the species and the strains used or the doses vary a lot. But FR considers that all the positive studies contribute to the weight of evidence and cannot be				

dismissed.

FR also agrees, as mentioned in the CLH report, especially in Tyl *et al.* (2008) that some of the effects observed seem to suggest an effect during lactation. Nevertheless FR chooses to address the effects on fertility only and for resources problems couldn't address the effects that could occurre on development. But FR would welcome another MS initiative to cover this point.

Concerning the epidemiological studies, FR agrees that the existing literature is still limited and requires further investigation. Epidemiological studies were included, as mentioned, as supportive evidence but, as stated in the report, FR suggest only a discussion on a possible classification as 1A. Please see the detailed answer about human information in response to COM no. 7.

About the assessment of effects on development, please refer to response to COM no. 1.

FR thanks BE for their editorial comments although the CLH report will not be updated by FR at this stage as mentioned in the new process.

RAC's response

Noted.

Date	Country	Organisation	Type of Organisation	Comment number
11.10.2013	Sweden		MemberState	30

Comment received

The Swedish CA agrees that there is sufficient evidence from studies in animals for concluding that bisphenol A (Cas No 80-05-07) produces adverse effects on the male and female reproductive system that warrants classification in Repr. 1B; H360F.
Based on the weight of evidence of numerous animal studies it is concluded that bisphenol A affects the male reproductive system in rats and mice after in utero exposure (in absence on major maternal toxicity). Effects were also seen after neonatal or peri-pubertal exposure as well as after exposure of adult animals. The observed effects on the reproductive system varied with the age of the animal at the time of exposure and included effects on fertility, effects on the seminiferous tubuli, effects on reproductive organ weights as well as effects on sperm production, sperm quality and level of the reproductive hormones. Bisphenol A also produces adverse effects on the female reproductive system. A large number of recent studies is available and several kinds of effects which may impair the fertility were observed. We agree with the notion presented in the report that this leads to consider the weight of evidence instead of basing the conclusion on few key studies limited to very specific models. Effects were observed following exposure during the prenatal, postnatal and adult stage, including increased occurrence of ovarian cysts and disturbance of the estrous cycle, which were observed in all of the animal studies presented in the report, and decrease in the number of pregnancies and implantations. All these findings support that a classification in Repr. 1B (H360F) is warranted. In addition, epidemiological studies also indicate that bisphenol A could be adverse for human reproduction and therefore the SE CA would welcome a discussion within RAC weather the data from these studies are robust enough to support a Repr. 1A (H360F) classification.

Dossier Submitter's Response

FR would like to thank SE CA for its support to the proposal and as indicated in the CLH dossier would welcome a discussion on the robustness of the epidemiological studies.

Conclusion of RCOM : Various hypothesis could explain the effects seen in the different studies which report effects of BPA on male or female fertility.

- As suggested in the CLH report, one explanation could be the pre-implantation loss:" When exposure occurs during adulthood, BPA induces an increase in total number of resorption after intragastrically administration (Al Hiyasat *et al*., 2004) or induces a decrease of the number of pregnancies and implantations following subcutenaous injections (Berger et al., 2007, 2008 and 2010). This effect was also found after a postnatal exposure (Bosquiazzo *et al*., 2010 and Varayoud *et al*., 2011) and can thus be considered as proven for these two windows of exposure. In the 3-generation study performed by Tyl, although the percent of post-implantation loss was unaffected, the number of implants, total pups, and live pups per litter were significantly reduced at 7500 ppm for all generations but not in the 2nd study up to 3500ppm (Tyl *et al*., 2008). The authors do not explain this difference. The decrease observed is probably due to pre-implantation loss that could not be evaluated in this study, by the time the parental females were scarified. However, as demonstrated in the studies performed by Berger *et al*., decrase in litter size seems to be mediated by a disruption of intrauterine blastocyst implantation rather than by a post-implantation effect. Therefore it can't be excluded that the decrease in implants and the reduced live litter size at birth observed in this study may result from a pre-implantation disruption".

- The complex effects on reproductive hormones could also explain part of the effects seen. IT stated: "The effects of BPA are more complex than being simply "estrogenic" and hint to modes of action modulated by dose, sex and lifestage; species- and strain-related susceptibility have been observed, mice appearing as less susceptible. Indeed, the results obtained from the 90day NCTR study show that BPA could have an non-monotonic androgenic/ anti-androgenic effect. This interplay among factors modulating BPA effects may partly explain why some studies contradict the majority of findings. This could maybe also explain why the levels of circulating estradiol in the NCTR study in animals treated with BPA are more affected than animals exposed to EE_2.

The comments obtained helped us to sort out the sata which tend to indicate that rat are la less sensitive species than mice and that gavage often leads to negative (or positive only at high dose) studies.

