Committee for Risk Assessment
RAC

Annex 2
Response to comments document (RCOM)
to the Opinion proposing harmonised classification and
labelling at EU level of

Anthraquinone

EC Number: 201-549-0
CAS Number: 84-65-1

CLH-O-0000001412-86-86/F

Adopted
4 December 2015
**ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON ANTHRAQUINONE**

**COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION**

Comments provided during public consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

All comments and attachments including confidential information received during the public consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the public consultation and are also published together with the opinion (after adoption) on ECHA’s website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties.

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**Substance name: Anthraquinone**  
**CAS Number: 84-65-1**  
**EC Number: 201-549-0**  
**Dossier submitter: Germany**

**GENERAL COMMENTS**

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Comment received

**COMMENTS SUBMITTED TO ECHA**

**BY CHEMICAL PRODUCTS CORPORATION**
**AND ITS SUBSIDIARY, CPT PULP AND PAPER, LLC**

Comments to ECHA regarding the CLH Report, “Proposal for Harmonized Classification and Labeling – Substance Name: Anthraquinone; EC Number: 201-549-0; CAS Number: 84-65-1; Version number: 2.0; Date: January 2015.”

The CLH report submitted to ECHA recommends that Anthraquinone (AQ) be considered for inclusion as “Carc. 1B, H350” on Annex VI to Regulation (EC) No 1272/2008, table 3.1, with regard to Article 42. Chemical Products Corporation and its subsidiary, CPT Pulp and Paper, LLC, submit the following information to demonstrate that the recommended classification of Anthraquinone contained in the above-named CLH report is not supported by sound science and should not be adopted. Chemical Products Corporation and its subsidiary, CPT Pulp and Paper, LLC, are headquartered in the United States. CPT Pulp and Paper, LLC produces an Anthraquinone aqueous suspension for use as a yield-enhancing catalyst in the manufacture of paper pulp.

The statement on page 7 of the CLH report, “AQ was not clearly demonstrated as mutagenic in the available tests.” is incorrect and misleading. As will be discussed in the following comments, Anthraquinone has been conclusively demonstrated to be a nonmutagen and should be classified as such at CLP Annex I reference 3.5. The recommendation in the CLH report for classification of Anthraquinone in CLP Annex I reference 3.6 as Carc. 1B would only be appropriate for the material tested in animals by the U.S. National Toxicology Program (NTP) in the 1990s – Anthraquinone contaminated with mutagenic nitroanthracene impurities resulting from manufacture using the nitric acid oxidation of anthracene process.
This is the material purchased from Zeneca FineChemicals by NTP in the early 1990s. None of the Anthraquinone in commerce today is manufactured by the nitric acid oxidation of anthracene process, so the Carc. 1B classification is not appropriate for the Anthraquinone now in commerce. None of the Anthraquinone in commerce in 2015 contains mutagenic nitroanthracene impurities.

We are confident that ECHA is committed to the application of sound science in its classification activities. We respectfully submit the following comments which will demonstrate that the recommendations in the CLH report are not based upon sound science and should not be adopted by ECHA.

1. The CLH report incorrectly implies that Anthraquinone might be mutagenic; it has been well established that Anthraquinone is a non-mutagen as detailed below. The CLH report on page 7 makes the incorrect and misleading assertion, “AQ was not clearly demonstrated as mutagenic in the available tests.”, and recommends no classification at Annex I 3.5 Germ cell mutagenicity with the comment, “Conclusive but not sufficient for classification”. U.S. National Toxicology Program Technical Report 494 [Attachment I] (the final published report is referred to herein as NTP 2005, while draft versions are referred to as draft TR-494); the IUCLID data set for Anthraquinone, Year 2000 edition [Attachment II]; Tikkanen et al. [Attachment VII]; and the Butterworth et al. assays presented in the CLH report demonstrate that Anthraquinone is not mutagenic. We submit that the available data is both conclusive and sufficient for classification of Anthraquinone as a non-mutagen. The draft TR-494 disseminated for public review and comment prior to peer review in February 2004 [Attachment III] states at page 116, “In the present study, we have confirmed the nonmutagenicity of pure anthraquinone, suggesting that the positive results reported previously were due to the presence of mutagenic contaminant(s).” NTP 2005 states at page 91, “In addition, although anthraquinone itself is not a bacterial mutagen...”. We respectfully submit that (1) the NTP conclusions reported in NTP 2005 [Attachment I] and the draft TR-494 [Attachment III], (2) the mutagenicity assays in the IUCLID dataset [Attachment II] beginning on page 32, (3) the negative mutagenicity assays reported by Tikkanen et al. [Attachment VII], and (4) the Butterworth et al. assays presented in the CLH report are sufficient to conclusively classify Anthraquinone as a non-mutagen.

2. The CLH report incorrectly bases its recommendation to classify the Anthraquinone now in commerce as Carc. 1B based upon animal studies conducted by the U.S. National Toxicology Program employing test material manufactured by the nitric acid oxidation of anthracene which was contaminated with mutagenic 9-nitroanthracene and which is no longer an article of commerce. There is no indication that the Anthraquinone in commerce in 2015 is a potential human carcinogen; ECHA should reject the proposed classification in the CLH report on the basis that it is not supported by sound scientific evidence. The CLH report states in Section 2.1 at page 7, “Based on the results of two carcinogenicity studies (oral administration of AQ to mice and rats; NTP 2005) it was concluded by IARC in 2012 that there is sufficient evidence in the experimental animals for carcinogenicity. Therefore, AQ was evaluated as possible carcinogenic to human (group 2B). Since this evaluation no further animal data has become available. The same experimental studies are re-evaluated for justification of classification of AQ as carcinogenic according to CLP Regulation.” The CLH report further states in Section 2.2 on page 8, “Overall, the NTP studies are valid and there is sufficient evidence for carcinogenicity.” We will demonstrate that the CLH report’s re-evaluation of the NTP studies is incorrect and that the NTP studies do not provide justification for the classification of the Anthraquinone in commerce in 2015 as carcinogenic according to CLP Regulation. Respected toxicologists dispute the validity of applying the results of the NTP studies to pure Anthraquinone because of mutagenic contamination in the NTP test material. Boobis et al. state in Toxicologic Pathology , “The data for anthraquinone
ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON ANTHRAQUINONE

are considered suspect because other carcinogenicity studies were negative, and the NTP carcinogenicity study used a batch of anthraquinone contaminated with the potent mutagen 9- nitroanthracene .... (A purified sample was negative in the Ames test.)” [Attachment IV - Boobis et al.; Toxicologic Pathology; Vol. 7, No. 6; page 719; 2009]. Professor Alan R. Boobis is a Fellow of the British Toxicology Society. Mutagenic contamination in the NTP test material went undetected until years after completion of the animal testing. The first draft TR-494 had been accepted by a NTP peer review panel in 1999 before NTP was alerted to the presence of mutagenic contamination. When mutagenic contamination of the NTP test material was detected, the U.S. National Institute of Environmental Health Sciences withdrew the first draft TR-494. In his letter to Chemical Products Corporation announcing his decision to withdraw the 1999 draft TR-494 [Attachment V], Dr. Samuel H. Wilson, the Deputy Director of the U.S. National Institute of Environmental Health Sciences stated, “Process: In the course of my review, I have reviewed the HHS and NIH Guidelines for Insuring the Quality of Information Disseminated to the Public, read draft TR-494, and read Chemical Products Corporation's letters and the NTP's responses to those letters. I have consulted with NIH and HHS staff familiar with the Information Quality process. I also have reviewed data and ongoing tests with the NIEHS' Environmental Toxicology Program who were responsible for the NTP studies and draft report. I have been assisted in these efforts by staff from the NIEHS Office of Policy, Planning, and Evaluation.

Conclusions: Following the process outlined above and after careful review of the information described above, I have reached the following conclusions:

1. The sample of Anthraquinone used in the 2 year NTP studies was contaminated with 9-nitroanthracene at a level of about 0.1%.

2. The presence of this contaminant raises doubt as to the effect(s) of Anthraquinone itself, or its metabolites, and confounds interpretation of the NTP studies referenced in TR-494....

3. The abstract of draft TR-494 will be immediately removed from the NTP website.” This letter is presented on the U.S. government website at http://aspe.hhs.gov/infoquality/requests.shtml. (it is entry 5.d.)

3. Anthraquinone contaminated with nitroanthracenes is no longer an article of commerce, so classification by ECHA of Anthraquinone contaminated with these mutagenic compounds is not an issue. Yet animal studies on this obsolete material is the basis of the the CLH report's recommended classification. Current manufacturing processes for Anthraquinone are not capable of introducing nitroanthracene contaminants into the product. Until the 1990s; some Anthraquinone in commerce was manufactured by the nitric acid oxidation of anthracene; NTP obtained its test material manufactured by the nitric acid oxidation of anthracene from Zeneca Fine Chemicals. This manufacturing process is no longer employed anywhere in the world. It is not possible for nitroanthracene contamination to result from any of the currently-practiced manufacturing processes for Anthraquinone. Classification of Anthraquinone by ECHA should not be based upon the carcinogenic influence of strongly mutagenic contaminants were present in some of the Anthraquinone in commerce until the 1990s, but which are not present in today's Anthraquinone article of commerce.

4. NTP 2005 and the CLH report incorrectly ascribe observed cancers to metabolites of Anthraquinone. After withdrawal of its 1999 draft TR-494 which ascribed carcinogenicity observed in its animal studies to Anthraquinone, and recognition that Anthraquinone is not a mutagen, NTP searched for metabolites of Anthraquinone which might be responsible for the observed carcinogenicity. NTP 2005 and the CLH report ascribe the carcinogenicity observed in NTP's animal studies to the action of 2-hydroxyanthraquinone which is
Annex 2 - Comments and response to comments on CLH Proposal on Anthraquinone

incorrectly characterized as “the primary metabolite of Anthraquinone”. In fact, Sato et al. (1959) reported that 2-hydroxyanthraquinone is not the primary metabolite of Anthraquinone and is present in only very small quantities in fresh rodent urine as it is expelled. Both NTP 2005 and the CLH report cite Sato et al. (1956) Metabolism of anthraquinone. I. Isolation of 2-hydroxyanthraquinone from the urine of rats. J. Biochem. 43 (1), 21-24. Neither NTP 2005 nor the CLH report cite Sato et al. (1959) Metabolism of anthraquinone. II. Sulfate Conjugate of 2-hydroxyanthraquinone. J. Biochem. 46 (8), 1097-1099. Sato et al. (1959) [Attachment VI] states, “In a previous study anthraquinone was fed to rats and 2-hydroxyanthraquinone was recovered from the urines (1). However, its quantity was found to be very small when freshly voided urine was examined...”. The actual constituent of rodent urine as it is excreted was found to be the sulfate conjugate of 2-hydroxyanthraquinone which decomposes on standing to sulfate and 2-hydroxyanthraquinone. Both NTP 2005 and the CLH report incorrectly refer to the supposed large quantity of 2-hydroxyanthraquinone in rat urine and its purported mutagenicity as an explanation for the cancers observed. NTP 2005 attempts to discount the biological significance of the mutagenic 9-nitroanthracene contamination in NTP’s test material by stating at page 111, “The identification of 2-hydroxyanthraquinone as a major urinary metabolite of anthraquinone is in agreement with results reported previously by Sato et al. (1959)...the animals would be exposed to a substantially greater amount of 2-hydroxyanthraquinone than 9-nitroanthracene over a typical 24-hour period.” If Sato et al. (1959) had been cited by NTP and critically considered by NTP's December 2004 peer review panel (or other reviewers), the conclusions presented in NTP 2005 would likely have been recognized as applying only to Anthraquinone contaminated with nitroanthracenes.

5. The small amount of 2-hydroxyanthraquinone in fresh rat urine may not be mutagenic. NTP incorrectly implies that 2-hydroxyanthraquinone was conclusively determined to be mutagenic in Salmonella typhimurium TA98 without metabolic activation based upon its positive mutagenicity assay in strain TA98 without metabolic activation. The conflicting negative assay of Anthraquinone in TA98 without S9 activation reported by Tikkanen et al. [Attachment VII] at page 301 is not cited, in fact, NTP 2005 specifically states that it does not exist (at page 91 NTP 2005 states, concerning Tikkanen et al., “testing was not done without S9”). Tikkanen et al. state, “None of the compounds were mutagenic in strain TA98, with or without metabolic activation.” Thus, evidence regarding mutagenicity of the 2-hydroxyanthraquinone in fresh rat urine is inconclusive; the CLH report does not address this issue. We submit that the argument in NTP 2005, accepted by the CLH report, that mutagens other than the nitroanthracene contamination caused the observed cancers is not persuasive in light of Sato et al. (1959) and Tikkanen et al.

