Committee for Risk Assessment

RAC

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

30 November 2018

CLH-O-0000001412-86-256/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **2,4-dinitrophenol**

EC Number: **200-087-7**

CAS Number: **51-28-5**

The proposal was submitted by **Germany** and received by RAC on **26 October 2017.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Germany has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **12 February 2018**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **13 April 2018**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Helena Polakovičová**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **30 November 2018** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	609-041-00-4	2,4-dinitrophenol	200-087-7	51-28-5	Acute Tox. 3 * Acute Tox. 3 * Acute Tox. 3 * STOT RE 2 * Aquatic Acute 1	H331 H311 H301 H373 ** H400	GHS06 GHS08 GHS09 Dgr	H331 H311 H301 H373 ** H400			
Dossier submitters proposal	609-041-00-4	2,4-dinitrophenol	200-087-7	51-28-5	**Retain** Acute Tox. 3 * Aquatic Acute 1 **Modify** Acute Tox. 3 Acute Tox. 2 STOT RE2	**Retain:** H331 H311 H400 **Modify** H300 H373	**Retain** GHS06 GHS08 GHS09 Dgr	**Retain:** H331 H311 H400 **Modify** H300 H373		**Add** dermal: ATE = 550 mg/kg bw oral: ATE = 30 mg/kg bw	
RAC opinion	609-041-00-4	2,4-dinitrophenol	200-087-7	51-28-5	**Retain:** Acute Tox. 3 * Aquatic Acute 1 **Modify:** Acute Tox. 2 Acute Tox. 3 STOT RE 1	**Retain:** H331 H311 H400 **Modify:** H300 H372	**Retain** GHS06 GHS08 GHS09 Dgr	**Retain:** H311 H331 H400 **Modify:** H300 H37**2**		**Add** dermal: ATE = 300 mg/kg bw oral: ATE = 30 mg/kg bw	
Resulting Annex VI entry if agreed by COM	609-041-00-4	2,4-dinitrophenol	200-087-7	51-28-5	Acute Tox. 3 * Acute Tox. 3 Acute Tox. 2 STOT RE 1 Aquatic Acute 1	H331 H311 H300 H372 H400	GHS06 GHS08 GHS09 Dgr	H300 H311 H331 H372 H400		**Add** dermal: ATE = 300 mg/kg bw oral: ATE = 30 mg/kg bw	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Based on the information from REACH registration documents, 2,4-dinitrophenol is used as an industrial chemical for the manufacture of other substances and for the manufacture of textiles, leather or fur.

The substance has historically been used in dyes, wool preservatives, herbicides and explosives since the early 20th century. During the 1930s, 2,4-dinitrophenol was also used extensively for dieting in tablet form in the US and several cases of poisoning were reported in connection with this use. The effect is attributed to the suppression of ATP (energy) production by uncoupling the oxidative phosphorylation of adenosine diphosphate in mitochondria leading to fatal hyperthermia before death and other adverse effects (e.g. an increase in basal metabolic rate, loss of weight, dizziness, and cataracts). Following the ban of this use in 1938 by the US FDA, the number of incidents decreased. However, it should be noted that 2,4-dinitrophenol is still being sold mostly over the internet under a number of different names as a weight loss/slimming aid. After the year 2000, an increased number of fatal overdoses, including cases of intended suicide, occurred in the UK and Germany.

2,4-dinitrophenol already has an entry in Annex VI of Regulation (EC) No 1272/2008/EC (CLP) and is currently classified as Acute Tox. 3*(H301), Acute Tox. 3* (H311), Acute Tox. 3*(H331), STOT RE 2* (H373)** and Aquatic Acute 1 (H400).
The current harmonised classification of dinitrophenol is the translation of the DSD classification: T; R23/24/25, R33, N; R50.

The intention of the CLH proposal was to re-evaluate an existing minimum classification for acute toxicity (oral and dermal routes) and a minimum classification for specific target organ toxicity – repeated exposure in order to comply with the CLP criteria. The results of the analysis of fatal human poisoning and LD_{50} values from studies in several species have shown that the current classification of the substance for acute oral toxicity is not appropriate and should be upgraded into a higher category.

Other hazards were not addressed in the CLH report.