We therefore propose that a classification Repr. 1B–H360F is warranted (Repr. Cat. 2; R60 according to Directive 67/548/EEC). Classification in Repr. 2 is not appropriate since there is more than some evidence of effects in animals or humans, the effects observed are sufficiently convincing to propose at least a classification in category 1B.

RAC's response

RAC considers that the adverse effects on fertility and on male and female reproductive organs reported in the guideline studies and supported by the non-guideline studies are considered not to be a secondary non-specific consequence of other toxic effects. The MoA is considered to be relevant to humans. Human data also provides some evidence of the BPA effects on fertility. RAC considers that the criteria for Repr. 1B for adverse effects on sexual function and fertility is fulfilled.

ATTACHMENTS RECEIVED

1. **Annex A - BPA REACH Consortium CLH male and female endpoints 10-10-2013** [Filename: Annex A - BPA REACH Consortium CLH male and female endpoints 10-10-2013] submitted by:
   Dow Europe GmbH (on 11/10/2013)
   Momentive Specialty Chemicals B.V. (on 10/10/2013)
   Bayer MaterialScience AG (on 10/10/2013)
   ReachCentrum BPA Consortium (on 10/10/2013)
   [Please refer to comments 19, 20, 21, 27]

2. **Annex B - BPA REACH Consortium CLH information on Tyl 10-10-2013** [Filename: Annex B - BPA REACH Consortium CLH information on Tyl 10-10-2013] submitted by:
   Dow Europe GmbH (on 11/10/2013)
   Momentive Specialty Chemicals B.V. (on 10/10/2013)
   Bayer MaterialScience AG (on 10/10/2013)
   ReachCentrum BPA Consortium (on 10/10/2013)
   [Please refer to comments 19, 20, 21, 27]

3. **Annex C - BPA REACH Consortium CLH summary NCTR-2013 10-10-2013** [Filename: Annex C - BPA REACH Consortium CLH summary NCTR-2013 10-10-2013] submitted by:
   Dow Europe GmbH (on 11/10/2013)
   Momentive Specialty Chemicals B.V. (on 10/10/2013)
   Bayer MaterialScience AG (on 10/10/2013)
   ReachCentrum BPA Consortium (on 10/10/2013)
   [Please refer to comments 19, 20, 21, 27]

4. **Annex D - BPA REACH Consortium CLH overview relevant studies for BPA classification 10-10-2013** [Filename: Annex D - BPA REACH Consortium CLH overview relevant studies for BPA classification 10-10-2013] submitted by:
   Dow Europe GmbH (on 11/10/2013)
   Momentive Specialty Chemicals B.V. (on 10/10/2013)
   Bayer MaterialScience AG (on 10/10/2013)
   ReachCentrum BPA Consortium (on 10/10/2013)
   [Please refer to comments 19, 20, 21, 27]

5. **Annex E - BPA REACH Consortium CLH review epidemiology studies described in the CLH proposal 10-10-2013** [Annex E - BPA REACH Consortium CLH review epidemiology studies described in the CLH proposal 10-10-2013] submitted by:
   Dow Europe GmbH (on 11/10/2013)
   Momentive Specialty Chemicals B.V. (on 10/10/2013)
   Bayer MaterialScience AG (on 10/10/2013)
   ReachCentrum BPA Consortium (on 10/10/2013)
   [Please refer to comments 19, 20, 21, 27]

6. **BPA REACH Consortium comment on CLH proposal on BPA prepared by ANSES 10-10-2013_final** [Filename: BPA REACH Consortium comment on CLH proposal on BPA prepared by ANSES 10-10-2013_final] submitted by:
   Dow Europe GmbH (on 11/10/2013)
   Momentive Specialty Chemicals B.V. (on 10/10/2013)
   Bayer MaterialScience AG (on 10/10/2013)
   ReachCentrum BPA Consortium (on 10/10/2013)
   [Please refer to comments 19, 20, 21, 27]

7. **Bisphenol classification** [Filename: Bisphenol classification] (submitted by Italy on 11/10/2013. *Contents of attachment copied under Comment number 16*)

8. **Proposal for harmonised classification and labelling : Bisphenol A** [Filename: Bisphenol A_ Public Consultation] (submitted by Belgium on 11/10/2013) *[Please refer to comment 29]*

9. **Comments on the proposal for harmonized classification and labeling of Bisphenol A.** [Filename: BPA com CLH submitted to ECHA at 11 Oct 2013] (submitted by the Netherlands on 11/10/2013) *[Please refer to comments 1,15]*

10. **Background information on new literature research of BPA by TEDX** [Filename: Background information human studies TEDX] (submitted by ChemSec on 10/10/2013) *[Please refer to comment 24]*

11. **Bisphenol A and Human Health: A review of the literature, Accepted Manuscript** [Filename: Rochester2013AcceptedManuscript] (submitted by ChemSec on 10/10/2013) *[Please refer to comment 24]*