6. NTP's peer review panel recognized that the results of the NTP animal studies applied only the material tested prior to being provided false information by NTP staff. The February 2004 NTP peer review panel directed that the term “anthraquinone” in the draft TR-494 it reviewed should be specified to refer only to “anthracene-derived anthraquinone”. After the February 2004 peer review panel restricted the conclusions presented in NTP 2005 to “anthracene-derived anthraquinone”, NTP added a negative mutagenicity assay for a sample designated “A07496” to the draft TR-494 study report. Sample A07496 is described in NTP 2005 as an aliquot of the NTP test material; the test material is identified at its Battelle storage facility in Columbus, Ohio as “Lot No. 5893”. There is no valid documentation that sample A07496 is an aliquot of Lot No. 5893. NTP presented the negative mutagenicity assay of sample A07496 to the December 2004 NTP peer review panel as justification for removal of the restriction on the conclusions in NTP 2005 required by the February 2004 NTP peer review panel. When a member of the December 2004 peer review panel asked NTP staff if mutagenic impurities in the NTP test material might have decomposed during the 8 year period between conclusion of animal testing and the
Laboratories had no means of determining that the sample labeled only “A07496” in Laboratories Test Article Receipt and Transfer Report test material showing that a sample labeled “Anthraquinone, Attachment IX. emailed back “confirming” the test article identities I’m uncertain as to what NTP, asking San at BioReliance Laboratories, the NTP can produce only a single email to Documents obtained through Freedom of Information Act Columbus, Ohio since the 1990s; there it is identified sample repository. positive contamination in the NTP test material of sample A07496 is not sufficient to demonstrate the 5893, in the flawed peer review process conducted by NTP. Because the December 2004 peer review panel relied upon false information, the conclusions to be drawn from the animal test data presented in NTP 2005 should be critically re-evaluated. The re-evaluation presented in the CLH report appears to have accepted the conclusions in NTP 2005 as valid without considering the implications of the 7. The negative mutagenicity assay for sample A07496, described in NTP 2005 as its test material, undoubtedly played a significant role in convincing the December 2004 NTP peer review panel to accept NTP 2005. The identity of the sample is unsubstantiated. The December 2004 NTP peer review panel erred in approving NTP 2005 which contains NTP assertion that A07496 is an aliquot of its test material, and accepting NTP’s contention that mutagenic impurities in the NTP test material were not biologically significant. NTP 2005 improperly presents sample A07496 as being an aliquot of NTP’s test material, Lot No. 5893, in the absence of any supporting documentation. The negative mutagenicity assay of sample A07496 is not sufficient to demonstrate the insignificance of mutagenic contamination in the NTP test material. NTP improperly discounted the validity of earlier positive mutagenicity assays of samples labeled “Lot #5893” obtained from the Battelle sample repository. The NTP test material has been stored at NTP contractor Battelle in Columbus, Ohio since the 1990s; there it is identified as “Anthraquinone, Lot No. 5893”. Documents obtained through Freedom of Information Act requests demonstrate that NTP can produce only a single email to support its assertion that sample A07496 is an aliquot the NTP test material, Lot No. 5893. In the fall of 2004, Kristine Witt at NTP emailed Richard San at BioReliance Laboratories, the contractor which performed mutagenicity assays for NTP, asking for confirmation that A07496 was Lot No. 5893 stating, “without confirmation of the test article identities I’m uncertain as to what the results are telling us.” Richard San emailed back “confirming” that sample A07496 was Lot #5893. These emails are included in Attachment IX. Attachment IX also contains (1) the Battelle Bulk Chemical Shipment Report showing that a sample labeled “Anthraquinone, Lot No. 5893” was shipped from the Battelle test material repository in Columbus, Ohio on June 1, 2004; and (2) the BioReliance Laboratories Test Article Receipt and Transfer Report documenting receipt of a sample labeled only “A07496" in Rockville, Maryland on June 2, 2004. Richard San at BioReliance Laboratories had no means of determining that the sample received on June 2, 2004 labeled
"A07496" was the NTP test material maintained at Battelle in Columbus, Ohio labeled "Lot No. 5893". Richard San had no factual basis upon which to confirm to Kristine Witt at NTP that sample A07496 was an aliquot of Lot No. 5893; his motivation for making such an unsupported confirmation is unknown. Boobis et al. [Attachment IV] states at page 719, "The data for anthraquinone are considered suspect because other carcinogenicity studies were negative...Certainly, it can be said that the material used by the NTP was mutagenic ..." (underline added). As noted earlier, Professor Alan R. Boobis is a Fellow of the British Toxicology Society. The IUCLID dataset 2000 [Attachment II], at page 35, contains a mutagenicity assay of an anthraquinone sample “known to contain 0.032% 9-nitroanthracene”. This is only about one third as much 9-nitroanthracene contaminant as the NTP test material has been acknowledged to contain; this sample exhibited significant mutagenicity. The Battelle Bulk Chemical Shipment Report in Attachment IX presents the assay of Lot No. 5893 as "99.4%", so that material could contain more nitroanthracene than has been acknowledged by NTP. The December 2004 NTP peer review panel modified the decision of the February 2004 NTP peer review panel based upon faulty information, and approved a modified draft TR-494 without the requisite public opportunity for comment. The February 2004 NTP peer review panel's recognition that the conclusions in TR-494 are not generally applicable to Anthraquinone in commerce should be accepted by ECHA.

8. The conclusions in draft TR-494 disseminated for public review and comment before peer review on February 17, 2004 were restricted to apply only to “anthracene-derived anthraquinone” by that NTP peer review panel to acknowledge that the results of the NTP animal studies were impacted by the presence of nitroanthracene impurities and are not applicable to Anthraquinone free of nitroanthracene impurities. ECHA should consider this peer review panel decision authoritative because it was reached in the absence of the false and unsupported information provided to the December 2004 NTP peer review panel. We respectfully submit that ECHA should also recognize that the NTP animal study results do not apply to the Anthraquinone in commerce in 2015 which is, without exception, free of nitroanthracene impurities.

9. It would be a perverse misapplication of the Precautionary Principle to apply the results of animal studies testing a material contaminated with nitroanthracenes to material in commerce which is not contaminated with nitroanthracenes. We are confident that ECHA will apply the Precautionary Principle appropriately to provide protection against actual chemical hazards identified through the application of sound scientific principles. The animal studies reported in NTP 2005 do not demonstrate that the Anthraquinone in commerce in 2015 represents a possible human cancer hazard. The proposed classification in the CLH report is not appropriate and should not be adopted.

In summary, Anthraquinone has been conclusively demonstrated to be a non-mutagen. The Carc. 1B, H350 classification recommended by the CLH report would only be appropriate for the material evaluated in NTP's animal studies - Anthraquinone manufactured by nitric acid oxidation of anthracene containing mutagenic nitroanthracene contaminants. This manufacturing method is no longer practiced in Europe or anywhere else in the world. None of the Anthraquinone in commerce at the present time is contaminated with nitroanthracene impurities, so classification of the article of commerce based upon the NTP animal studies makes no sense. There is no sound science to justify the CLH report's recommendation that a "Carc. 1B, H350" classification is appropriate for the Anthraquinone in commerce at the present time or the foreseeable future. We are confident that ECHA's commitment to sound science will result in its determination that the CLH report recommendation is not the appropriate classification for the Anthraquinone in commerce in 2015. If I can provide any additional information or documentation regarding these comments, please email me at jcook@cpc-us.com.
Respectfully submitted by,
Jerry A. Cook
Chief Technical Officer

ECHA note: The following attachment was provided with the comment above [Attachment 1]

COMMENTS SUBMITTED TO ECHA BY CHEMICAL PRODUCTS CORPORATION AND ITS SUBSIDIARY, CPT PULP AND PAPER, LLC

Dossier Submitter’s Response

Mutagenicity

To the commentator’s view that the statement “AQ was not clearly demonstrated as mutagenic in the available tests” is incorrect and misleading and the proposal that AQ should be classified as non-mutagen:

A classification for the absence of hazardous properties is not intended by the CLP Regulation. It is true this sentence in the introductory part of the CLH report could be misleading. For clarification of the mutagenicity database and in response to the several comments:

1. Testing of AQ (guideline-compliant studies with purity ≥ 99% and stated as free of 9-NA) was negative for mutagenicity in vitro in bacterial gene mutation tests and in mammalian cell cultures (mouse lymphoma assay, chromosome aberration test) as well as in vivo in soma cells (micronucleus test). Data for induction of mutagenic effects in germ cells are not available.

In vitro-testing on AQ with and without S9 was negative. This may raise the question why, while the major metabolites were mutagenic, no effect was seen in these tests with S9 mix. A negative response cannot be interpreted that the metabolites were not produced. One interpretation could be that the amount of metabolites in the in vitro systems was not sufficiently high to produce a positive result. In line with the observation of negative in vitro tests with S9 mix, the negative in vivo micronucleus demonstrates that AQ (including the metabolites that have been produced in the organism) was negative (or may not have reached the bone marrow in sufficiently high concentrations).

2. However available tests on the metabolites give some indication on possible mutagenic effects. 2-OH-AQ and 1-OH-AQ are positive in guideline compliant bacterial gene mutation tests. Other bacterial tests for 2-OH-AQ and 1-OH-AQ are of minor significance due to methodological insufficiencies. No further tests are available.

The comment refers to inconsistencies between the response to Salmonella typhimurium strain TA98 for AQ and its metabolites. In general differences in the testing of the pure substance and of the metabolites may occur, e.g. due to differences in testing concentrations and cytotoxic concentrations. The positive Salmonella typhimurium assay on TA98 with or without S9 mix with 2-OH-AQ does not conflict the negative outcome of AQ in the same strain. A negative response in TA98 on AQ seems to be reliable based on the study of Táublová (2009) which after
submission of additional information during the public consultation is now accepted as key study (see DS’ response to comment 19). The negative Tikkanen et al. study (1983) on AQ is not robust due to major defaults in the documentation and methodology (see Table 15 of the CLH report). The negative Ames test for AQ does not invalidate the positive Ames test on 2-OH-AQ.

3. In in vitro tests carried out similarly to the corresponding guideline, 9-NA induced mutagenic effects in bacterial gene mutation tests and was weakly positive (Durant et al. 1996) or inconclusive regarding the induction of gene mutations in mammalian cell cultures (Butterworth et al. 2004). In this study, the result of mouse lymphoma assay in L5178Y cells should be considered as equivocal, because with S9 mix positive effects were only observed at concentrations that induced strong to extreme toxic effects. Two further bacterial gene mutation tests are of minor significance due to methodological insufficiencies. The public consultation revealed additional information from two somatic mutation and recombination tests with Drosophila melanogaster (positive result without dose-dependency and equivocal result) that are of minor significance due to methodological insufficiencies.

In conclusion from the tests available, AQ without 9-NA is negative for mutagenicity. Some indications on a mutagenic potential from positive bacterial tests are given for the metabolites, but no follow-up testing has been conducted. The impurity 9-NA was also positive in bacterial tests, and mammalian cell tests indicate positive or equivocal results.

Based on the available positive in vitro data the level of concern on a mutagenic potential is roughly comparable for the metabolites and 9-NA. A final conclusion on the mutagenic potential is not possible for any of these substances due to the lack of reliable data from further in-vivo testing.

Carcinogenicity
The purity in the NTP carcinogenicity study (NTP 2005) was 99.8% (99.9% according to the letter of September, 2003). On request of the commentator in November, 2002 (and from letters in 2000)¹, NTP examined for unidentified contaminants and confirmed the presence of 0.1% 9-NA in the AQ sample used for the 2-year study and in the Salmonella mutagenicity tests giving positive results (Letters of March 19 and September 8, 2003).

