Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

2,4-dinitrophenol

EC Number: 200-087-7
CAS Number: 51-28-5

CLH-O-0000001412-86-256/F

Adopted

30 November 2018

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: 2,4-dinitrophenol
EC number: 200-087-7
CAS number: 51-28-5
Dossier submitter: Germany

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
13.04.2018	Belgium		MemberState	1
Comment received				
BE CA welcomes this proposal for harmonized classification and labelling and would like to thank the DE CA for this CLH proposal dossier. We support the inclusion of ATE values in the Annex VI of CLP since it will be a great help for both industries and authorities to correctly and consistently calculate the classification of mixtures containing 2,4-dinitrophenol. ECHA note – An attachment was submitted with the comment above. Refer to public attachment 2,4 dinitrophenol_STOT RE.docx				
Dossier Submitter's Response				
DE CA appreciates the BE comment.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
13.04.2018	France		MemberState	2
Comment received				
ANSES support the proposed revision of classification as discussed below.				
Dossier Submitter's Response				
DE CA appreciates the support of the FR CA.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
13.04.2018	Belgium		MemberState	3

Comment received

Acute tox oral
BE CA agrees with the Acute tox. 2 classification for oral toxicity of 2,4-dinitrophenol, mainly based on a GLP acute toxicity study on rat (Spencer et al, 1948, rel. 2). 9 to 40 rats per dose were exposed to 10-20-23-25-27-30-40-50-60-70-80 and 100 mg/kg bw dinitrophenol per gavage. This study demonstrated that dinitrophenol has a very steep dose-response. 37% (11/30) and 90% (19/20) mortality occurred respectively at 30 and 40 mg/kg bw. The LD50 is estimated between 30-40 mg/kg bw. These observations are supported by the further reports in rat (LD50 = 30 mg/kg bw ; Schafer, 1972, rel. 4), in rabbit (LD50 = 30 mg/kg bw according to RTECS-Website and HSDB database, rel. 4) and in dog (LD50 = 20-30 mg/kg bw according to RTECS-Website).
Guinea pig seems to be little less sensitive than rat, rabbit and dog for oral toxicity, with a LD50 of 81 mg/kg bw (RTECS website).
Various cases of human oral poisoning to dinitrophenol are described in literature. The fatal dose range include findings from 6,2 mg/kg bw during 4 days (McFee et al, 2004) and 7 mg/kg bw during 5 days (ATQDR, 1995). The lowest reported suicidal dose is 40 mg/kg bw. These observations cannot be used for the setting of an ATE either because of repeated exposure or extreme dosing due to suicidal purpose. Nevertheless, they suggest the need for a conservative ATE setting based on animal data's.
BE CA is of the opinion that the Spencer report should be considered as the key study on the classification process but weight-of-evidence tends to indicate that 30 mg/kg bw/day is a critical threshold in both rat, rabbit and dog. We also note that 90% mortality occurred at 40 mg/kg bw whereas 11/30 rats died at 30 mg/kg bw in the Spencer study. Considering the very steep dose-response we support an ATE oral of 30 mg/kg bw.

Acute tox dermal
BE CA agrees with the Acute tox. 3 classification for dermal toxicity of 2,4-dinitrophenol based on available data in the CLH proposal dossier. One acute dermal toxicity is available in Guinea pig (Spencer et al., 1948, rel. 2). Alcoholic solution of Dinitrophenol was applied dermally to 5 Guinea pigs per dose group (100-200-300-400-500-700 and 1000 mg/kg bw). 1/5 animal died at 300 and 400 mg/kg bw whereas 40% mortality occurred at 500 mg/kg bw. All animals died at the two highest doses. LD50 is therefore estimated between 500 and 700 mg/kg bw and dinitrophenol demonstrated a steep dose-response after dermal exposure.
Although they have been demonstrated to be more sensitive than Guinea pig after oral exposure, no data is currently available for acute dermal toxicity of rat or rabbit.
Considering this uncertainty, the low number of animals per dose group used in the Spencer study and the very steep dose-response, BE CA considers that the setting of the ATE should be very careful. We suggest to set the ATE at 550 mg/kg bw for dermal exposure.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment 2,4 dinitrophenol_STOT RE.docx