12. **Table 1. Studies on Bisphenol A (BPA) and Health Effects. From Rochester JR, Bisphenol A and Human Health: A review of the literature, Reproductive Toxicology (2013 in press)** [Filename: Rochester2013Table1] (submitted by ChemSec on 10/10/2013) *[Please refer to comment 24]*

13. **Review of the epidemiology studies described in the ANSES 2013 report on harmonized classification and labeling of Bisphenol A** [Filename: Review of the epidemiology studies] (submitted by Exponent on 10/10/2013)
    [Please refer to comments 9, 25]

CONFIDENTIAL ATTACHMENT

14. **Bisphenol A and Human Health: A review of the literature** [Filename: BPAAccepted] (submitted by the European Environmental Bureau (EEB) on 11/10/2013)
    [Please refer to comment 14]

Annex 1:

As indicated by the BPA REACH Consortium (please refer to the third attachment of this COM: Annex C), the report of a study conducted by the US NCTR (National Center for Toxicological Research) entitled "Evaluation of the toxicity of Bisphenol A (BPA) in Male and Female Sprague-Dawley Exposed Orally from Gestation Day trough Postnatal Day 90" was recently finalized. This preliminary study to a perinatal 2-year guideline chronic rodent toxicity study on BPA is a part of the CLARITY-BPA program, through which «NIEHS has established an unprecedented level of collaboration among extramural grantees and regulatory researchers. By drawing upon the strengths of academic and regulatory expertise and research approaches, CLARITY-BPA represents a potential new model for filling knowledge gaps, enhancing quality control, informing chemical risk assessment, and identifying new methods or endpoints for regulatory hazard assessments».

In this preliminary study, BPA was administered by oral gavage from gestation day 6 through the start of labor and then directly to pups from postnatal day (PND) 1 (day of birth = PND 0) until termination at PND 90 ± 5 to Sprague-Dawley rats from the NCTR breeding colony (Sprague-Dawley/CD23/NCTR BR). BPA doses were 2.5; 8; 25; 80; 260; 840; 2,700; 100,000 and 300,000 µg/kg body weight (bw)/day. The pups were directly dosed due, as explained by the authors, to their expectation that transfer to pups through milk would be low. In was demonstrated in a parallel study (Doerge *et al*., 2010) that the serum levels of total BPA in the nursing pups were approximately 300-fold lower than the serum levels in the dams.

Vehicle (0.3% carboxymethylcellulose) and naïve control groups were included to assess any effects of the gavage procedure on the endpoints measured. Two doses (0.5 and 5.0 µg/kg bw/day) of the synthetic estrogenic substance ethinyl estradiol (EE_2) were also included as the reference estrogen.

Various effects were observed in this study but for most of them only at highest doses of BPA used in the study meaning 100 and 300 mg BPA/kg bw/day.

First both the 100 and 300 mg BPA/kg bw/day doses statistically depressed gestational body weight gain by 11% and 16%, respectively. As observed in the positive control (reference estrogen) used (Ethinyl estradiol) for which a reduction of the gestational body weight gain by 7 and 14% respectively for 0.5 and 5.0 µg EE_2/kg bw/day. Therefore, effects of high doses of BPA on gestational BWG was coherent with what observed with EE2.
Some effects were observed on the mammary gland like a ductal adenocarcinoma in the 2.5 µg BPA/kg bw/day group. Moreover the incidence of mammary gland ductal hyperplasia was increased at 100 and 300 mg BPA/kg but reached the statistical significance for the 300 mg BPA group only. No significant effects were observed in the low BPA dose range. This effect on the mammary gland may explain the reduction of pups bodyweight during the lactation period, considering that the bw at birth was not affected. Same reduction during the lactation period was observed in mice pups in the Tyl *et al.* study (2008) at the highest dose.

Various effects were observed in the pups:

Effects on the reproductive tract in females:

A reduced post-wean bodyweight (8-9% for the 100 and 6-13% for the 300 mg BPA groups). The pre-wean bodyweight was also affected in the 300 mg BPA group (decline of 12-16%), as well as the survival of the pups (72.4%).