In their response of September 8, 2003, US National Institute for Environmental Health Services concluded that “The presence of this contaminant raises doubt as to the effect(s) of anthraquinone itself, or its metabolites, and confounds interpretation of the NTP studies referenced in draft TR-494.” Further investigations initiated by NTP in 2003 confirmed 1-OH-AQ and 2-OH-AQ in urine samples of rats feed for 7 days with four lots of 7500 ppm AQ from the three production processes² (data see Attachment Doc 2.1 to Comment 9, thought to be the same study as Graves, 2005) with a concentration of 1-OH-AQ is about 2% of 2-OH-AQ. For 7500 ppm AQ-OX the urine concentration of 2-OH-AQ was double than for the other AQ from the other two production processes. Further tests on lower concentrations (938 and 3750 ppm for 9 days) revealed similar levels of 2-OH-AQ for 3750 ppm treated rats for AQ from all three production types, and even lower urine concentrations at 938 ppm for the AQ-OX in comparison to AQ-FC and AQ-DA (see Doc 2.2 of comment 9).

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¹ [http://aspe.hhs.gov/infoquality/requests.shtml](http://aspe.hhs.gov/infoquality/requests.shtml)

² Oxidation of anthracene, AQ-OX (with HNO₃), Friedel-Crafts acylation AQ-FC (from phthalic acid anhydride and benzene), Diels-Alder reaction, AQ-DA (from p-benzoquinone and 1,3-butadiene)
In line with the US National Institute, the DS has the view that no conclusion on the contribution of AQ, its metabolites or the impurity 9-NA can be drawn. Optionally all of them, some of them or a single compound (e.g. one metabolite) could be responsible for the tumour response. Each of them may have contributed to the tumour responses seen in rats and mice, but the available data do not allow to attribute the carcinogenic response to one of them. The DS noted that the US Governmental Institute did not confirm that 9-NA is the responsible carcinogen. In their follow up investigations on purified samples they found that the urinary metabolite 2-OH-AQ was a strong mutagen and was 10-fold more potent than 9-NA (letter of March 19, 2003). This observation was opposite to the conclusions drawn by Butterworth\(^3\), who conducted several of the mutagenicity studies and considered 9-NA as potent as benzo(a)pyrene (more details in the CLH report 4.10.4 Summary and discussion of carcinogenicity). The DS observed that the metabolites of AQ could be more potent in the bacterial test system than 9-NA. However, the DS is reserved with regards to whether the potency in in vitro tests is applicable to the in-vivo situation.

Mutagenicity (as far as known from in vitro gene mutation tests for the metabolites and the impurity 9-NA) may have a role or may not have any role in the carcinogenicity of AQ. It was shown that AQ alone does not have mutagenic properties in soma cells. However a mutagenic activity resulting from administration of AQ by its metabolites and impurities cannot be excluded. It is to note that the mutagenicity data on AQ, its metabolites and impurity 9-NA are part of the considerations on the mode of carcinogenic action but the mutagenicity data due to the preliminary conclusions with regards to the mutagenic potential are not (solely) decisive for the proposed classification on carcinogenicity.

Considering the overall information there is no proof of evidence that 9-NA was the only carcinogenic substance in the NTP studies. 9-NA has not been tested in a carcinogenicity study. In line with the view of the NTP Board of Scientific Counselors, it is not possible to determine to what extent, if any, the impurity 9-nitroanthracene (9-NA) has influenced the outcomes of the NTP carcinogenicity study on AQ. Overall, it is considered unlikely that the carcinogenic response could solely be attributed to 9-NA. The low exposure level (based on the 0.1% concentration) makes it unlikely that 9-NA was the only agent that was responsible for the carcinogenic response. The weak mutagenicity indicates that 9-NA has mutagenic properties in vitro. ‘Weak’ means the effect in bacterial gene mutation tests was positive (but not strong related to the increase in mutation rates) and the effects in somatic cell gene mutation tests were equivocal for L5178Y cells and marginal positive (only doubling of the mutation frequency’) for h1A1v2 cells. This information alone does not give a clear hint on the contribution to the tumour response.

The comment argues that the nitric acid production which results in 9-NA impurity are no longer used. The comment also points to the NTP peer review from February 17, 2004 where the panel acknowledged that the results of the NTP study refer to anthracene-derived AQ. As there is no evidence that 9-NA is the only responsible carcinogen in the NTP cancer study, it is of lower significance for the classification whether the production of AQ have changed or which production type\(^4\) is used. Cancer studies on AQ from the other production

\(^3\) Public comment to

\(^4\) Oxidation of anthracene, AQ-OX (with HNO\(_3\)),
Friedel-Crafts acylation AQ-FC (from phthalic acid anhydride and benzene),
Diels-Alder reaction, AQ-DA (from p-benzoquinone and 1,3-butadiene)
types (Friedel-Crafts technology (AQ-FC) or Diels-Alder chemistry (AQ-DA)in comparison to the production derived from oxidation of anthracene (AQ-OX) that is probably used for the NTP studies) are not available. On mutagenicity there were bacterial test data on AQ from all three production types. All of them were negative.

No specification on the synthesis could be found in the NTP report on how AQ was produced at Zeneca Fine Chemicals who supplied the test substance for the NTP studies, while in the comment No. 5 the information was given that Zeneca Fine Chemicals produced with nitric acid oxidation of anthracene. In a meeting of the NTP Board of Scientific Counselors on December 9, 2004\(^5\) it was argued that 2-OH-AQ, the major metabolite, will be produced regardless of the method of manufacture (confirmed in studies, see above the DS’ response with reference to Attachment Doc 2.1 and Doc 2.2 that were attached to Comment 9)). The final conclusion of the Board meeting in December 2004 was to defeat an amendment ‘anthracene-derived’.

The DS concluded that the carcinogenic responses in animal studies are attributable to AQ (including its active metabolites and possible impurities) and thus, AQ is considered to be carcinogenic. A contribution of the carcinogenic response to one of the substances (either for AQ, its metabolites or possible impurities) cannot be made.

AQ itself (highly purified and without 9-nitroanthracene as impurity) was not demonstrated as mutagenic in the available tests. The question of a contribution of mutagenicity (e.g., by the metabolites or impurities) cannot be answered at the current state of knowledge.

As a monoconstituent substance is defined by one main constituent that is present at concentrations ≥80%, AQ at purities above this level fulfils the criteria for a monoconstituent that may contain up to 20% additional constituents as impurities. Impurities above ≥1% should be specified. In practice impurities at lower concentration are often/not always specified. As it remains unclear whether AQ, its metabolites and possible impurities exerts the carcinogenic action, the formulation of a characteriser of AQ for the entry into Annex VI towards its impurities is not useful at this stage of knowledge. The entry in Annex VI should refer to the main constituent.

AQ is registered for concentrations of AQ at ≥90% and ≤100%, typical concentration ≥99%. The impurities were not characterised. The CSA also states ≥90% and ≤100%, with impurities <1% (range 0-10%). A remark is given that no specified impurity is below 1%, no CMR substance at ≥0.1%.

**RAC’s response**

RAC agrees with the DS. AQ is not considered mutagenic based on the available data. As to the carcinogenicity response, the DS is quite reasonable in their assessment that AQ is the substance to be considered as carcinogenic. It is impossible to acknowledge the contributions made by minor components such as 9-NA to the overall carcinogenicity profile which is clearly evident from the NTP studies because of the limited amount of data available. Industry has been aware of these results since the first preliminary NTP draft reports yet no new relevant long-term investigations have been performed on AQ derived from other process technologies. Similarly, the contribution of metabolism to the carcinogenicity profile is difficult to access. There is a lack of clear metabolic data for AQ and the few mutagenicity investigations with the hydroxyl AQ metabolites suggest there is mutagenic potential at least equal to if not more than any other component found in the

AQ-OX technical material. In the absence of new data, the lack of 9-NA in the technical material is not sufficient reason per se to invalidate the tumour responses observed in the NTP bioassays.

For clarification, the statement in Boobis et al. (Toxicologic Pathology), “The data for anthraquinone are considered suspect because other carcinogenicity studies were negative, and the NTP carcinogenicity study used a batch of anthraquinone contaminated with the potent mutagen 9-nitroanthracene...” is not particularly helpful. There is no reference given for “other carcinogenicity studies” or discussion on their potential robustness, there is no data to substantiate other findings for the investigation of carcinogenicity with exposure to AQ. Also, it is erroneous to describe 9-NA as being particularly potent relative to other potentially mutagenic components. There is no data to provide supporting evidence that 9-NA is as potent as the positive controls used in the investigations described by NTP (2005). In fact, NTP make a brief comparison illustrating that the positive controls are many times more potent in producing replicants per µg than 9-NA and in some circumstances 2-OH-AQ is more “potent” than 9-NA.

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Comment received

Usefulness of anthraquinone

Anthraquinone has been used commonly as an intermediate in the manufacture of dyes since the late 19th century. In 1972, Bach et al. in East Germany found that anthraquinone increased pulp yield, and ever since it has been used also as a cooking additive in the production of paper pulp, with anthraquinone being now widely used in pulp-making mills around the world. In Europe, anthraquinone is also used as a pesticide, but the extent of this usage has been very limited compared with the usage for dyes or as a cooking additive in the pulping process.

The volume of anthraquinone currently used in pulp mills worldwide is estimated to be at least approximately 20,000 tons/year. This amount of anthraquinone as a cooking additive in the pulping process is estimated to reduce the use of wood chips by 13 million tons/year, which is equivalent to a forest area of 207 km²/year.

Furthermore, this reduction in the consumption of wood chips is associated with reduction of carbon dioxide derived from the burning of fossil fuels that would be required for transportation of the wood chips. More specifically, an estimated reduction of 4,330 thousand tons/year of carbon dioxide is attained by the above-mentioned decrease in the consumption of wood chips.

As described above, anthraquinone is greatly contributing to forest conservation and global warming prevention, in addition to increasing the pulp yield and thereby the profits of pulp-making mills. Many studies on cooking additives in pulping process have found no alternative to anthraquinone. Inappropriate regulation on anthraquinone to limit its use would have a major impact on the social economy stated above.

We would appreciate a sound judgment of the responsible personnel based on good sense regarding this matter.
ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON ANTHRAQUINONE

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<td>Thank you for your comment. Please note that the data are not relevant for the decision finding on classification.</td>
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<tr>
<td>For information, the use of AQ as a pesticide is no longer authorised within the EU (EFSA Opinion, 2012).</td>
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<tr>
<td>RAC agrees with the DS, that these are useful data but not directly relevant to the classification discussion.</td>
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Comment received

Comments regarding the migration of AQ from paper into foodstuff

The CLH report states on page 7, “AQ can be applied in the production of cellulose fibres. In case of use of such fibres for manufacturing of paper and board for food contact any traces of the substance that remain in the cellulose can transfer to foodstuffs. ” But this dossier submitter does not show any evidence and makes the misleading and incorrect assertion.

The German Federal Institute for Risk Assessment (BfR) also stated in their published Opinion No.005/2013 that: “the BfR has information on cases where the permitted residue limit value for anthraquinone was exceeded in tea which can be attributed to the anthraquinone levels contained in the paper and cardboard used as packaging materials.”. [Attachment 1] However, they did not disclose more detailed information.

For an example, a search result in the Rapid Alert System for Food and Feed (RASFF), which is managed by DG SANCO of the European Commission indicates that AQ was detected from dry food like tea and chili pepper. In addition, almost all cases were detected from tea.

In order to confirm the possibility of migration of AQ from paper into dry foodstuff like tea or chili pepper, we commissioned a laboratory to perform a migration test. [Attachment 2] The test was performed in accordance with the COMMISSION REGULATION (EU) No10/2011 on plastic materials and articles intended to come into contact with food because there was not the similar standard for paper. Experimental conditions were as follows;

Samples: Two unbleached kraft papers, (A) and (B). AQ content in sample were (A)5.7 ppm and (B)1.6ppm.
Food simulant: TENAX (poly(2,6-diphenyl-p-phenylene oxide)
Migration time and temperature: 10 days at 60C degree, corresponding to more than 6 months at room temperature.
Ratio of sample surface area to volume of food simulant: 1dm2/4g
Solvent for extraction of AQ: acetone
Determination of AQ: GC-MS (limit of detection: 0.005ppm)

Results of measurement of AQ extracted in acetone were as follows;

Sample name Peak area of AQ
Paper (A) 0 (not detected)
Paper (B) 0 (not detected)
BLANK 0 (not detected)

Therefore, we can conclude that AQ was not migrated from paper into dry food. The test results also indicate that the detected AQ listed in RASFF were not caused by the transfer from paper for food contact. According to this consideration, the statement of the CLH report above should be corrected.

