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 misleading

and incorrect. We submit that the scientific record presented in these comments shows that Anthraquinone has been conclusively demonstrated to be a non-mutagen and should be classified as non-mutagenic 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 contaminated with mutagenic nitroanthracene impurities employed in animal tests by the U.S. National Toxicology Program (NTP) in the 1990s. This unique Anthraquinone material, contaminated with mutagenic nitroanthracene impurities resulting from manufacture by the nitric acid oxidation of anthracene process, is no longer an article of commerce. NTP purchased its test material from Zeneca Fine Chemicals in the early 1990s. Neither Zeneca nor any other manufacturer employes the nitric acid oxidation of anthracene process in 2015, 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 or any other mutagenic impurities.

We are confident that ECHA is committed to the application of sound science in its classification activities. We respectfully submit the following comments to 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 misleading and incorrect 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 (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 I]; Tikkanen et al. [Attachment VI]; 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 II] 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 Anthraquinone is conclusively demonstrated to be a non-mutagen by:

(1) the conclusion reached by NTP after careful consideration

reported in NTP 2005 and the draft TR-494 [Attachment II],
(2) the mutagenicity assays in the UCLID dataset [Attachment I] beginning on page 32,
(3) the negative mutagenicity assays reported by Tikkanen et al. [Attachment VI], and
(4) the Butterworth et al. assays presented in the CLH report.

2. The CLH report incorrectly bases its recommendation to classify the Anthraquinone now in commerce as Carc. 1B upon animal studies employing test material which was contaminated with mutagenic 9-nitroanthracene resulting from manufacture by the nitric acid oxidation of anthracene process (reported in NTP 2005). Its re-evaluation of the NTP 2005 studies is flawed.

Anthraquinone containing 9-nitroanthracene is no longer an article of commerce. There is no indication that the Anthraquinone in commerce in 2015 is a potential human carcinogen; it is not mutagenic and does not contain mutagenic impurities. 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 submit that the CLH report's re-evaluation of the NTP studies is flawed and incorrect; the following information demonstrates 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 the NTP test material contained mutagenic contamination. Boobis et al. state in 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 .... (A purified sample was negative in the Ames test.)" [Attachment III - Boobis et al.; Toxicologic Pathology; Vol. 37, 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, presenting Anthraquinone as the mutagen responsible for the observed cancers, 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 confirmed, the U.S.

National Institute of Environmental Health Sciences withdrew the first draft TR-494. In his letter to Chemical Products Corporation announcing withdraw of the 1999 draft TR-494, 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." [Attachment IV]

This letter is available on the U.S. government website at http://aspe.hhs.gov/infoquality/requests.shtml. (it is item 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; no other mutagenic impurities have been found in the Anthraquinone of commerce in 2015.

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 influence of strongly mutagenic contaminants which 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 Anthraquinone metabolite 2-hydroanthraquinone.

After withdrawal of its 1999 draft TR-494 which ascribed carcinogenicity observed in its animal studies to Anthraquinone, NTP determined that Anthraquinone is not a mutagen. NTP then began evaluation of metabolites of Anthraquinone which might be mutagenic and 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 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 V] 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.* (1956)...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 9-nitroanthracene.

5. 2-hydroxyanthraquinone, present in a very small quantity in fresh rat urine, has not been conclusively demonstrated to 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 VI] at page 301 is not cited. Tikkanen et al. state, "None of the compounds were mutagenic in strain TA98, with or without metabolic activation." Remarkably, NTP 2005 states at

page 91, concerning Tikkanen et al., "testing was not done without S9". Thus, evidence regarding mutagenicity of the 2-hydroxyanthraquinone present in very small quantities in rat urine is inconclusive and is not examined in the CLH report. We submit that the contention 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. False and unsupported information was added to NTP 2005 after NTP's February 2004 peer review panel recognized that the results of the NTP animal studies applied only to the contaminated material tested. The February 2004 NTP peer review panel recognized the impact of mutagenic contamination and directed that the term "anthraquinone" in the draft TR-494 it reviewed should be restricted 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 presented the negative mutagenicity assay for a sample designated "A07496" to the December 2004 NTP peer review panel in a successful effort to remove the "anthracene-derived anthraquinone" restriction imposed 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 mutagenicity assay of sample A07496, NTP's Dr. Cynthia Smith replied that decomposition of impurities was unlikely because sample A07496 had been stored frozen under argon throughout this extended period. Records obtained by Chemical Products Corporation after publication of NTP 2005 demonstrated that Dr. Smith's assurance was false; all of the NTP test material, stored as "Lot No. 5893", has been stored at room temperature under air since completion of animal testing. The influence of this false information on the peer review panel's deliberations was likely to have been significant; the validity of the December 2004 peer review panel's acceptance of the conclusions presented in NTP 2005 is called into question by the false information they relied upon in making their decision.