Cycle

The cyclicity of the females' pups was assessed first during PND 69-90 and also through PND 150-170. In the 300 mg BPA group 63% of the females were asynchronous, in comparison 94% and 100% of the reference estrogen group (0.5 and 5.0 µg EE_2 respectively) were found asynchronous. And in the 100 and 300 mg BPA groups females had abnormal estrous cycles. For the 100 mg BPA it was for the period PND 150-170 only (100% of the females). And for both periods for the 300 mg BPA group (90 and 100% of the females respectively). It should be noted that for both period, control vehicle group was also affected since 7 females out of 20 and 10 females out of 16 had abnormal cycles for PND 69-90 and PND 150-170 respectively. For the low doses of BPA there was a significant increase in vaginal metestrus prevalence in the 2.5 and 25 µg BPA/kg bw/day dose groups with a significant change in the overall stage prevalence profile at the latter dose. Changes in other organs of the female reproductive tract were not seen at any BPA doses. The only finding was a decreasing trend in estrus prevalence in the uterus. Nevertheless this effect on the estrous cycle is coherent with what was observed by Kato *et al*. (2003) in Sprague-Dawley rats subcutaneously injected with an equivalent of 26, 105 or 427 mg BPA/kg bw/day from PND 0 to PND9. 2 out of 8 in the mid dose and 0 out of 6 in the high dose had a normal estrous when assessed during PND 61-94. Same was observed in the study by Fernandez *et al*. (2009) in which altered estrous cyclicity was reported, with the high dose causing permanent estrus. In this study Sprague-Dawley rats were treated from PND1-10 with doses ranging from 2.5 to 62.5 mg BPA/kg bw/day. Contrary to Kwon *et al.* study in which SD rats were exposed by gavage to 3.2, 32 or 320 mg BPA/kg bw/day and no effects were reported on the estrous cycle. Mendoza-Rodriguez *et al*. (2011) showed also in rats received BPA at approximately 1.2 mg/kg bw/day during gestation and lactation that almost 80% of F1 offspring had irregular estrous cycles. The other multi-generations studies in rats such as Tyl *et al*. (2002) or Ema *et al.* (2001) do not reported any effects on the estrous cyclicity, even at high doses (see below for details). This shows that guideline studies can also be incoherent.

Cysts

In the NCTR study, cysts in the ovaries were observed at the 300 mg BPA dose, in 14 out of 19 animals with a high severity profile. No cysts were observed at the lower doses. This effect is often observed following a perinatal exposure to BPA, since some were observed in the Kato *et al.* (2003) and Fernandez *et al.* (2010) studies. Effects were observed at 25 mg BPA/kg onward for the Fernanadez *et al.* study. In Kato *et al.* study, SD rats were given sc BPA et 0.25, 1 or 4 mg/pup from PND0 to PND9. In this study early vaginal opening, irregular estrous cycles, a decrease in the area occupied by the corpora lutea (CL) in the ovary, and multiple cystic follicles in the ovary (4 animals out of 8 compared to 0 in controls) were found in the animals treated neonatally with 1mg BPA. Additionnaly to these effects (with 5 animals out of 5 with polycystic ovaries) at 4 mg BPA/pup unusual body weight gains (bw was significantly less than control from PND9 to 30 – corresponding to the

lactation period, but greater on any day after PND61) and lack of CL were noted. Several other studies reported cysts in ovaries following an exposure to BPA in rats (Adewale *et al*., 2009) or in mice (Newbold et al., 2009; Signorile *et al*., 2010 ; Karavan *et al*., 2012). In Newbold *et al*. study, cysts in ovaries were observed at doses as low as 1 µg BPA/kg bw/day.

Cysts were also observed, in the NCTR study, on the follicles as well as an increase of the depletion of the corpus luteum and a depletion of the antral follicles. Effects on the follicles were also observed in other studies like in Karavan *et al.* study (2012) where the development of the follicles was impaired. In Adewale *et al.* (2009) some hemorrhagic follicles were observed in rats treated with 50 µg BPA or 50 mg BPA. In this study the number of corpus luteum was reduced at 50 mg BPA. In the study by Newbold *et al.* (2007) a dose dependent (mice were treated with 10, 100 or 1000 µg/kg bw/day) decrease in the number of corpus luteum as well as in Fernandez *et al.* study (2010) in which Sprague Dawley rats were treated with low doses of BPA. In this study an impairment of the development of the follicles was also reported meaning a reduced antral follicles and increased number of atretic follicles. In the study by Rodriguez *et al.* (2010) the development of the follicles was also affected. And finally in Kato *et al*. study (2003) a decrease in the number and area of the corpus luteum was observed.

Then, the different effects observed on the ovaries in the NCTR study were also reported in previous studies in which animals were exposed to BPA *in utero* and/or during the post-natal period (before weaning), although effects were generally observed at lower doses in the existing studies. Why this new study provided by NCTR does not reproduce these effects at lower doses is unknown.