**ECHAs note: The following attachments were provided with the comment above [Attachments 2 and 3]**

- Report – Migration test of 9,10-anthraquinone in the Unbleached kraft paper.
  February 13, 2004
- BfR removes anthraquinone from its list of recommendations for food packaging.

**Dossier Submitter’s Response**

Thanks for this information. Please note that these data are not relevant for the classification proposal on the inherent hazardous properties.

**RAC’s response**

RAC agrees with the DS, that these are useful data but not relevant for the classification discussion.

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<td>DEZA, a.s.</td>
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Comment received

We agree with all comments submitted by CHEMICAL PRODUCTS CORPORATION AND ITS SUBSIDIARY, CPT PULP AND PAPER, LLC; Wibax; KAWASAKI KASEI CHEMICALS LTD nad VUOS, a.s. We will not repeat all arguments already submitted by above mentioned companies to RAC. We do believe that RAC will evaluate carefully all submitted comments and will reject Annex XV proposal for CLH.

We would like to note that Annex XV dossier submitter (Germany) discredited our Lead Registrant dossier for the substance Anthraquinone by stating "not reported" or "testing guidance not followed" in the Annex XV proposal without any evidence. All the data needed for preparation of Annex XV dossier is disseminated on ECHA dissemination portal (you can check it even now). If the dossier submitter (Germany) is not sure about the source of information, why he did not contacted data holder (Lead Registrant)? We reject all these vague statements and ask dossier submitter to correct the information in Annex XV dossier.

It is remarkable that CLH proposal contains conclusions from US NTP 494 report, but the report s such do not contain such conclusions.

We are aware that this Annex XV proposal for CLH was chosen as "pilot test" for TTIP agreement. We want to believe that also RAC is aware of this fact.

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7 http://www.bfr.bund.de/cm/349/bfr-removes-anthraquinone-from-its-list-of-recommendations-for-food-packaging.pdf
We suggest RAC will reject Annex XV proposal for Anthraquinone prepared by Germany.

Dossier Submitter’s Response
Noted. Some additionally supplied information on mutagenicity testing has been considered (e.g. see response to comments No. 16 and 19).

RAC’s response
Noted.

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<td>Sweden</td>
<td>WIBAX AB</td>
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Comment received

General comments

Manufacturing methods and purities of Anthraquinone (AQ):

9,10 Anthraquinone (AQ) used today as pulp production additive are manufactured by the methods below:

1. Friedel Crafts Reaction: Synthesis from phthalic anhydride and benzene
2. Diels -Adler reaction: Naphthalene process
3. Vapor-phase oxidation of anthracene with air

1. Phthalic anhydride process (Friedel Craft reaction):
   Synthesis of AQ from phthalic anhydride and benzene where aluminum chloride and sulfuric acid is used in the oxidation process [Vogel, 1985]. The purity of the final product is typical 99.2% and the AQ product is free from impurities as aromatic and nitroanthracenes [Butterworth, et al 2001].

2. Diels Adler reaction
   Reaction of 1,4 naphtoquinone with 1,3 butadiene followed by oxidation in aqueous solution. [Chung 1978]. Kawasaki Kasei Chemicals use a three step process that begins with oxidation of naphthalene to napthoquinone, which in turn is reacted with butadiene in a Diels-Adler reaction. The product is then oxidized to AQ in aqueous solution [Vogel 1985]. The resulting product is free from mutagenic impurities [Butterworth et al 2001]

3. Vapor-phase oxidation of anthracene with air
   Catalytic oxidation of anthracene with preheated air. After passing through the oxidation reactor the product is lead to the condensation section [Vogel, 1985]. This method is preferred for easy continous process both in oxidation and condensation phase. The AQ produced in Europe is manufactured with this method and the purity of the final product is minimum 98,5%, typical 99,2% and the AQ product is free from mutagenic substances, see Attachment 1: GC Analysis of Anthraquinone produced with vapor phase oxidation method.

This process should not be mistaken for the process which involves oxidation of anthracene in Nitric acid.

AQ used in NTP 2005 study
The AQ used in the NTP 2005 study [NTP, 2005] was purchased from Zeneca Fine Chemicals and produced with nitric acid oxidation of anthracene and was contaminated with 9 nitro anthracene. AQ manufactured with this method consists of the mutagenic impurity 9-nitro anthracene. The nitric acid oxidation method is no longer used for production of AQ.

Function of AQ in the pulp manufacturing process.
AQ works as a catalyst in the cooking process. AQ reduces the peeling break down reactions of the carbohydrates. AQ also increase the delignification rate. The results are an increased wood yield, a reduced need of alkali and sulfides for the delignification and reduced temperature needed for the cooking. The main benefits are:

- Less need of wood to produce the pulp
- Less need of cooking chemicals which reduce the load of the recovery plant
- Increased strengths properties of the pulp
- Energy savings, due to reduced temperature and need of alkali

For more details, see Attachment 2, Anthraquinone: An important additive for pulp manufacturer

Environmental benefits of AQ in the pulp manufacturing process.
The increase in yield and reduction of load has a direct positive impact on our environment by reduced consumption of wood and pulping chemicals. In the Nordic countries only, AQ saves several million trees every year, resulting in a positive impact to the environment through less fuel needed for the wood transports and reduced CO2 emissions. The saved trees will instead contribute to the absorption of CO2.

A single pulp and paper industry which produces 350 000 Adt pulp per year, reduces the wood consumption in the range of 360 000 trees per year and substantial environmental benefits due to reduction of truck transports, fuel and CO2 emissions. The yearly savings will be:

1600 truck transports of wood
35 truck transports of fuel
195 m3 fuel
650 tons CO2

For more details, see Attachment 2, Anthraquinone: An important additive for pulp manufacturer

References not included in the CLH report


Butterworth et al: international Journal of Toxicology, 23, 335-344, 2004

References in Attachment 2. “Anthraquinone: An important additive for pulp manufacturer” which are not included in CLH report


EFSA, European Food Safety Authority, 2012. Reasoned opinion on the review of existing maximum residue levels (MRL’s) for anthraquinone according to Article 12 of Regulation (EC) No 296/2005.


Reference lists are shown in the attached files.

*Comments Anthraquinone 2015 from WIBAX AB
*Anthraquinone: An important additive for pulp manufacturer

**ECHA note: The following attachments were provided with the comment above:**

- COMMENTS SUBMITTED TO ECHA BY WIBAX AB. [Attachment 7]
- Attachment 1 GC Analysis of Anthraquinone produced with vapor oxidation method. Confidential attachment. [Attachment 4]
- Attachment 2 Anthraquinone an important aditive for pulp manufacturer 2015-03-31. Confidential attachment. [Attachment 5]

Dossier Submitter’s Response

Thank you for the information. The information from the confidential attachments is not essential for the decision on the classification.

RAC’s response

Noted.

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Comment received

MS FR agrees with the classification proposal for human health (Carc 1B – H350).

Dossier Submitter’s Response

Thank you for your support.

RAC’s response

Noted.
Comment received

The NTP 2005 study is carried out on AQ originating from the nitric acid oxidation method i.e. a completely different manufacturing method with a different composition of AQ than the methods used today and is therefore not relevant for AQ produced and used today.

The study by Delgado-Rodrigues et al (ref) is not cited in the CHL report although it gives relevant information i.e. reporting of mutagenic potential of 9-NA in bacteria assay, a mammalian cell line and a Drosophila wing spot mutagenicity assay.

The findings of Sato et al (ref) has not been considered in the CHL report i.e. the findings that 1-hydroxy-AQ could not be detected in rat urine and furthermore that 2-hydroxyAQ is likely to be formed from another substance in the urine in the presence of air/oxygen during the isolation/analysis process. Hence there is no evidence that 1-hydroxyAQ and 2-hydroxyAQ are metabolites of AQ.

EFSA recommendation to set an MRL of 10 ppb in food was based on lack of data to enable a correct risk assessment rather than data giving evidence that AQ has carcinogenic properties (EFSA, European Food Safety Authority, 2012. Reasoned opinion on the Review of existing maximum residue levels (MRLs) for anthraquinone according to Article 12 of Regulation (EC) No 296/2005)

9,10 Anthraquinone (AQ) used today as pulp production additive is manufactured by the methods below:

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3. Vapor-phase oxidation of anthracene with air

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3. Vapor-phase oxidation of anthracene with air
Catalytic oxidation of anthracene with preheated air. After passing through the oxidation reactor the product is lead to the condensation section. This method is preferred for easy continuous process both in oxidation and condensation phase. The purity of the final product is minimum 98,5%, typical 99,2% and the AQ product is free from mutagenic substances [Vogel, 1985].

These processes should not be mistaken for the process which involves oxidation of
anthracene in Nitric acid which was used in former times. This method generates AQ with 9-nitroanthracene (9-NA) as an impurity. AQ produced and used today originate from method 1-3 above which result in AQ of higher purity i.e. AQ used today is not containing any mutagenic or carcinogenic substances, such as 9-nitroanthracene.

AQ used in NTP 2005 study
The AQ used in the NTP 2005 study [NTP, 2005] was purchased from Zeneca Fine Chemicals and produced with nitric acid oxidation of anthracene and was contaminated with 9 nitro anthracene which is a known mutagen. The nitric acid oxidation method is no longer used for production of AQ hence AQ produced and used today have a higher purity and do not contain any mutagenic impurities such as 9-nitroanthracene and the results from the NTP 2005 study is not relevant/fully applicable to AQ used and produced today.

There are important reports/studies on AQ which has not been considered in the CHL report (1-4 below). It’s very important that the classification of AQ is based on the right AQ product, correct executed studies and that the studies are evaluated in a scientifically correct way and we call for the inclusion of this information in the decision on classification of AQ.


Dossier Submitter’s Response
Thank you for the information, see response to comment 1.

The studies of Delgado-Rodriques and Sato are now considered (see response to comment 17).

To Sato et al. (1959):
Of higher relevance are more recently conducted studies of Graves et al. (2005\(^8\)) (see also Doc 2.1 and 2.2 to comment 9) who confirmed that 1-OH-AQ and 2-OH-AQ were found in samples of F344 rats that were fed with formulations of 4 lots of AQ, produced by three different synthetic routes: (AQ-OX, AQ-DA and AQ-FC\(^9\)). 1-OH-AQ, 2-OH-AQ and AQ were found in all samples from the dosed animals (more details see response to comment 1).

EFSA in their opinion did neither assess the toxicological properties of AQ nor did they recommend a classification on the carcinogenicity, but they highlighted the potential for

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\(^9\) Production types (Friedel-Crafts technology (AQ-FC), Diels-Alder chemistry (AQ-DA), from oxidation of anthracene (AQ-OX)
carcinogenicity in their opinion (Reasoned opinion on the review of the existing maximum residue levels (MRLs) for anthraquinone according to Article 12 of Regulation (EC) No 396/2005).

RAC’s response

Noted. RAC agrees with the DS. The study by Delgado-Rodriques et. al., (1995) was a useful test for somatic mutation and recombination in an in-vivo eukaryotic assay. Several compounds were tested including 9-NA. The results for 9-NA in both the ST (standard) cross and the HB (high bioactivation, a surrogate index for metabolic activation) cross were inconsistent and did not show a dose-response relationship. This paper did not provide much evidence for genotoxic activity for 9-NA; at some test concentrations there were positive results, at others negative results for small single spots. The test substance naphthalene was also tested in this study (along with positive controls) and showed clear positive responses especially following metabolic activation, thus illustrating that the test system was competent at detecting mutagenic substances. The study by Delgado-Rodriques et.al., (1995) did not add or reduce any support for 9-NA as a mutagen.

CARCINOGENICITY

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Comment received

We agree that that the criteria for classification in Carc. 1B, H350 for Anthraquinone are met. The proposal is very clear and well justified.

Dossier Submitter’s Response

Thank you for your support.

RAC’s response

Noted.