The CLH dossier is based on available data in the REACH registration dossier for 2,4-dinitrophenol and other information available in the scientific literature including an analysis of data on fatal human poisoning cases.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute toxicity: oral

In the CLH report two acute oral toxicity studies in animals (one in rats and mice and one in rats only) and a summary of acute toxicity data on different species published in the literature were presented. The study by Spencer *et. al.* (1948) performed in rats had been identified by the DS as the key study on acute oral toxicity of 2,4-dinitrophenol. All other reports and information presented in the CLH report indicated the LD_{50} values and the test species, but generally all other experimental details were lacking. Due to the information gaps, they had been given Klimisch

scores of 4 (not assignable) and the LD_{50} values from these sources were used only as supporting information.

In a study similar to OECD 401 (Spencer *et al.,* 1948), young mature white rats of both sexes (9 to 40 animals per dose group) had been treated by gavage with 2,4-dinitrophenol in olive oil containing a 5 – 10% gum Arabic solution at dose levels of 10, 20, 23, 27, 30, 40, 50, 60, 70, 80 and 100 mg/kg bw. No mortality had occurred at doses of 10, 20, 23 and 27 mg/kg bw. Deaths had been reported at doses ≥37 mg/kg bw an hour or two after the treatment with 2,4-dinitrophenol. It was concluded that 2,4-dinitrophenol had been a rapidly acting agent. A survival dose of 27 mg/kg bw and a lethal dose of 100 mg/kg bw had been defined. This study had demonstrated that 2,4-dinitrophenol has a very steep dose-response curve: no mortality had occurred at doses of 10 – 27 mg/kg bw, 37% (11/30) mortality had occurred at 30 mg/kg bw, 90% (18/20) mortality had occurred at 40 mg/kg bw and 100% (20/20) mortality had occurred at 100 mg/kg bw. The LD_{50} value had not been calculated, but it was estimated to be between 30 and 40 mg/kg bw.

The results from other oral studies in rats indicated LD_{50} values between 30 and 71 mg/kg bw. The results of an acute oral toxicity using other species (mouse, rabbit, guinea pig, dog and cat) indicated LD_{50} values between 20 and 81 mg/kg bw.
The LD_{50} value that had been estimated in the key study was supported by data from two study reports in white rats (no further details) that had been treated once by gavage: An LD_{50} of 30 mg/kg bw (Dow Chemicals (1950); cited by the ATSDR (1995)) and an LD_{50} value which had been estimated to lie between a 100% survival dose of 20 mg/kg bw and a 100% lethal dose of 60 mg/kg bw (Dow Chemicals (1940) cited according to ATSDR (1995)). The publication of Shafer (1972) referred also to the LD_{50} of 30 mg/kg bw in rats, however, without any further information regarding the author of the study.
The study by Kaiser, 1964 (Klimisch 4) presented the LD_{50} of 71 mg/kg bw for weanling male rats of the Sherman strain, and the LD_{50} of 72 mg/kg bw for weanling male C.F. 1 white mice (no further details); the range of doses had applied and a number of investigated animals was not reported.
Additional information on oral acute toxicity was available from the HSDB database from the rat (LD_{50}: 30 mg/kg bw), rabbit (LD_{50}: 30 mg/kg bw) and dog (LD_{50}: 20-30 mg/kg bw)) and on the RTECS website from the mouse (LD_{50}: 45 mg/kg bw), rabbit (LD_{50}: 30 mg/kg bw), guinea pig (LD_{50}:81 mg/kg bw) and cat (LD_{50}: 75mg/kg bw)). The DS propose classification in category 2 with an ATE of 35 mg/kg bw. The DS agreed after comments in PC that an ATE of 30 would be more relevant.

Human information

The ATSDR (1995) compiled several cases of oral poisoning by dinitrophenol in humans from the 1930s leading to a fatal outcome with doses of dinitrophenol in the range of less than 1 mg/kg bw (an intake of 6 weeks) and 7 mg/kg bw (an intake of 5 days).

Recent fatal case reports have been published, one withan estimated lethal dose was 6.2 mg/kg bw/d during 4 days (McFee *et al*, 2004) and seven cases after one dose in a dose range of 40 to 57 mg/kg bw. The lowest reported suicidal dose was 40 mg/kg bw.

The acute toxic effects of 2,4-dinitrophenol observed in humans before the death were: fatal hyperthermia, increased basal metabolic rate, nausea, vomiting, sweating, dizziness, headache, loss of weight and cataracts.