Dossier Submitter's Response

As stated in the dossier, for the calculation of the ATE the Spencer study with the average of the dose below and above the LD_{50} has been used and yielded a value of 35 mg/kg bw for acute toxicity oral and a value of 600 mg/kg bw for acute toxity dermal. However, the arguments of the BE CA were considered by DE CA. With respect to the steep dose-response in both cases (oral and dermal) the DE CA agrees to an ATE oral of 30 mg/kg bw

and an ATE dermal of 550 mg/kg bw.
RAC's response
RAC notes the support for the proposed classification as Acute Tox 2 –H300 and Acute Tox 3 – H311 and supports the DS conclusion regarding an ATE (oral) of 30 mg/kg bw. As for the dermal route, there are uncertainties/deficiences, and the very steep dose-response relationship in the key study, therefore for derivation of the ATE value the conservative approach using the converted acute toxicity point estimate supplied in CLP Annex I, Table 3.1.2. for category 3, i.e. 300 mg/kg bw is applied. RAC notes the support for the proposed classification as Acute Tox 2 –H300 and Acute Tox 3 – H311 and agrees with the DS conclusion regarding an oral ATE of 30 mg/kg bw and a dermal ATE of 550 mg/kg bw.

Date	Country	Organisation	Type of Organisation	Comment number
13.04.2018	France		MemberState	4
Comment received				
By oral route, the experimental data indicates that 2,4-dinitrophenol has a LD50 between 20 and 80 mg/kg in several species with a steep dose-response. Although the reliability of available studies is variable, the most robust study (Spencer 1948) points toward a LD50 in rats between 30 and 40 mg/kg and this is consistent with the proposed classification Acute Tox 2 – H300. Human data also supports that mortality may occur from subacute exposure to doses as low as 1.03 mg/kg (ATSDR 1995) although death is not systematic at such level of doses. Lethality has been observed at multiple occasions from single doses of 40 mg/kg. ANSES therefore agree that the database supports a classification Acute Tox 2 –H300 by oral route. By dermal route, ANSES agrees that the available data support the classification Acute Tox 3 – H311. The Spencer study (1948) is considered to be an appropriate study to derive the ATE for both oral and dermal routes. However, calculation of the ATE by interpolation should be considered.				
Dossier Submitter's Response				
DE CA appreciates the fundamental support of FR CA. DE CA has reflected the comments of the BE CA (comment No 3) and came to the conclusion that with respect to the steep dose response in both cases (oral and dermal) the DE CA agrees to an ATE oral of 30 mg/kg bw and an ATE dermal of 550 mg/kg bw.				
RAC's response				
RAC notes the support for the proposed classification as Acute Tox 2 –H300 and Acute Tox 3 – H311 and agrees with the DS conclusion regarding an oral ATE of 30 mg/kg bw. As for the dermal route, there are uncertainties/deficiences, and the very steep dose-response relationship in the key study, therefore for derivation of the ATE value the conservative approach using the converted acute toxicity point estimate provided in CLP Annex I, Table 3.1.2. for category 3, i.e. 300 mg/kg bw is applied.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
13.04.2018	Belgium		MemberState	5
Comment received				
A subacute oral toxicity was performed on rat (similar to OECD TG 407, Koizumi et al, 2001, rel. 1). Six animals per dose group were respectively exposed to 0-3-10-30-80 mg/kg bw/day during 28 days. There was no mortality or histopathological changes observed up to 30 mg/kg bw/day although symptoms of toxicity (decrease in locomotor activity and				