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Comment received

The decision on classification of anthraquinone as carcinogen 1B is solely supported by two carcinogenicity studies conducted by NTP in rat and mice (pag. 7 of BAuA report). Those studies have been challenged by the nature of the test item that has been demonstrated to contain not just 9-NA but also other relevant impurities.

This report comments upon BAuA report and would like to highlight the following facts:
1. Lack of data on 9-NA:
   The potential hazard of 9-NA was solely assessed by in vitro mutagenicity test (OECD 471 and 476).

No data is available for in vivo test for 9-nitroanthracene. Fu et al found out different metabolism of 9-NA depending on test conditions (aerobic or anaerobic). It may impact in different outputs for in vitro and in vivo test.
No data is available for chromosome aberration for 9-nitroanthracene in mammal cells (neither in vitro nor in vivo). This mechanism of action was not investigated in mammals. Furthermore Dihl et al (2008) investigated 9-NA for genotoxicity in the wing somatic mutation and recombination test (SMART) of Drosophila. Results demonstrated that 9-NA induced genetic toxicity causing increased incidence (75%) of homologous somatic recombinations. The evidence indicating that the major effect observed in this study is an increased frequency of mitotic recombination emphasizes another hazard that could be associated to NPAHs--the increment in homologous recombination (HR).

2. Metabolites 1-OH AQ and 2-OH AQ
BAuA report postulates that these metabolites may be responsible for the carcinogenetic effects seen in NTP studies. These are certainly main metabolites of AQ. However Graves et al.(2005) found out quantitative differences on the production of these metabolites depending on the origin of AQ. A metabolism study was conducted using male Fischer 344 rats in which they were fed formulations of 4 lots of anthraquinone, produced by three different synthetic routes: (AQ-OX, AQ-DA and AQ-FC). 1 and 2-hydroxyanthraquinone and anthraquinone were found in all samples from the dosed animals, with 2-hydroxyanthraquinone ten folds the concentration of 1-hydroxyanthraquinone. Both metabolites were found to be 65% less concentrated in AQ-FC than in AQ.OX. Original report is attached to this comment (DOC 2).

3. Purity of NTP samples
It is clearly stated that AQ impurities are different depending on the manufacture origin. Some of these impurities may be relevant to genotoxicity effect. Butterworth et al. demonstrated that NTP studies were carried out with AQ containing impurities. BAuA report refers to 9-NA which has been reported to be at 0.1%.

Furthermore Chemical Products Corporation send a comments letter to NTP in 2004 (DOC 3) concerned by the fact that Technical Report 494 of NTP itself recognized the presence of 0.1% mutagenic 9-Nitroanthracene in the test material, but also states on page 31 that high performance liquid chromatography/ultraviolet detection analysis indicates that the TR494 test material may contain up to 0.5% contaminants.

An analysis performed by Arkion Life Sciences indicates 0.6% contaminants in an aliquot of the TR494 test material; all of the contaminants detected in the Arkion Life Sciences analysis could be expected to confound the results of the TR494 studies. Draft TR494 does not identify any contaminants other than 0.1% 9-Nitroanthracene. The quantity and identity of the remaining contaminants in the TR494 test material is uncertain. (DOC 3)

Chemical Products Corporation’ letter to NTP reads: “CPC dissolved an aliquot of TR494 test material in concentrated sulfuric acid, and then reprecipitated it (the long-standing gravimetric technique for determining Anthraquinone purity). The reprecipitated material contained no detectable 9-Nitroanthracene, yet this refined TR494 test material still retained mutagenic activity. CPC provided this information to NTP in October 2000. CPC found evidence that there were impurities present in the TR494 test material as discrete particulates. These may or may not be uniformly distributed in each aliquot; CPC reported to NTP in late 2000 that the refined aliquot of the NTP test material exhibited a dark gray color as opposed to the pale yellow color of refined Anthraquinone. NTP does not present a comprehensive characterization of the identities and distributions of impurities in the TR494 test material.

BAuA report states “The low exposure level, the bioavailability, and the weak mutagenicity
make it unlikely and implausible that 9-NA was solely and totally responsible for the carcinogenic response” (page 55). We would add that metabolites 1-OH AQ and 2-OH AQ would also not be solely responsible too in the light of genotoxicity available test. CPC analytics demonstrated the mutagenicity activity of the test item after extraction of 9-NA but other impurities still remained.

This is extremely important point taking into account that proposal of classification is just supported by theses test, which has been demonstrated to be done with contaminated test item.

4. Some controversial affirmations
In the report there are some points which are controversial by being contradictory and sometimes biased explanation of the facts.

a) Genotoxicity test for AQ are negative both in vitro and in vivo (with purified item). However in page 55 the report reads: “Hence, AQ has the potential to act through a mechanism involving mutagenicity, and 9-NA is not a necessary component of this action.”

b) There is any data on carcinogenicity for either 9-Na nor 2-OH-AQ. However in page 55 reads: “The NTP concluded that 2-OH-AQ is an in situ metabolite of AQ that, based on experimental mutagenicity data, is as likely to be of comparable carcinogenicity or (with higher probability) more carcinogenic than 9-NA. “ Furthermore 2-OH-AQ is mutagenicity is only based in Ames test.

c) Page 54: “Moreover, measurements in the male rat urine showed, that 2-OH-AQ is systemically present at several-fold higher amounts than it is theoretically possible for the 0.1% 9-NA contamination, even if the latter was 100% bioavailable (NTP 2005, p. 92 table 23).” It is assumed that 2-OH-AQ is a metabolite of 9-NA which is quite surprising (see Fu et al 1985 for 9-NA metabolites identification).

However it links with Graves et al (2005) findings with respect to a difference of 65% less 2-OH-AQ in AQ FC than in AQ OX which seems to be the AQ quality used in NTP test. This seems to be one more piece of evidence of diverse effects of different AQ origins.

d) BAuA recognises that no carcinogenicity test is available for pure AQ in page 54: “In fact, it should be taken into account further that carcinogenicity has not been examined for 100% pure AQ or AQ-DA or AQ-FC. Thus, it is to conclude that AQ as tested in the NTP studies (containing 0.1% 9-NA) was carcinogenic. A possible contribution of the impurity cannot be assessed.”

This last may be true since no data for 9-NA is available, as stated in point 1 of this report; following this approach it is also true that a possible contribution of AQ cannot be assessed as well, due to the demonstrated composition of the tested sample with plausible confounding effects from co-exposure to other chemicals. Therefore the principle for classification “a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms” is not meet.

e) Comparison with CLP criteria.
First point is not meet: “two or more species of animals or in two or more independent studies in one species carried out at different times or in different laboratories or under different protocols”; Studies are not independent since the sample is the same and also the laboratory. However it seems to match pretty much with some criteria for category 2, if any: “(b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of
the studies; “

5. Mode of action
As a result of the output of carcinogenicity studies conducted by NTP, different authors have been trying to find out a plausible explanation and elucidate the mode of action of AQ in carcinogenesis. BAuA report also intends to do so.

Genotoxicity test are negative both in vitro and in vivo (with purified item). Therefore no genotoxic mode of action can be postulated. However in page 55 the report reads: “Hence, AQ has the potential to act through a mechanism involving mutagenicity, and 9-NA is not a necessary component of this action.”

Page 55 reads. “In addition, the lack of activity in mutagenicity assays does not give proof of non-carcinogenicity of AQ, because carcinogenicity of AQ could also be mediated by other mechanisms.”. It is well known that carcinogenicity may be due to epigenetics. However there is a piece of information to take into account that is contradictory with this hypothesis: a negative output for A mammalian cell transformation test in Chang (human liver) cells and BHK-21 C13 (baby Syrian hamster kidney)

6. Analogues.
BAuA report mentions in page 55 results for so-called analogues reported in NTP 2005 report. Those chemicals are halogen, amino or nitro substituted which are known alert structures. Nelson et al (1982) explained the structure-activity relationship for nitroaromatic compounds.

Ar = Any aromatic/heteroaromatic ring
- Chemicals with ortho-disubstitution, or with an ortho carboxylic acid substituent are excluded.
- Chemicals with a sulfonic acid group (-SO3H) on the same ring of the nitro group are excluded.

Metabolic activation of nitroaromatic compounds to toxic metabolites, involve initial one-electron reduction of the nitro group to yield a resonance-stabilized nitro anion radical. Under aerobic conditions, the radical can reduce molecular oxygen to form superoxide anion, which can generate various toxic and DNA-reactive oxygen species. In a more anaerobic environment, the nitro anion radical can be reduced further to nitroso, hydroxylamine, and amine (Nelson 1982). Further activation of hydroxylamine produces the reactive nitrenium ions (see SA25 and SA28).

Therefore should not be considered valid analogues. However these is used as a rationale for third criteria of CLP by BAuA: “In additional considerations there are structurally related substances (from the NTP data base) with shown carcinogenic potential as well as a possible contribution of mutagenicity (in particular of metabolites) as a mode of action. “

However the BAuA report also mentions emodin ((1,3,8-trihydroxy-6-methylanthraquinone) and we would also add chrysophanol (1,8-dihydroxy-3-methylanthraquinone) as analogues. Metabolites of emodin are v-hydroxyemodin and 2-hydroxyemodin (Mueller 1998; Murakami 1987; Masuda, 1984) and chrysophanol is transformed, in a cytochrome P450-dependent oxidation, to aloe-emodin (1,8-dihydroxy-3-hydroxymethylanthraquinone) as the major product formed (Stephan et al. 1998).
Metabolic pathway of emodin and chrysophanol (from Stephan et al. 1998)

Summary of Available data for Emodin (see all details in DOC 1. Point 4)

- Genetic toxicity in vitro: Chromosomal aberrations in mammalian cells:
  Chromosome aberrations were induced in cultured CHO cells in the absence of S9 activation and in the presence of S9; the response observed without S9 was stronger than with S9.

- Genetic toxicity in vitro: Mammalian cell micronucleus test:
  The test substance did not reveal any micronuclei inducing activity in either human lymphocytes or in Hep-G2.

- Genetic toxicity in vitro: Gene mutation in mammalian cells:
     The substance induced a moderate increase in mutant fraction (it was only tested without metabolic activation).
     Treatment with the test substance did not result in an increase in the number of colonies resistant to 6-TG. Exposure in suspension in the presence of liver homogenate was also negative.
     In the V79-HGPRT mutation assay, the test substance emodin was highly mutagenic without metabolic activation. However, the increase in the induction of mutation was observed at highly toxic concentrations. Furthermore, a low plating efficiency was observed in the negative and positive controls.

- Genetic toxicity in vivo: Mammalian erythrocyte Micronucleus test:
     There was no statistically significant enhancement in the frequency of micronucleated PCEs in comparison to the negative controls at both preparation intervals.
  2. NTP (National Toxicology Programme) (1992): Test method equivalent to OECD guideline 474.
     In peripheral blood samples from mice in the 14-week feed study, an increase in the frequency of micronucleated NCEs was seen in females, but not in males. The small increase in NCEs observed in the female mice was statistically significant (P=0.001), but no individual exposed group value differed significantly from the control value; the result in female mice was concluded to be weakly positive.

- Genetic toxicity in vivo: Mammalian bone marrow chromosome aberration test:
  1. NTP (National Toxicology Programme) (1993a): Test method equivalent to OECD guideline 475.
     No increases in the frequencies of micronucleated erythrocytes were observed in any of the treatment groups (only males were used).
  2. NTP (National Toxicology Programme) (1993b); Witt KL, Knapton A, Wehr CM, Hook GJ,
ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON ANTHRAQUINONE

Mirsalis J, Shelby MD and MacGregor (2000): Test method equivalent to OECD guideline 475. No increases in the frequencies of micronucleated erythrocytes were observed in any of the treatment groups (males and females were used).

- Carcinogenicity
Rats: There was no evidence of carcinogenic activity of emodin in male rats. There was equivocal evidence of carcinogenic activity in female rats.
Mice: There was equivocal evidence of carcinogenic activity in male mice. There was no evidence of carcinogenic activity in female mice.

CONCLUSIONS
- Proposal for classification is solely based on NTP carcinogenicity test. It has been demonstrated that NTP test item was not pure AQ and therefore tests results are not matching with all previous mutagenicity test. No evidence for mutagenicity was found for AQ in vitro or in vivo.
- Those tests should be withdrawn and considered not valid for AQ assessment.
- Mechanism for carcinogenicity has not been elucidated; this is the result of intending create a link between all available data on AQ with NTP test. Pattern of effects seen in NTP studies seems to be done by a direct acting mutagen that AQ demonstrated no potential for.
- Further investigation is required before proceeding with decision on classification of AQ.