However, it should be noted that none of these effects on estrous cyclicity and cysts in ovaries was reported in the Tyl *et al.* study (2002), in which males and females CD Sprague Dawley rats were exposed to BPA (approximately the same range of doses that the latest study from NCTR) at any dose for any generation or at a non significant level, although the highest dose was much higher than in the NCTR study. Estrous cycle were not modified either in the Ema study (Ema *et al*., 2001) with 92-100% of females with normal estrous cycles except at 20 mg BPA in F1 with only 76% of the females with normal estrous cycles, while ovaries morphology seems not to have been evaluated. The study ran by NTP in 1985 on mice by continuous breeding did not assess these endpoints. However Tyl *et al*. in 2008 assessed these endpoints in mice exposed by gavage. No cysts were reported although histopathology of ovaries was evaluated. These data show that there is inconsistencies of effects observed in the guideline studies at high doses. On the other end, the guideline studies are consistent in the fact that they do not describe any finding at low doses. Why effects at these low doses are therefore exclusively described in scientific non GLP studies can also not be explained. Route of exposure seems to play a role as gavage studies being most of the time negative or mildly positives. However, this prarameter cannot per se explain the discrepancies observed between the studies and specie/ strain, window of exposure, dosage… also impact the response to BLA.

Moreover in the NCTR study a statistically significant dose-dependent increase of cystic endometrial hyperplasia in the uterus for the highest doses of BPA (significant for 300 mg only). For the low doses there was only a low incidence of this hyperplasia of the uterus and the effect was not dose-dependent (5% in the vehicle, 22% in 8 µg BPA/kg bw/day, 9.5% in 25 µg BPA/kg bw/day, 10% in 840 µg BPA/kg bw/day, and 5% in 2,700 µg BPA/kg

bw/day) and is significant for the 8 µg dose only. This effect has been observed in other studies like in Newbold *et al.* (2007 and 2009) in which cystic endometrial hyperplasia was observed at 100 µg/ kg bw/day in CD-1 mice. Signorile *at al.* (2010) also reported this effect at 100 and 1000 µg/ kg bw/day in mice also. This parameter has not been assessed in any of the Tyl *et al.* studies, in fact no multigeneration study mentioned this type of effect.

In the NCTR study, the hormone levels were also affected by the BPA exposure since estradiol (assessed at PND80) and TSH (assessed at PND90) levels were increased in both 100 and 300 mg BPA groups. This is not in line with previous results reported from guideline study (Ema *et al*., 2001) where no treatment related changes were observed in any of the serum hormone levels measured except significant decreases in serum LH levels at 0.2, 2, and 20 mg/kg and in serum T3 levels at 200 mg/kg were found in F0 females. No significant differences in any serum hormone levels of male and female F1 adults were noted between the control and BPA-treated groups. In the NCTR study both hormone were not affected at all.
Additionally, it is very surprising to see that in the NCTR study, the level of circulating estradiol in females at PND80 in the reference estrogen group were lower than in high doses of BPA groups: 13.76 and 13.21 pg/mL for 0.5 and 5.0 µg EE_2/kg respectively versus 17.11 and 21.87 pg/mL for 100 and 300 mg BPA/kg respectively. Moreover, there is no dose dependent increase of the circulating estradiol with increasing dosing with EE_2, since the circulating level of estradiol are quite the same for both doses of EE_2 used as reference estradiol and is even lower for the highest dose. It is therefore possible to consider the choice of this reference estrogen as questionable or at least finding the results surprising.

In the NCTR study, cholesterol level was reduced in 100 mg BPA group so did the progesterone and prolactin in the 300 mg BPA group. These hormone levels weren't affected in Mendoza-Rodriguez *et al.* study (2011) despite the fact that authors reported irregular estrous cycles when Wistar rats were treated with 1.2 mg BPA/kg bw/day from GD6 to PND21 in drinking water.

Therefore several effects were observed in female pups exposed in utero and post-natally to BPA, mostly in high doses. In one hand theses effects are observed almost in high doses groups only in another hand the effects reported in this preliminary study are generally not observed in previous GLP compliant multigeneration studies at similar or higher doses.

Effects on the reproductive tract in males:

As for the females, in the NCTR study, the pre-wean survival and bodyweight were affected in the 300 mg BPA group with a decline of 9-12% in the pre-wean bodyweight and 73.8% of survival. There was also a reduction in the post-wean bodyweight for this group.

Concerning the hormones, following an exposure to 100mg BPA/kg bw/day the T_3 level was increased at PND15 and the cholesterol level was decreased at PND90. About the testosterone, in this study the serum testosterone level were measured at PND80 following an exposure from GD6 to PND90 and there was first a trend to increase of the level from

2.5 to 25 µg/kg bw/day compared to the vehicle control and then a decreased from 80 µg/kg bw/day to 300 mg/kg, indicating that effect of BPA on testosterone serum level might follow a non-monotonic distribution. The serum level observed in both doses of EE_2 (reference estrogen used) was between the 100 and 300 mg BPA groups. In most of the studies, regardless of the dose or the period of exposure, an exposure to BPA leads to a decrease of the serum testosterone level except in Watanabe *et al*. (2003) in which in rats exposed by gavage to 4, 40 or 400 mg BPA/kg an increase of the Testosterone level was reported at 4 mg only.