NTP's December 22, 2006 response to a Request for Correction submitted by Chemical Products Corporation (available on the U.S. government website http://aspe.hhs.gov/infoquality/requests.shtml as item 28.b3.) was addition of an erratum to NTP 2005 correcting this "error". NTP 2005, page 20, now states, "Dr. Klaunig asked if the samples assayed were the original test material and if any degradation might have occurred during the interval. Dr. Smith replied that this was the same material used in the animal studies, and it was stored frozen under argon, so degradation was unlikely. See Erratum."(underline added), and the last page of NTP 2005 (inserted unnumbered after page 358) states, *"Erratum – replacement text for NTP TR-494, page 20, column 2, paragraph 3: Dr. Klaunig asked if the*

samples assayed were the original test material and if any degradation might have occurred during the interval. Further examination of the shipment information for the sample from the 2-year bioassay sent to BioReliance Corporation for genetic toxicology testing in Salmonella showed that it was from archived bulk material. Following completion of the bioassay, this material was stored as received at room temperature (approximately 25°C), protected from light, and without inert gas headspace...."

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 CLH report re-evaluation of NTP 2005 is flawed; NTP 2005 does not meet current sceintific standards and should not be the basis of classification of Anthraquinone.

7. The negative mutagenicity assay for sample A07496; presented for the first time to the December 2004 NTP peer review panel and described in NTP 2005 as an aliquot of NTP's test material, Lot No. 5893; undoubtedly played a significant role in convincing the December 2004 NTP peer review panel to remove the "anthracene-derived anthraquinone" restriction imposed on the conclusions in TR-494 by the February 2004 NTP peer review panel. The December 2004 NTP peer review panel accepted NTP's contention that the negative mutagenicity assay of sample A07496 demonstrated that mutagenic impurities in the NTP test material were not biologically significant. Earlier positive mutagenicity assays of samples labeled "Lot #5893" were

dismissed.

NTP cannot document that sample A07496 is, in fact, an aliquot of its test material, Lot No. 5893, the identity under which it is stored by NTP contractor Battelle in Columbus, Ohio since conclusion of animal testing in the mid-1990s.

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 Corporation, the contractor which performed mutagenicity assays for NTP, asking for confirmation that A07496 was Lot No. 5893, and stating, "without confirmation of the test article identities I'm uncertain as to what the results are telling us." Richard San sent an email to NTP "confirming" that sample A07496 was Lot #5893. These emails are included in Attachment VII.

Attachment VII 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; (2) the BioReliance Testing Laboratories (BTL) Test Article Receipt and Transfer Report documenting receipt of a sample labeled only "A07496" in Rockville, Maryland on June 2, 2004; and (3) emails between Kristine Witt at NTP and Richard San at BioReliance.

The BTL Test Article Receipt and Transfer Report demonstrates that Richard San had no means of determining that

the sample received by BioReliance Corporation on June 2, 2004 was the NTP test material maintained at Battelle in Columbus, Ohio labeled "Lot No. 5893"; it was labeled only "A07496" upon receipt at BioReliance Corporation; his emailed confirmation is not valid.

Boobis et al. [Attachment III] 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 stated previously, Professor Alan R. Boobis is a Fellow of the British Toxicology Society.

The IUCLID dataset 2000 [Attachment I], 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 December 2004 NTP peer review panel modified the decision of the February 2004 NTP peer review panel based upon incorrect information, and approved the NTP 2005 in a substantially modified form without the requisite opportunity for receipt and consideration of public comments. The CLH report re-evaluation of NTP 2005 is flawed; the December 2004 NTP peer review panel's approval of NTP 2005 should not be accepted by ECHA because they resulted from false and unsupported information provided in December 2004.

8. The conclusions in the draft TR-494 disseminated for public review and comment before peer review on February 17, 2004 [Attachment II] were restricted to apply only to "anthracene-derived anthraquinone" because of recognition that the results of the NTP animal studies were significantly impacted by the presence of 9-nitroanthracene contamination and are, thus, 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 which approved NTP 2005. 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 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 chemical hazards which can be rationally anticipated from scientifically sound information. The animal studies reported in NTP 2005 do not demonstrate that the Anthraquinone in commerce in 2015 represents a possible human cancer hazard, thus 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, contrary to the ambiguous language in the CLH report.

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 and 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, or any other mutagenic impurities. Classification of the Anthraquinone in commerce based upon the NTP animal studies is not scientifically sound.

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,

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