SEE ATTACHED DOC 1-3

ECHA note: the following attachment was provided with the comment above [Attachments 8 – 14-]:
- Letter from Nopco on the comments submitted during the public consultation
- COMMENTS TO CLH REPORT FOR CLASSIFICATION PROPOSAL
- Doc 1 – Anthraquinone. Genotoxicity and carcinogenicity potential of different manufacturing origins
- Doc 2.1 - BIOLOGICAL SAMPLE ANALYSIS REPORT. ANALYSIS OF URINE SAMPLES FOR 1-AND 2-HYDROXYANTHRAQUINONE
- Doc 2.2 - BIOLOGICAL SAMPLE ANALYSIS REPORT. ANALYSIS OF URINE SAMPLES FOR 1-AND 2-HYDROXYANTHRAQUINONE
- Doc 2.3 - BIOLOGICAL SAMPLE METHOD DEVELOPMENT REPORT. ANTHRAQUINONE
- Doc 3 – Letter to NTP on Comments concerning NTP draft Technical Report 494

Dossier Submitter’s Response

See response to comment 1.

It has been recognised in the CLH report that the substituents could determine the metabolism, toxicity including carcinogenicity and the target organs involved.

RAC’s response

Noted.

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Comment received

The substance is not carcinogenic. In case you agree on lack of information you have to
follow REACH legislation and ask Lead Registrant for new testing proposal for endpoint carcinogenicity and wait for results of the test. We did not submitted testing proposal for this endpoint, because we do not see any gap. There is enough information available to prove that the substance is not carcinogenic.

Dossier Submitter’s Response

Please see the response to comment 1 and consider the explanation on the definition of a mono-constituent at the end of the response.

RAC’s response

Noted.

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Date | Country | Organisation | Type of Organisation | Comment number
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07.04.2015 | Sweden | WIBAX AB | Company-Downstream user | 11

Comment received

Comments regarding Carcinogenicity

The CLH report proposal for CLP classification of AQ as category 1B carcinogen, H 350 (May cause cancer) are mainly based on three main factors:

1. Results from the NTP study
2. That the metabolite 2-hydroxi-AQ is formed in high content
3. That the metabolite 2-hydroxi-AQ is much more mutagen than the impurity 9-nitro anthracene

According to our research the facts are not correct and its contradictions in the literature. It is major doubts regarding all three factors, see detailed comments below.

1) AQ used in the NTP study was consisting of mutagenic impurities. The results of tests for AQ with the impurity 9-nitro anthracene vary from none to a dose dependent mutagenicity [Butterworth, 2001], [NTP, 2005]. Tests with AQ without mutagenic impurities (9 nitro anthracene) or AQ with high purity was not mutagen in the test system used. Thus, this clearly indicates that the AQ sample used by the NTP study may be affected by an unknown pattern of toxic impurities.

It’s not a clear link between the Substance AQ and the carcinogenicity. The production samples of AQ used in the NTP 2005 study containing 9-nitro anthracene resulted in carcinogenic activities in rat and mouse [NTP, 2005]. This has been an important argument in the CLH proposal to suggest AQ in Category 1B with the hazard phrase “H350: May cause cancer”. According to the CLP-regulation it is correct to use animal data on carcinogenicity for this category “if there is a clear link between the substance and the carcinogenicity” [ECHA, 2013]. However, there are impurities involved here which implies there is not a clear link for ‘one’ substance to the toxic effects. In the NTP study it has not been fully investigated what role in the observed effects the impurities may have.

The AQ used in the NTP study was produced by the nitric acid production method which no longer are used. The AQ used today don’t consist of any mutagenic impurities as 9-nitro anthracene. The AQ produced in Europe today are manufactured with the vapor oxidation process and has a purity of typically 99,2 % without any mutagenic impurities.

2) Formation of 2-hydroxi-AQ is not verified. In the discussion for carcinogenicity the CLH
ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON ANTHRAQUINONE

The report says that the 2-hydroxy-AQ is the primary metabolite and that this substance is much more mutagen and therefore contributing more to cancer compared with 9-nitroanthracene. In fact shown by Sato et al. (1956) the isolation of 1-hydroxy-AQ was not successful, and the 2-hydroxy-AQ was interpreted as a major metabolite of AQ in rat urine present in low levels compared to the AQ-dose given in the diet, but only after air/oxygen contact in the chromatography process used for identification purposes. Later work [Sato et al., 1959], rats feed with AQ and receiving an i.c. 35SO4-injection, indicated that 2-hydroxy-AQ was liberated from a “substance” in urine and concluded that the substance was a sulfate conjugate of 2-hydroxy-AQ. The fact that the formation of 2-hydroxy is very low, the argumentation in both NTP 2005 and CLH reports regarding the contribution of this metabolite for the carcinogenicity of AQ are not correct. Sato et al 1959 was not cited in neither the NTP study nor CLH reports.

The presence of this sulfate-2-hydroxy-AQ conjugate was not considered in the CLH or NTP-reports

No studies are done of the biotransformation of AQ to metabolites 1-hydroxy-AQ and 2-hydroxy-AQ. Nor publications reporting that AQ is bio transformed to 1-hydroxy-AQ and 2-hydroxy-AQ in humans.

3) The grade of mutagenicity of 2-hydroxy-AQ is not fully verified. Butterworth et al reported that the mutagenicity of 2-hydroxy-AQ was negative, while that of 9-nitro anthracene as positive [Butterworth et al: international Journal of Toxicology, 2004]. This outcome contradicts the description from “4.10.4 Summary and discussion of carcinogenicity” CLH report p.54, “Butterworth et al, 2004 estimated that 2-OH-AQ is a bacterial mutagen twice as potent as the impurity 9-NA “

Difference between IARC classification and CLH report proposal of CLP classification

The CLH-classification of AQ also goes beyond the IARC expert classification. In contrast, the IARC classification of AQ is in IARC-Group 2B: “Possibly carcinogenic to human” [IARC Monograph, 2012]. This reflects that this international expert organ on cancer issues, finds the available data less convincing for the mother substance AQ to be classified in Group 2A: “Probably carcinogenic to humans”. The distinction between the terms “possibly” and “probably” is important and means that there are doubts about the carcinogenicity of AQ in humans.

Conclusion
Based on the comments above Wibax AB cannot agree on the CLH reports proposed 1B, H350 “May cause cancer) classification for AQ

ECHA note: The following attachments were provided with the comment above:

- COMMENTS SUBMITTED TO ECHA BY WIBAX AB. [Attachment 7]
- Attachment 1 GC Analysis of Anthraquinone produced with vapor oxidation method. Confidential attachment. [Attachment 4]
- Attachment 2 Anthraquinone an important additive for pulp manufacturer 2015-03-31. Confidential attachment. [Attachment 5]
## Annex 2 - Comments and response to comments on CLH Proposal on Anthraquinone

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### Comment received

Not relevant as the studies performed are based on AQ that was produced according to a manufacturing method not used today. The AQ was contaminated with mutagenic substances.

### Dossier Submitter's Response

Please see the response to comment 1.

### RAC's response

The studies are relevant. They are the only robust 2-year bioassays for carcinogenicity available for assessment of the carcinogenic potential of AQ.

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### Comment received

Comments to ECHA regarding the animal test results in CLH Report

1. Introduction

We scrutinized the substance of CLH report, proposal for harmonized classification and labelling, based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2 (Substance Name: Anthraquinone). As the result, we found incorrect descriptions in this report, so that it seems to be insufficient evidence to propose classifying anthraquinone (AQ) into carcinogenicity 1B. We suggest the following pointing as evidence.

2. The incorrect descriptions in CLH report regarding the cancer in rats

The description in Table 21 on page 46 of the CLH report disagrees with the description in Table 23 on page 49-50 based on the result in NTP report.

2.1 Cancer of the kidney and urinary bladder in rats

The result of F344/N Rats50M/50F in Table 21 described “AQ caused cancer of the kidney and urinary bladder in M/F rats”. On the other hand, in Table 23, neoplastic effects of the kidney and urinary bladder in male rats were observed NOT in cancer BUT in renal tubule adenoma or transitional epithelial papilloma.

2.2 Cancer of the liver in female rats

The result of F344/N Rats50M/50F in Table 21 indicated “AQ caused cancer (omitted) of liver in F rats”. On the other hand, in Table 23, neoplastic effects of the liver in female rats were observed NOT in cancer BUT in hepatocellular adenoma.

3. The animal study data reported by NTP

The National Toxicology Program (NTP) reported data from 2-year feed studies on the...
carcinogenicity of AQ (purity, 99.8%) in F344/N rats and B6C3F1 mice (NTP 2005). Data on the carcinogenic effects are summarized in Table 1[Attachment 1] by organ and tumor type.

[Attachment 1]
Table 1 Summary of carcinogenic effects

As summarized in [Attachment 1] regarding liver tumors, the incidences of adenoma, adenocarcinoma, and blastoma were all clearly increased in male and female mice. In rats, however, liver adenoma was only infrequently observed in males and females. The incidence of liver adenoma in rats showed no clear dose-related trend. In a 14-week study, however, the incidence of hepatocellular hypertrophy was high in both rats and mice.

As for kidney tumors, adenoma was observed in male and female rats and carcinoma was observed only in female rats. None of the mice developed adenoma or carcinoma. Of note, rats showed accumulation of hyaline droplets in the renal tubules even in the 14-week study.

Urinary bladder or thyroid gland tumors were observed in either rats or mice (of either sex), but not in both animal species.

From the viewpoint of the extrapolatability of the data to humans, the observed variability of the endpoint does not adequately support Carcinogenicity Category 1B.

4. The incorrect description of mutagenicity in CLH report

We also refer to 2-hydroxyAQ (2-OH-AQ) which is a metabolite of AQ and 9-nitroanthracene (9-NA) which is an impurity of AQ sample used by NTP. We doubt strongly the below description from “2.2 Short summary of the scientific justification for the CLH proposal” on page 7 of the CLH report, “Due to biotransformation processes mutagenic metabolites of AQ appear, which are at least five times more potent and present at systemically higher concentration than 9-NA.”.

We illustrate the reasons using specific activity as an index of mutagenic for 2-OH-AQ and 9-NA.

Specific activity (SA) is calculated by the following formula.

$$SA = \frac{(X - Y) \times 1000}{Z}$$

SA: Specific activity (revertants/mg)

X: The number of revertant colonies per plate (In case of plural value, the average value is substituted)

Y: The number of colonies in the negative control

Z: Amount of test substance per plate (ug/plate or ug/plate)

Specific activity calculated by the result of mutagenicity test using 2-OH-AQ or 9-NA from NTP report (2005) is shown in Table 2 [Attachment 2]. It indicates that the specific activity of 2-OH-AQ was higher than that of 9-NA in only case using the bacteria TA98(-S9). On the other hand, test results using TA98(+S9), TA100(-S9), and TA100(+S9) indicates that the specific activity of 9-NA was higher than that of 2-OH-AQ.

[Attachment 2]
Table 2 Comparison of the specific activity between 2-OH-AQ and 9-NA based on NTP report (2005)

Furthermore, Butterworth et al. reported that the mutagenicity of 2-OH-AQ was negative, while that of 9-NA was positive (Butterworth et al.: International Journal of Toxicology, 23,
ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPSAL ON ANTHRAQUINONE

Needless to say, specific activity of 9-NA is higher than that of 2-OH-AQ. This outcome contradicts the below description from "4.10.4 Summary and discussion of carcinogenicity" on page 54 of the CLH report, “Butterworth et al. (2004) estimated that 2-OH-AQ is a bacterial mutagen twice as potent as the impurity 9-NA.”.

By above our consideration, the mutagenicity of 9-NA should be more intense than that of 2-OH-AQ.

Genotoxicity of AQ was examined in many studies. The current conclusion is that AQ itself is not genotoxic or mutagenic, but 2-OH-AQ, the major urinary metabolite in rats, is apparently mutagenic in the Ames test using Salmonella typhimurium strains. However, a 100% pure AQ showed no mutagenic activity in the Ames test with or without rat S9 metabolic activation enzymes. Although genotoxicity or mutagenicity of the major metabolite of AQ, 2-OH-AQ, may play a role in the carcinogenesis in animals, this has not been demonstrated by animal studies.