Additionally, the AGDI (Anogenital Distance Index i.e. AGD divided by the cube root of body weight) was increased when measured at PND90 in the 300 mg BPA group by 6.5% compared to control. Nevertheless the AGD remained unaffected. Both parameters remained unaffected at PND1. Therefore this previous observation on AGDI at PND90 was considered by the authors as chance observations of no biological significance. These parameters were also not modified in a bunch of scientific studies: In 2011, LaRocca *et al.* exposed C57/Bl6 mice via oral gavage to either vehicle (sesame oil; n=12), 50 µg (n=11) or 1000 µg /kg (n=14) of BPA (purity > 99%) from GD 10 through 16. The positive control group (n=14) received 2µg/kg of DES. Only the DES treated group had an AGD increased. Kobayashi *et al.* (2012) saw no effects neither on AGD nor on AGD index in twenty males and twenty female weanling pups from female Sprague-Dawley rats (9 weeks of age) treated with 0 (control), 0.33, 3.3 or 33 ppm (corresponding to approximately 0.05, 0.5 and 5 mg/kg/d) of BPA (purity > 99.6%) in the diet from GD 6 trough PND 21 (n=12).

However, AGD parameter but not AGDindex was modified in a previous guideline study. The effect of BPA on fertility was evaluated in an oral two generation reproduction toxicity study in Crj;CD (SD) IGS rats (Ema *et al.*, 2001). by gavage up to 200 µg/kg/day BPA during a premating period of 10 weeks for males and 2 weeks for females and a 2-week mating period. Compared to controls, a statistically significant decrease (<5%) in AGD was seen in F1 males at 0.2, 20 and 200 µg/kg/day, and F2 males at 20 and 200 µg/kg/day. These decreases were not statistically significant when the ratio of the AGD to body weight was determined (the AGD is correlated with body weight).

The age of the testicular descent was modified for the 300 mg BPA group, it occurred 2 days later than in controls. The 260 µg BPA was also significantly affected for this parameter. There were no difference-in body weights at the time of the testicular descent in both affected groups. This effect has been reported in the study by Tyl *et al*. (2008) at 600 mg.

AGD, AGD index and testicular descent have been evaluated in epidemiological studies. In Miao *et al*. (2011) dose-response relationship has been observed with increased BPA exposure levels in pregnancy associated with greater magnitude of shortened AGD in male offspring, with a statistically significant trend for the association ($p = 0.008$). In another study by Fénichel *et al.* (2012) authors showed no correlation between fetal *in utero* exposure to unconjugated BPA (as measured in cord blood) and the physiopathology of undescended testes unlikely.

The sperm parameters were not significantly affected in any of the BPA groups. Nevertheless, increased incidence of seminiferous tubule giant cells was reported at a single

dose (2.5 µg/kg bw/day). This effect was reported in other study such as Tan *et al.* (2003) in which Sprague-Dawley rats exposed from PND 23-53 at 100 mg/kg bw/day via their food and giant cells were observed in the seminiferous tubules. Iida *et al*. (2002) also noticed effects of a BPA exposure on the histology of the seminiferous tubules. In this study ddY mice were exposed to 1, 10 or 100 mg of BPA by oral route from GD10 to GD17. These histopathological abnormalities were observed at PND 60 and 120 at all doses.

Concerning the organs, few statistically significant effects were observed. There was a decrease in the weight of the epidydimal fat pad for both high doses but it was only significant for the 300 mg BPA group. There were also a dose dependent decrease of the seminal vesicles weight from 2.7 mg BPA onward but these changes were not statistically significant. Similarly, a decrease in the ventral prostate weight by almost 20% occurred in the highest dose group but this change was not statistically significant.

Other effects:
Concerning the systemic toxicity reportedBPA exposure induced some cysts in the renal tubules. This effect was dose dependent (according to the statistical analysis) for both highest doses of BPA since there was 1 animal affected (out of 20) in the vehicle control group, 8 (out of 21) in the 100 mg BPA group and finally 12 (out of 19) animals affected in the 300 mg BPA group with a higher severity profile. Nevertheless, others doses of BPA were also affected since for all low doses between 5 and 8 animals were affected, then the significance was reached for the 25, 80, 260 and 2,700 µg BPA groups. Surprisingly, the lowest dose of EE_2 (0.5 µg/kg) induces cysts in the renal tubules (7 out of 20 animals) but not the highest dose (5.0 µg/kg – 2 animals affected out of 20). But one could again note that the effects observed in the "reference estrogen" group are really comparable to what is observed for high doses of BPA, and explain these effects rather as a direct effect on these organs like on reproductive organs rather than a global systemic toxicity.