An additional ten fatal poisoning cases without information on the applied doses of dinitrophenol demonstrated an increase of fatalities.

Acute toxicity: dermal

The DS pointed to one study in guinea pigs for an evaluation of acute dermal toxicity of 2,4-dinitrophenol (Spencer *et. al.,* 1948), Klimisch 2. Guinea pigs of both sexes (5 animals per dose group) had been exposed dermally (on the clipped abdomen) to 100, 200, 300, 400, 500,700 and 1000 mg/kg bw of 2,4-dinitrophenol in an alcoholic solution for 4 hours.

Results:

100 and 200 mg/kg bw - no mortality

300 and 400 mg/kg bw – 20% mortality (1/5)

500 mg/kg bw - 40% mortality (2/5)

700 and 1000 mg/kg bw - 100% mortality (5/5)

Conclusion:

2,4-dinitrophenol has a steep dose-response curve. The LD_{50} had not been calculated, but its value could be estimated to lie between 500 and 700 mg/kg bw. The DS propose classification in category 3 with an ATE of 600 mg/kg bw. The DS agreed after comments in PC that an ATE of 550 would be more relevant.

Human information:

Very limited data was available on non-oral poisoning. Only one case report from China indicated that a dermal exposure can lead to fatal effects, but sufficient details are not available.

Comments received during public consultation

Two Member States (MS) supported the proposed classifications for acute oral and dermal toxicity. One of these MS agreed that the ATE could be derived on the basis of the Spencer study (1948) for both the oral and dermal routes (ATE oral of 35 mg/kg bw, ATE dermal of 600 mg/kg bw) and suggested considering calculation of the ATE by interpolation. The other MS pointed to cases of human oral poisoning by dinitrophenol presented in the literature (McFee *et al,* 2004), ATSDR (1995), BfR (2015)). The observations from humans could not be used to set an ATE because they involved repeated exposure or extreme dosing (suicide), but they suggested the need for setting a conservative ATE based on the animal data. The study of Spencer *et al.* (1948) was considered the key study for acute oral toxicity classification of the substance. The weight of evidence assessment of the DS had indicated that 30 mg/kg bw is a threshold for lethality in the rat, rabbit and dog following oral exposure, while the guinea pig is less sensitive (LD_{50} of 81 mg/kg bw). Considering that a very steep dose - response relationship in the key study, an oral ATE of 30 mg/kg bw was supported. Only one study in guinea pigs was presented in the CLH report which was relevant for classification for acute dermal toxicity. In acute oral toxicity studies it was demonstrated that rats and rabbits were more sensitive than guinea pigs, but there were no data to support the sensitivity of different species by the dermal route. Taking into account the low number of animals per dose group in the key dermal study leading to a very steep dose response relationship for mortality, care should be taken in setting the ATE. In conclusion, a dermal ATE of 550 mg/kg bw was proposed.

The DS had considered the arguments of the MS agreed that the oral ATE should be established at 30 mg/kg bw and the dermal ATE at 550 mg/kg bw.

Assessment and comparison with the classification criteria

Acute toxicity: Oral

The key acute oral study in rats (Spencer *et al.*, 1948) yielded a range from 30 to 40 mg/kg bw for the LD_{50} value.

This range fulfils the criteria for Cat. 2 and is supported by the LD_{50} value of 30 mg/kg bw referred to in the additional studies conducted in the rat, rabbit and dog. RAC concludes that **classification of 2,4-dinitrophenol as Acute Tox. 2; H300 (Fatal if swallowed) is warranted**.

The DS originally proposed an ATE value of 35 mg/kg bw for the classification of mixtures, on the basis of the Spencer *et al.* (1948) study as an average calculated from the doses below and above the LD_{50}. However, taking into account the steep response relationship observed in the key animal study, agreed on an oral ATE of 30 mg/kg bw.

RAC concludes that the approach of the DS to select the lowest ATE value for classification of mixtures is appropriate and justified based on the evidence. RAC concludes that for 2, 4-dinitrophenol **an ATE value of 30 mg/kg bw is warranted for acute oral toxicity**.