5. Carcinogenicity data in humans
“...The Working Group identified a series of publications on dye and resin workers in a single facility in the USA who were potentially exposed to AQ during its production or its use to produce AQ intermediates. These publications reported on findings from the initial cohort, nested case-control analyses of lung cancer and central nervous system tumours, and updated findings on an expanded cohort. An excess risk of mortality from lung cancer was found among workers employed in the AQ dye production area in both nested case-control and cohort analyses. Workers in this production area were potentially exposed to AQ, AQ dye intermediates, anthracene, vanadium pentoxide and epichlorohydrin. Within the AQ dye production area, a 12-fold increased risk for lung cancer was found for workers producing AQ itself, but this was based on only a few exposed cases. The increased risk did not appear to be due to cigarette smoking, or exposure to asbestos or epichlorohydrin. An excess incidence of central nervous system tumours was also found among workers employed in the AQ dye production area, but this was based on only three exposed cases who may also have been exposed to epichlorohydrin, which was also associated with an increased risk of these tumours. The major limitations of these studies were that: (1) risk estimates were calculated for men employed in AQ and AQ dye production, but exposure to AQ per se was not evaluated; (2) the statistical power to detect effects for specific cancers was limited because of the small number of exposed cases; and (3) the ability to evaluate potential confounding from other occupational exposures was also limited."

In light of the above, there is insufficient evidence of the carcinogenicity of AQ in humans.

6. Conclusion
Upon the comprehensive evaluation of the above, we cannot agree with this proposal to classify AQ into carcinogenicity 1B "It is presumed to have carcinogenic potential for humans, classification is largely based on animal evidence.”.

< NOTE >
We attached following 3 pdf files in a zip named
"Attachments(Comments_to_ECHA_regarding_the_animal_test_results_in_CLH_Report).zip"
+ Attachment_1(Table1_Summary_of_carcinogenic_effects).pdf
+ Attachment_2(Table2_Comparison_of_the_specific_activity_between_2-OH-AQ_and_9-
ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON ANTHRAQUINONE

ECHA note: The following attachment were provided with the comment above [Attachments 4 -6]:
- Table 1, Summary of carcinogenic effects
- Table 2, Comparison of the specific activity between 2-OH-Aq and 9-NA based on NTP report (2005)
- Butterworth, Mathre, Ballinger, Adalsteinsson. Contamination is a frequent confounding factor in toxicology studies with anthraquinone and related compounds

Dossier Submitter’s Response
Thank you for your comment and documents. For many points see the response to comment 1.

To point 2
All benign and malignant tumours are to be assessed.

To point 4
From the calculations the comment presents in Table 2 of the attachment one could take both conclusions – the bacterial test systems may indicate that 2-OH-AQ is more potent than 9-NA or it is less potent. Should the most sensitive substance be estimated on the basis of the lowest positive concentration or based on the highest activity? Finally the relevance of each of the numbers could not be estimated and is not relevant as based on the data available they do not allow to disregard one of both substances with mutagenic properties and are uncertain with regards to their interpretation for the in vivo activity.

RAC’s response
The DS is correct. All types of tumours are assessed with regard to classification and considering a weight of evidence approach.

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Comment received
The NL CA has some doubts on the proposed classification for carcinogenicity, Carc. 1B (H350). For Carc. 1B (H350) classification, there needs to be an increase in malignant or a combination of malignant and benign neoplasms in two or more species of animals. In the 2-year feed study by the NTP (2005), there was some evidence of carcinogenic activity of anthraquinone in male F344/N rats based on increased incidences of renal tubule adenoma and of transitional epithelial papillomas of the kidney and urinary bladder (Table 23, CLH Report). There was clear evidence of carcinogenic activity of anthraquinone in female F344/N rats based on increased incidences of renal tubule adenomas and carcinomas. Increases in the incidences of urinary bladder transitional epithelial papilloma and carcinoma (combined) and of hepatocellular adenoma in female rats were also related to anthraquinone exposure (Table 23, CLH Report). However, the actual increase in malignant tumours was absent in males and very limited in females. There was clear evidence of carcinogenic activity in male and female B6C3F1 mice based on increased incidences of liver adenomas and carcinomas. Hepatoblastomas were also observed in male mice (Table 23, CLH Report). Given that there are unresolved questions with regards to contamination with 9-nitroanthracine (anthraquinone purity of 99.8%), and that there is an increase of only
benign tumors in rat, and liver carcinomas in mice, a classification of Carc. Cat. 2 (H351) might be considered more suitable. The comparison with the criteria should better illustrate with more detail why classification for carcinogenicity in category 1B is warranted. This should include the additional factors in paragraph 3.6.2.2.4-9 of the CLP criteria.

**Dossier Submitter’s Response**

Treatment-related tumours if benign should also be considered for classification proposals. It is the overall weight of evidence from two species and tumours in several organs that supports the classification as Carc Cat 1B.

The uncertainties with regards to 9-NA and their relevance for classification are discussed in the response to comment 1.

**RAC’s response**

There are clear treatment responses as testified by the numerous tumour profiles even though they do not follow strict monotonic dose responses. Comparison with concurrent controls and historical control data is quite important in this case and as the DS rightly points out, an overall weight of evidence approach is the fairest way to judge the data and propose a classification. There is progression from adenoma to carcinoma and also low incidences of very rare malignant tumours. Overall RAC has concluded that the evidence is in favour of classification as Carc. 1B in this case.

**MUTAGENICITY**

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Comment received

The NL CA agrees for no classification for mutagenicity, but clarification is recommended on why the in vivo 3-fold increase in single-strand DNA breaks (comet assay, p. 39, CLH Report) in liver and kidney is not relevant for classification given that both organs are target tissues for carcinogenicity.

**Dossier Submitter’s Response**

Thank you for the statement regarding the non-classification for mutagenicity of AQ.

In the publication of Cesarone et al. (1982) it is informed on the induction of single strand breaks (in vivo Comet assay). Only one dose was tested per substance in a kind of screening of various substances. The purity of tested AQ batch is unknown. Furthermore, a positive control as an essential reference parameter for the reliability of a test result was not taken into account. All in all the DS is of the opinion that the 3-fold increase in single-strand DNA breaks in liver and kidney cells of mice after i.p. injection of AQ cannot be assessed with sufficient certainty. Therefore this result is not considered for a discussion on classification of AQ.

**RAC’s response**

RAC agrees with the NL and the DS that no classification for mutagenicity is warranted.

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The substance is not mutagenic. Please check the Lead Registrant dossier on dissemination portal with all relevant information. In case you will need background information, we can send you study reports of all submitted studies.

Dossier Submitter’s Response

Thank you for your comment.

The final reports ‘Bacterial reverse mutation test’ (Täublová 2009), ‘In vitro mammalian cell gene mutation test’ (Bednáříková 2010) and ‘In vitro mammalian chromosome aberration test’ (Lazová 2010) are now available on a confidential basis. The negative results of the three guideline-compliant in vitro studies support the conclusion of the DS, who evaluated AQ as non-mutagenic based on the available data.

RAC’s response

RAC agrees with the DS, that no classification for mutagenicity is appropriate. These reports were judged as acceptable and were considered in the assessment of AQ mutagenicity.

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<td>Sweden</td>
<td>WIBAX AB</td>
<td>Company-Downstream user</td>
<td>17</td>
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</table>

Comment received

Comments regarding Mutagenicity

1. AQ without impurity of 9 nitro anthracene is not mutagen
   Based on the references in the CLH report [Butterworth, 2001], [NTP, 2005], it’s clear that AQ without mutagenic impurities (9 nitro anthracene) or AQ with high purity was not mutagen in the test system used. The results of tests for AQ with the impurity 9-nitro anthracene vary from none to a dose dependent mutagenicity. Thus, this clearly indicates that the AQ sample used by the NTP 2005 study may be affected by an unknown pattern of toxic impurities.

2. Mutagenicity of 9 nitro anthracene is proven
   All studies referred in the CLH report regarding mutagenicity of 9 nitro anthracene was weakly positive to positive [Butterworth, 2004], [Fu et al, 1985], [NTP, 2005], [Pitts 1982], [Zeiger 1988] and [Durant, 1996]. An additional mutagenicity study performed by [Delgado-Rodrigues et al, 199510], which is not cited in the CLH report, also reported a mutagenic potential of 9 NA in bacteria assay, a mammalian cell line and a Drosophila wing spot mutagenicity assay.

3. The degree of mutagenicity of 2-Hydroxi AQ is not fully verified
   Butterworth et al reported that the mutagenicity of 2-hydroxi AQ was negative, while that of 9-nitro anthracene as positive [Butterworth et al: international Journal of Toxicology, 2004]. This outcome contradicts the description from “4.10.4 Summary and discussion of carcinogenicity” CLH report p.54, “Butterworth et al, 2004 estimated that 2-OH-AQ is a bacterial mutagen twice as potent as the impurity 9-NA “

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4. Question marks regarding the formation of the metabolites 1-hydroxy AQ and 2-hydroxy-AQ.

The mutagenicity of the mother compound depends on the biotransformation to metabolites, of which 1-hydroxy-AQ and 2-hydroxy-AQ are debated in both CLH report and the NTP study. As shown by [Sato et al., 1956] the isolation of 1-hydroxy-AQ was not successful, and the 2-hydroxy-AQ was interpreted as a major metabolite of AQ in rat urine present in low levels compared to the AQ-dose given in the diet, but only after air/oxygen contact in the chromatography process used for identification purposes. Later work [Sato et al., 1959], rats fed AQ and receiving an i.c. 35SO4-injection, indicated that 2-hydroxy-AQ was liberated from a "substance" in urine and concluded that the substance was a sulfate conjugate of 2-hydroxy-AQ.

The presence of this sulfate-2-hydroxy-AQ conjugate [Sato et al., 1959] was not considered in the CLH or NTP-reports.

**ECHA note: The following attachments were provided with the comment above:**

- COMMENTS SUBMITTED TO ECHA BY WIBAX AB. [Attachment 7]
- Attachment 1 GC Analysis of Anthraquinone produced with vapor oxidation method. Confidential attachment. [Attachment 4]
- Attachment 2 Anthraquinone an important aditive for pulp manufacturer 2015-03-31. Confidential attachment. [Attachment 5]

**Dossier Submitter's Response**

Thank you for your comments that will be answered as follows:

Comment 1:
AQ is not mutagen when high purity charges without 9-nitroanthracene as impurity were tested.

Comment 2:
The results of the Somatic mutation and recombination tests with Drosophila melanogaster (Delgado-Rodriques et al. (1995); Mutation Research 341, pp 235 – 247) with 9-nitroanthracene is assessed as inconsistent by the authors. In principle, the test result is of questionable relevance due to the unknown purity of the tested substance and the lack of a positive control.

See 3:
The DS agrees to the statement that the degree of mutagenicity of 2-OH-AQ is not fully verified (see table 20 of the CLH report).

The estimation of Butterworth et al. (2004) that 2-OH-AQ has a higher mutagenic activity in bacteria than 9-nitroanthracene results from collected test data (Butterworth et al. 2004; International Journal of Toxicology 23, pp 335 - 344). The authors report in their publication on positive bacterial gene mutation tests for 2-OH-AQ with metabolic activation as well as for 9-NA without metabolic activation. 2-OH-AQ is positive in the tester strain TA 1537 (also Salmonella typhimurium strains TA 98, TA 100, TA 1535 and E. coli WP2 uvrA were tested). A positive effect was observed in

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the dose range of 1.0 – 333 µg/plate with metabolic activation (see page 341 of the publication). The highest mutation frequency ($F_{\text{max}}$) of 14.2 was obtained at a concentration of 10.0 µg/plate.

In the same publication also a positive bacterial gene mutation test is described for 9-nitroanthracene (see page 342 of the publication). Both tested Salmonella typhumirium tester strains TA 98 and TA 100 were weakly positive without metabolic activation: TA 98 from 0.3 up to the highest tested concentration of 10 µg/plate ($F_{\text{max}} = 3.5$ at 10 µg/plate); TA 100 at the highest tested concentration of 10 µg/plate ($F_{\text{max}} = 2.0$).