Others effects in kidneys were also reported like nephropathy but first this effect was not dose-dependent since the significance was reached for the 25 µg and 2,700 µg BPA groups only. Moreover the vehicule control was also affected since 12 animals out of 20 showed this pathology, and highest doses of BPA were affected similarly to the vehicule control group (13 out of 21 and 12 out of 19 for the 100 and 300 mg BPA groups respectively).

Effects of BPA on kidney have already been described in various studies:
In the Fertility Assessment by Continuous Breeding" (NTP, 1985b), CD-1 mice showed effects at necropsy of the F0 generation (controls and top dose group only). Treatment-related effects were seen at the highest dose level; for both sexes relative liver weight was increased about 28% and relative combined kidney weight increased 10-16% compared to controls. At necropsy of the F1 generation, treatment-related effects of similar magnitude were generally observed in males and females; compared to controls, increased relative liver weights (6-29%) and kidney weight (13-20%) were observed in all treated groups.
The 2-generation study (OECD 416) (Tyl *et al.*, 2008), where mice were exposed by gavage showed increased kidney and liver weight from 300 ppm and onward for F0 males, from 0.018 ppm in F1 parental males, in F0 and F1 females and in F1 & F2 pups (male and females) at 3500 ppm. Concerning the liver, in the Tyl *et al*. study (2002) the absolute weight was not affected in females except for the F3 generation for the females (significant

decrease p<0.001 at 7500 ppm); F0 and F2 were significantly affected when the liver was expressed as a percent of the bodyweight (increase at 7500 ppm only). In the NCTR study, the absolute liver weight was not affected in any treated group (except for the "reference estrogen" control for which both doses induced a significant rise of the absolute liver weight and the weight relative to the bw), but the 300 mg BPA induced an increase of the liver weight relative to the bw in females. Concerning males low doses of BPA do not exert any effect on the weight of liver or kidney. The EE_2 induces a significant increase of the relative liver weight. In the high doses of BPA, there was a significant decrease of the kidney and liver weights in the 300 mg BPA group only. The same effects was observed in Tyl study (2002) in rats at 50 and 500 mg. the opposite was observed in mice (Tyl *et al*., 2008) in which an increase of the liver and kidney weight was observed at 600 mg BPA/kg only for liver and from 50 mg BPA/kg for the kidney. This comparison shows that even on liver/ kidney effects of BPA, there is discrepancies between the guideline studies.
These results suggest rather a strong and direct effect of BPA on kidney than general over toxicity.
Moreover, there is no reason to believe that the effects observed on the fertility endpoints are secondary to BPA effects on kidney or liver.

Choice of the reference estrogen: Some results observed for the "reference estrogen" group were pretty surprising. First, as described previously the levels of circulating estradiol following the EE_2 exposure were lower than in the high doses BPA groups, moreover the level is the same for both EE_2 groups, as if a plateau has been reached. Despite some effects that were expected for example in females the effects on the ovary (cysts) and the effects on the cycle (anestrus and asynchrony), other are more unexpected like the fact that an effect is observed on the pituitary gland at 0.5 µg EE_2 but not at 5.0.

Concerning the vehicle used in this preliminary study there were few differences between the vehicle and naïve control groups that would suggest interference of the dosing method with study interpretation. The differences between naïve and vehicle controls included reduced prewean survival in vehicle males, a greater anogenital distance (AGD) and anogenital distance index (AGDI) in the naïve males, increased acinar cell degeneration and infiltration of lymphocytic cells in the pancreas of naïve males, and a higher incidence of renal cysts in naïve females. The latter difference did affect the interpretation of the treatment effects of BPA on the kidney of females, in that the incidence of lesions in the vehicle control group was lower than in any other treatment group.

In conclusion, the effects observed on the reproductive parameters occurred mostly at highest doses (but still relevant for classification and labeling as no major toxicity is observed) of BPA only (100 and 300 mg/kg bw/day) which is contradictory to what is observed in low doses studies included in the CLH dossier. The effects on the gestational bodyweight gain and on pups bodyweight (at 300 mg BPA/kg bw/day) are equivalent to those observed with the positive control group (EE_2). They are reasonable and do not show over general toxicity. Moreover, pretty same systemic toxicity were observed in NCTR study (2013) and in Tyl *et al*. study (2002) both conducted in Sprague-Dawley rats, since effects on the kidney and liver weight are reported in males at 50 mg for Tyl *et al.*'s study and at 300 mg for NCTR, nevertheless additional effects on reproductive parameters were observed in the NCTR study. This underlines the difficulty to be reproducible even in GLP/guideline compliant study.

It is possible that BPA induces cysts in various organs. It is possible that the effects observed on reproductive endpoints are attributable to pre-implantation loss. Many questions remained unsolved. Since this NCTR is a preliminary study only, we could hope that the long-term study and the whole CLARITY-BPA program will finally addressed all questions remaining on effects of BPA.