Acute toxicity: dermal

The key acute dermal study in guinea pigs (Spencer *et al.,* 1948) yielded a range from 500 to 700 mg/kg bw for the LD_{50} value ($500 < LD_{50} < 700$ mg/kg bw). This study is very old and was not conducted using methodology which is consistent with OECD guidelines (exposure period was only 4 instead of 24-hours), therefore the resulting LD_{50} value is probably underestimated but supports classification for dermal toxicity in category 3 ($200 < LD_{50} < 1000$ mg/kg bw). It is not expected that the deviation in exposure time (from 24 hours to 4 hours) in an acute dermal toxicity test could have an impact on this category.

RAC concludes that **classification of 2,4-dinitrophenol as Acute Tox. 3; H311 (Toxic in contact with skin) is warranted**.

The DS originally suggested an ATE value of 600 mg/kg bw for the classification of mixtures, on the basis of the Spencer *et al.* (1948) study as an average calculated on the basis of the doses below and above the LD_{50}. However, the DS reflected the arguments of one MS received during the public consultation and taking into account the very steep dose-response relationship of 2,4-dinitrophenol agreed to a dermal ATE of 550 mg/kg bw.

RAC agrees that a conservative approach for setting the ATE value for classification of mixtures is appropriate. However, RAC would like to point out that the exposure time in the Spencer *et al.* (1948) study was only 4 hours instead of the 24 hours recommended in the relevant OECD TG, and therefore the resulting LD_{50} value is probably underestimated. In addition, the acute oral studies indicate that the guinea pig is less sensitive than the rat or rabbit. Though not directly demonstrated for the dermal route, it seems very likely that the guinea pig is also less sensitive via the dermal route and the use of other species might have resulted in a lower LD_{50} value. Taking into account these deficiencies/uncertainties, and the very steep dose-response relationship observed in this study, RAC concludes that for derivation of the ATE value for 2,4-dinitrophenol, a 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 more appropriate than the value proposed by the DS.

RAC concludes that for 2,4-dinitrophenol **an ATE value of 300 mg/kg bw is warranted for acute dermal toxicity**.

No data were available and no change in the current classification for acute inhalation toxicity was proposed. RAC agrees to **retain the existing minimum classification as Acute Tox. 3* (H331)**.

RAC evaluation of specific target organ toxicity– repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

The DS proposed classification of 2,4-dinitrophenol with STOT RE 2 (H373: May cause damage to organs through prolonged or repeated exposure) on the basis of mortality that had been observed in rats at 80 mg/kg bw/day in one subacute oral toxicity study (Koizumi *et al.,* 2001).

The DS referred to a long history of oral poisoning by dinitrophenol in humans and also noted that the ATSDR (1995) compiled several studies from the 1930s with different doses. However, according to the DS these latter studies are not necessary to confirm the toxicological profile of dinitrophenol.

Comments received during public consultation

Comments were sent by two MS. One MS agreed with the proposed STOT RE 2; H373 classification based on mortality observed at a dose of 80 mg/kg bw/day in a 28-day study.

The other MS supported a STOT RE classification based on lethality which can be explained by a very well documented mechanism of an action of 2,4-dinitrophenol – suppression of the adenosine triphosphate (ATP) production by uncoupling the oxidative phosphorylation of adenosine diphosphate (ADP) in mitochondria. This mode of action indicated that the substance does not target a specific organ but induces a general failure of the organs, leading to death.

They pointed out that in humans, deaths after repeated exposure to dinitrophenol occurred at much lower doses than 30 mg/kg bw/day, suggesting that humans might be more sensitive than rats after the repeated exposure to dinitrophenol. They also noted the consistency between the observations in humans, including the relationship between the exposure duration and the oral dose as presented in the Table below.

***Table**: Fatal oral poisonings in human after the repeated exposure (compiled from Table 13 of the CLH report)*

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

They pointed to the uncertainties in a key rat study (Koizumi et al, 2001) as indicated by the significant gap between the two highest doses (30 and 80 mg/kg bw/day), considering that the

LD50 had been reported to be between 30-40 mg/kg bw/day for acute oral toxicity in this species and that a very steep dose response relationship for mortality is well known. They would have appreciated a clarification of the moment of death at the higher doses. They also noted that no cataracts had been observed. Dinitrophenol is known to induce cataracts in humans but also in animals. They would also have appreciated knowing if cataracts has been investigated and if any other information was available on this specific point. As the purity of the tested substance was 82.5% with no information about impurities provided, they asked if the tested doses were corrected for purity. Taking into account the uncertainties indicated in the Koizumi (DATE) study and the observation of greater sensitivity of humans than rats after repeated exposure, they were of the opinion that the reliability of the human cases should be carefully assessed, potentially leading to a STOT RE 1 classification based on the human data.