See 4:
We agree to the conclusion that the mutagenicity of the mother compound AQ could depend on the biotransformation to metabolites. For the exemplified discussed metabolites 1-OH-AQ and 2-OH-AQ only limited information is available on the induction of mutagenic effects (see table 20 of the CLH report). On the other hand the mutagenicity tests with the parent compound AQ (high purified and without 9-nitroanthracene as impurity) showed no mutagenic activity with and without metabolic activity at in vitro testing and in an in vivo micronucleus test (see Table 14 of the CLH report). Accordingly, the DS follows the conclusion of Butterworth et al. (2004) that the quantity of produced metabolites may not be sufficient for a possible induction of mutagenic effects. Therefore, further metabolites such as sulfate-2-OH-AQ conjugate [Sato et al., 1959] were not considered in the CLH report.

RAC’s response
RAC supports the comments of the DS.

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<td>Sweden</td>
<td>Swedish Forestry Industries</td>
<td>Industry or trade association</td>
<td>18</td>
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</table>

Comment received
Not relevant as the studies performed are based on AQ that was produced according to a manufacturing method not used today. The AQ was contaminated with mutagenic substances.

Dossier Submitter's Response
Thank you for the comment.

The available studies are relevant insofar as they enable an assessment of the mutagenicity of AQ depending on the purity of the tested batch. Based on the available data it can be concluded that AQ is not mutagenic in high purity batches without mutagenic contaminants such as 9-nitroanthracene.

RAC’s response
In support of the DS, RAC considers the studies relevant. The weight of evidence supports no classification for mutagenicity.

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Comment received
As no full studies were required from Lead registrant it seems that RSSs from REACH
registration were used for the evaluation of the reliability of studies only. It means that the reliability stated in CLH report is related to RSSs only, not to full studies. Why the submitter of CLH report didn’t require the full studies? How the reliability of information was evaluated, e.g. Durant et al. 1996 (reliability 2) vs. Lazová 2010 (reliability 4)?

The information on testing substance “purity: not known” is stated in CLH report for the following studies Täublová 2009, Bednáriková 2010 and Lazová 2010, although this information is available in registration dossier disseminated on ECHA website (it is public available information). Why?

Study Täublová 2009 – it is stated in CLH report that cytotoxicity was “not determined” although cytotoxicity was evaluated for strain S. typhimurium TA 100 (it is public available information again).

Study Lazová 2010 – this study was performed according to OECD 473. It is stated “not in accordance with OECD 473” in CLH report. Please, clarify it.

It is stated on the page 28 of CLH report: “For justification of classification/non-classification of AQ only those mutagenicity studies are of major relevance, which were carried out in accordance with the corresponding OECD test guideline. Mutagenicity tests whose test performance was carried out similar to the corresponding OECD test guideline should be also considered for the description of mutagenic effects.” Does it mean that studies performed according to methods in Regulation 440/2008 are not applicable for the classification purpose? It is in conflict with Art.13(3) of REACH Regulation, isn’t it? Please, clarify it.

We attach above mentioned studies (Täublová 2009, Bednáriková 2010 and Lazová 2010) to re-evaluate the information in CLH report.

**ECHA note:** The following confidential attachments were provided with the comment above:

- Täublová 2009, Anthraquinone – bacterial Reverse Mutation Test
- Lazová 2010 – Anthraquinone. Mutagenicity: In vitro Mammalian Chromosome Abberation Test (OECD 473)
- Bednáriková 2010 - Anthraquinone. Mutagenicity: In vitro Mammalian Cell Gene Mutation Test (OECD 476)

**Dossier Submitter’s Response**

Thank you for sending of following not publicated final reports: ‘Bacterial reverse mutation test’ (Täublová 2009), ‘In vitro mammalian cell gene mutation test’ (Bednáriková 2010) and ‘In vitro mammalian chromosome aberration test’ (Lazová 2010) on a confidential basis.

The negative results of the three guideline-compliant in vitro studies support the conclusion of the DS, who evaluated AQ as non-mutagenic based on the available data.

With respect to the paragraph cited from page 28 of CLH report ("For justification of classification/non-classification of AQ only those mutagenicity studies are of major relevance, which were carried out in accordance with the corresponding OECD test guideline. Mutagenicity tests whose test performance was carried out similar to the corresponding OECD test guideline should be also considered for the description of mutagenic effects.") the DS wants to respond to the following questions/statement:

(1) Does it mean that studies performed according to methods in Regulation 440/2008 are not applicable for the classification purpose?
(2) It is in conflict with Art.13(3) of REACH Regulation, isn’t it? Please, clarify it.

Obviously there is a misunderstanding regarding to the cited paragraph which refers to the quality (OECD guideline compliant, similar to OECD guideline, non-guideline compliant) of
the available mutagenicity tests. These tests are listed in Part B 'Methods for the determination of toxicity and other health effects' of the Regulation 440/2008. Thus the quoted passage is not in conflict with Art.13(3) of REACH Regulation.

RAC’s response
Noted. The three study reports submitted were evaluated and were considered acceptable and of high standard. The results of these studies support no classification of Anthraquinone for mutagenicity.

TOXICITY TO REPRODUCTION

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<td>DEZA, a.s.</td>
<td>Company-Manufacturer</td>
<td>20</td>
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Comment received
The substance is not reprotox. Please check the Lead Registrant dossier on dissemination portal with all relevant information.

Dossier Submitter’s Response
Thank you for your comment, but this toxicological endpoint was not evaluated for this dossier.

RAC’s response
Not applicable in this instance.

Date       | Country | Organisation | Type of Organisation       | Comment number |
------------|---------|--------------|----------------------------|----------------|
07.04.2015  | Sweden  | WIBAX AB     | Company-Downstream user    | 21             |

Comment received
Comments regarding Toxicity
According to animal test data, AQ show no acute and only minimal toxicity. NTP 2005 study [NTP, 2005] indicated early small effects on feeding and weight development in the 14 weeks and 2 years studies. Final animal weight did not differ between exposed and control. Survival was similar between exposed and control animals. The NTP 2005 study were in accordance with the Bayer studies [Bayer AG, 1976] and [Bayer AG, 1979] that are cited in the CLH-report.

ECHA note: The following attachments were provided with the comment above:
- COMMENTS SUBMITTED TO ECHA BY WIBAX AB. [Attachment 7]
- Attachment 1 GC Analysis of Anthraquinone produced with vapor oxidation method. Confidential attachment. [Attachment 4]
- Attachment 2 Anthraquinone an important aditive for pulp manufacturer 2015-03-31. Confidential attachment. [Attachment 5]

Dossier Submitter’s Response
Thank you for the comment but the reproductive toxicity was not evaluated for this dossier and repeated dose toxicity was not considered for classification purposes.
### ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON ANTHRAQUINONE

**RAC’s response**

Not applicable in this instance.

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<td>Sweden</td>
<td>Swedish Forestry Industries Federation</td>
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<td>22</td>
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</table>

**Comment received**

Not relevant as the studies performed are based on AQ that was produced according to a manufacturing method not used today. The AQ was contaminated with mutagenic substances.

**Dossier Submitter’s Response**

Thank you for the comment but this toxicological endpoint was not evaluated for this dossier.

**RAC’s response**

Not applicable in this instance.

### RESPIRATORY SENSITISATION

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<td>Company-Manufacturer</td>
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</table>

**Comment received**

The substance is not a Respiratory Sensitizer. Please check the Lead Registrant dossier on dissemination portal with all relevant information.

**Dossier Submitter’s Response**

Thank you for the comment but this toxicological endpoint was not evaluated for this dossier.

**RAC’s response**

Not applicable in this instance.

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</table>

**Comment received**

Not relevant as the studies performed are based on AQ that was produced according to a manufacturing method not used today. The AQ was contaminated with mutagenic substances.

**Dossier Submitter’s Response**

Thank you for the comment but this toxicological endpoint was not evaluated for this dossier.
ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON ANTHRAQUINONE

RAC’s response
Not applicable in this instance.

OTHER HAZARDS AND ENDPOINTS – Skin Sensitisation Hazard

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<td>Company-Manufacturer</td>
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Comment received

There is some evidence that the substance is Skin Sensitizer.

Dossier Submitter’s Response

Thank you for the comment but this toxicological endpoint was not evaluated for this dossier.

RAC’s response
Not applicable in this instance.

NON-CONFIDENTIAL ATTACHMENTS RECEIVED

1. COMMENTS SUBMITTED TO ECHA BY CHEMICAL PRODUCTS CORPORATION AND ITS SUBSIDIARY, CPT PULP AND PAPER, LLC. Submitted by an Individual on 12.03.2015. (Filename: 150310 Comments to ECHA including attachments.pdf) [Please refer to Comment number 1]
3. BfR removes anthraquinone from its list of recommendations for food packaging. Submitted by KAWASAKI KASEI CHEMICALS Ltd. On 31.03.2015. (Filename: Attachment_1(BfR_opinion No_0052013).pdf) [Please refer to Comment number 3]
4. Table 1, Summary of carcinogenic effects. Submitted by KAWASAKI KASEI CHEMICALS Ltd. On 02.04.2015. (Filename: Attachment_1(Table1_Summary_of_carcinogenic_effects).pdf) [Please refer to comment 13]
5. Table 2, Comparison of the specific activity between 2-OH-Aq and 9-NA based on NTP report (2005) Submitted by KAWASAKI KASEI CHEMICALS Ltd. On 02.04.2015. (Filename: Attachment_2(Table2_Comparison_of_the_specific_activity_between_2-OH-AQ_and_9-NA).pdf) [Please refer to comment 13]
6. Butterworth, Mathre, Ballinger, Adalsteinsson. Contamination is a frequent confounding factor in toxicology studies with anthraquinone and related compounds Submitted by KAWASAKI KASEI CHEMICALS Ltd. On 02.04.2015. (Filename: Attachment_3(report by Butterworth).pdf) [Please refer to comment 13]
7. COMMENTS SUBMITTED TO ECHA BY WIBAX AB. (Filename: Comments Anthraquinone from WIBAX 2015-04-04.pdf [Please refer to comments 5, 11, 17, 21]
8. Letter from Nopco on the comments submitted during the public consultation. Submitted by Nopco Paper Technology Holding AS. (Filename: NOPCO.pdf) [Please refer to comment 9]
9. COMMENTS TO CLH REPORT FOR CLASSIFICATION PROPOSAL (Filename: COMMENTS TO CLH REPORT FOR CLASSIFICATION PROPOSAL.pdf) Submitted by Nopco Paper Technology Holding AS. [Please refer to comment 9]


11. Doc 2.1 - BIOLOGICAL SAMPLE ANALYSIS REPORT. ANALYSIS OF URINE SAMPLES FOR 1-AND 2-HYDROXYANTHRAQUINONE. Submitted by Nopco Paper Technology Holding AS. (Filename: DOC 2.1.pdf) [Please refer to comment 9]

12. Doc 2.2 - BIOLOGICAL SAMPLE ANALYSIS REPORT. ANALYSIS OF URINE SAMPLES FOR 1-AND 2-HYDROXYANTHRAQUINONE. Submitted by Nopco Paper Technology Holding AS. (Filename: DOC 2.2.pdf) [Please refer to comment 9]

13. Doc 2.3 - BIOLOGICAL SAMPLE METHOD DEVELOPMENT REPORT. ANTHRAQUINONE. Submitted by Nopco Paper Technology Holding AS. (Filename: DOC 2.3.pdf) [Please refer to comment 9]


CONFIDENTIAL ATTACHMENTS RECEIVED

The following attachments were provided by an Academic institution on 01.04.2015. [Please refer to comment number 19]


The following attachments were provided by WIBAX AB on 07.04.2015 [Please refer to comments 5, 11, 17, 21]

4. Attachment 1 GC Analysis of Anthraquinone produced with vapor oxidation method (Filename: Attachment 1 GC Analysis of Anthraquinone produced with vapor oxidation method.pdf)

5. Attachment 2 Anthraquinone an important aditive for pulp manufacturer 2015-03-31 (Filename: Attachment 2 Anthraquinone an important aditive for pulp manufacturer 2015-03-31.pdf)

The following attachment was provided by a Company-Manufacturer on 07.04.2015

6. AQ-Internal grant study