It points out the fact that it is very difficult to compare all the effects observed since animals or even strains, doses and time of exposure are not the same and that even GLP-compliant study are difficult to reproduce.

This additional study shows reproductive effects from 100mg/ kg bw/d onward. This allows drawing a relative consistency in the effects seen at doses > 100 mg/kg bw/d. Why no statistically significant effects are seen in lower doses (and contradictory to numerous scientific publications) cannot be explained so far as animals or even strains, doses and time of exposure vary from on study to another. Therefore, consistency on a dose-response relationship cannot be established. From the database used in this report, the linearity of the dose-response relationship, considering additional factors such as strain and window of exposure cannot be established.

The conclusion of the CLH report was that based on the weight of evidence of numerous animal studies, it appears that BPA impacts the male reproductive system with effects on the seminiferous tubules, the reproductive hormones levels and the quantity and quality of sperm following an *in utero* exposure at doses that do not lead to major toxicity and in specific models that can predict human toxicity. We stated in the CLH report that in males, when the exposure occurs neonatally, effects on fertility and on the organs of the reproductive tract are observed. An exposure during the puberty leads to effects on the levels of the reproductive hormones, on the seminal vesicles, prostate, testis and epididymis weights, and on sperm quality. Finally, when exposed during adulthood BPA induces effects on the plasma testosterone levels, on the organs of the reproductive tract and on the sperm production and quality.
Based on human studies, it appeared that exposure to BPA affects men fertility and reproductive hormone levels in specific population.
In the NCTR study the effects observed in males are pretty with what we conclude in the CLH report: Despite no effects were observed in the NCTR study on the sperm parameters, and an effect on the seminiferous tubule was observed at one dose only, some effects were reported on the sex hormones and on the weight of some reproductive organs like the ventral prostate. Finally some markers of the sexual development were affected like the AGDI and the age of the testicular descent.

In female animals, our conclusions in the CLH report were that following a pre- and post-natal exposure only, an increased occurrence of ovarian cysts or disturbance of estrous cycles are observed in all the animal studies presented in the report (exhaustive literature search from 2002 to 2011). These observations corroborate risks identified in human through epidemiological studies. This effect was also reported in the NCTR study. This is then a pretty consistent effect reported in numerous species and also described in humans.
When the exposure occurs at the adult or postnatal age decrease in the number of pregnancies and implantations was systematically reported. This seemed to be contradicted

by multi-generation studies, although pre-implementation loss was not assessed. In fact, pre-implementation loss seems to be responsible of the effect of BPA on fecundity in rodents. Implantation failures were reported in a study conducted in women undergoing a medical-assisted procreation, and same kind of women seemed to have a worse ovarian response (number of ovocytes collected and amplitude of the preovulatory oestradiol peak) when the urinary levels of BPA were higher. And the pregnancy outcome seems to be also affected by an exposure to BPA because miscarriages and premature birth were observed in different studies.
In the few animal studies describing this endpoint, endometrial hyperplasia was observed. Concerning the epidemiological studies in women, endometriosis and hyperplasia were reported. Both of these effects were also reported in the NCTR study.
Finally, advancement of the age at puberty, or changes in the sex hormones levels were observed in animals but contradicted or not corroborated by epidemiological studies. In females pups of the NCTR study, the estradiol levels were significantly increased in the highest dose groups treated with BPA. The age of the vaginal opening occurred 2.5 days later (35.0 days) in the 300 mg BPA group compared to control but it was not statistically significant.

Doerge, D. R., Vanlandingham, M., Twaddle, N. C., Delclos, K. B. Lactational transfer of bisphenol A in Sprague-Dawley rats. *Toxicol Lett* **199**: 372-376, 2010.

Fernandez *et al.* (2010)

Kato, H., Ota, T., Furuhashi, T., Ohta, Y., iguchi, T. Changes in reproductive organs of female rats treated with bisphenol A during the neonatal period. *Reproductive Toxicology* **17**: 283–288, 2003.

Mendoza-Rodríguez CA, García-Guzmán M, Baranda-Avila N, Morimoto S, Perrot-Applanat M, Cerbón M (2011) Administration of bisphenol A to dams during perinatal period modifies molecular and morphological reproductive parameters of the offspring. *Reproductive Toxicology* **31**, 177-183.

Newbold RR, Jefferson WN, Padilla-Banks E (2007) Long-term adverse effects of neonatal exposure to bisphenol A on the murine female reproductive tract. *Reproductive Toxicology* **24**, 253-258.

Newbold RR, Jefferson WN, Padilla-Banks E (2009) Prenatal Exposure to Bisphenol A at Environmentally Relevant Doses Adversely Affects the Murine Female Reproductive Tract Later in Life. *Environmental Health Perspectives* **117**, 879-885.