salivation) were observed after the first dosing of 30 mg/kg bw/day. At 80 mg/kg 50% of females and 2/12 males died with various symptoms of toxicity (locomotor activity, prone position, ptosis, panting, crawling position, salivation, tonic convulsion). Relative liver weight was increased in both sexes and relative brain, kidney and testes were increased only in males. Histopathology reported mineralization of the corticomedullary junction in kidneys in both sexes.
We deeply regret the significant gap between the two highest doses, considering that the LD50 has been reported to be between 30-40 mg/kg bw and that the steep dose-response is very well known, as exposed in the previous sections. We would also appreciate further clarification about the moment of death for the reporting at higher dose.
Moreover, we note that not observation has been made about cataract. Dinitrophenol is known to induce this kind of pathology in human but also in animals. We would appreciate to know if this specific endpoint has been investigated and if any other information is available on this specific point.
Finally, the purity of dinitrophenol in this study is only 85,2% and no information is provided about impurities. Could you also inform us if the tested doses have been corrected for the purity?
The mechanism of action of dinitrophenol is very well documented. This compound suppresses the ATP production by uncoupling the oxidative phosphorylation of adenosine disphosphate in mitochondria. This mode of action indicates that dinitrophenol does not target a specific organ but induces a general failure of the organism, leading to death. Consequently, BE CA supports a STOT RE classification for lethality.
In human, deaths after repeated exposure to dinitrophenol occured at much lower doses than 30 mg/kg bw/day, suggesting that human might be more sensitive than rat after repeated exposure to dinitrophenol. BE CA also notes the coherence between these 6 observations, including the relation between the exposure duration and the oral dose.
Exposure duration (days) Dose (mg/kg bw/day) Ref.
4 6,2 Mc Fee et al, 2004
5 7 ATSDR 1995
14 2,66 ATSDR 1995
42 0,62 to 3,8 ATSDR 1995
42 2,9 to 4,3 ATSDR 1995
46 1,03 ATSDR 1995
Table 1 : Fatal oral poisonings in human after repeated exposure
Therefore, considering the uncertainty induced by the high dose gap in the Koizumi study and the observations of higher sensitivity of human than rat after repeated exposure, BE CA is of the opinion that the reliability of the human cases should be carefully assessed in Committee, potentially leading to a STOT RE 1 classification based on human data.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment 2,4 dinitrophenol_STOT RE.docx

Dossier Submitter's Response

The DE CA appreciates the questions of the BE CA. The Koizumi study gave no information about the moment of death. Furthermore, the study did not report on investigations of the eyes of the animals, therefor no information is available on the cataract forming potency of dinitrophenol in this study. Finally, the study gave details on the composition of the 2,4-dinitrophenol used: 2,4 dinitrophenol 85.2 %, 13.9 % water, 0.6 % 2,6-dinitrophenol and 0.3 % unkown compounds as impurities. However, no information was given, if a correction took place for the purity.

The DE CA also appreciates the suggestion of the BE CA to consider the human fatalities after repeated exposure for specific target organ toxicity after repeated exposure. The compilation of human fatalities made by the BE CA from table 13 of the dossier suggests a

higher sensitivity of human than rat after repeated exposure. However, due to the very limited information on the human fatalities the DE CA still supports the STOT RE 2 classification but it would accept a STOT RE 1 classification based on human data.
RAC's response
RAC appreciates the questions and comment of the BE CA. Although human data presented in the CLH report are not conclusive on their own for a STOT RE 1 classification, they show that significant toxic effects observed in animals are of relevance to human health and indicate that lethality after an oral exposure cannot be attributed to acute toxicity alone. The human data presented in the CLH dossier are limited, but indicate effects related to the uncoupling of mitochondrial oxidative phosphorylation by 2,4-dinitrophenol - weight loss, increased basal metabolic rate and perspiration, increased pulse, respiratory rate, and body temperature. There is an extensive database on the effects of dinitrophenols in humans which indicates that humans could be more sensitive than rodents (ATSDR, 1995). Considering the mode of action and the significant toxic effects in several organ/systems due to ATP depletion with the possible fatal consequences in humans, RAC agrees that classification of 2,4-dinitrophenol as STOT RE 1 is more appropriate.

Date	Country	Organisation	Type of Organisation	Comment number
13.04.2018	France		MemberState	6
Comment received				
Due to mortality at 80 mg/kg/d in a 28-day study, ANSES agrees that classification STOT RE 2 – H 373 is confirmed.				
Dossier Submitter's Response				
DE CA appreciates the support of the FR CA.				
RAC's response				
Noted.				

PUBLIC ATTACHMENTS
1. 2,4 dinitrophenol_STOT RE.docx [Please refer to comment No. 1, 3, 5]