The response of the DS is summarised as follows:

- the Koizumi (DATE) study gave no information about the moment of death,
- the study did not report on investigations of the eyes of the animals, therefore no information is available on the cataract forming potency of dinitrophenol in this study
- 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 % of unknown compounds as impurities. However, no information was available on whether the doses in the studies were corrected for the purity.

The DS appreciated the suggestion to consider the human fatalities after repeated exposure for STOT RE classification. Although the compilation of human fatalities made from Table 13 of the CLH report suggested a higher sensitivity of humans than rats after repeated exposure, there was very limited information about the human fatalities. The DS had proposed a STOT RE 2 classification, but would agree with a STOT RE 1 classification based on human data.

Assessment and comparison with the classification criteria

For specific target organ toxicity, the report from one subacute oral toxicity study was presented (Koizumi *et al*. (2001), Klimisch 1) and this was selected as the key study. In the Table below the effects reported in this study at doses relevant for classification are presented.

Table: *Summary of the repeat dose toxicity study with 2,4-dinitrophenol*

Study	Dose levels	Results/Effects at doses relevant for classification	Reference
Oral, 28 day (gavage) similar to OECD407 GLP Reliability 1 (reliable without restriction) Sprague-Dawley SPF rats (6/sex/group; additional 6/sex for recovery groups at two highest doses) Test material: 2,4-dinitrophenol (purity 85.2%) suspended in 1 w/v%	0, 3, 10, 30, 80 mg/kg bw/day (Recovery group: 0, 30, 80 mg/kg bw/day) Guidance value for classification Category 1: ≤30 mg/kg bw/day	30 mg/kg bw/day: Mortality: No Observation ↓ locomotor activity and salivation after first dosing only (not relevant for classification) **80 mg/kg bw/day**: **Mortality: 6/12 f and 2/12 m** Observation: ↓ locomotor activity, prone position, ptosis, panting, crawling position and salivation repeatedly in all animals,	Koizumi *et al.* (2001)

methylcellulose solution	Category 2: **≤300** mg/kg bw/day	- Tonic convulsion (2/12 m and 4/12 f) - Rigidity (2/12 m and 5/12 f) Organ weights: ↑ liver both sexes (relative), persisted throughout the recovery period ↑ brain, kidneys and testes in males (relative) Histopathology: mineralisation of corticomedullary junction in kidney in both sexes in the scheduled- sacrifice group and recovery group, but statistically significant only in males of scheduled- sacrifice group Haematology: ↑ haemoglobin and haematocrit during the treatment, ↓ red blood cell count, haemoglobin and haematocrit in the recovery group, limited to males	

As presented in the Table above, in the key 28-day oral toxicity study on rats, at 30 mg/kg bw/day no mortality and no histopathological changes were observed, although some symptoms of toxicity were observed after the first dose. At 80 mg/kg bw/day, 6/12 females and 2/12 males died and various symptoms of toxicity were observed. The relative liver weight was increased in both sexes and the relative weights of brain, kidney and testes were increased only in males. Histopathology reported mineralisation of the corticomedullary junction in the kidney in both sexes.

Although the 28-day oral toxicity study on rats was evaluated by the DS as reliable without restrictions, there were some uncertainties e.g. no information on when the observed mortality at 80 mg/kg bw/day occurred, inconsistency between the LD_{50} and the dose inducing mortality. The latter may be explained by the large dose spacing for this substance considering its rather steep dose-response curve. There was also no information on whether there was a correction for the purity. Considering the extrapolated guidance value range from 30 to 300 mg/kg bw/day for a 28-day oral study in rats, it is not expected that the majority of these uncertainties would have an impact on the classification in category 2, taking into account that at 30 mg/kg bw/day no mortality and no relevant toxic effects have been observed. However, without the data on the time of death it is difficult to evaluate whether the mortality in this study resulted from a sub-acute effect of the substance or not.

For classification for the acute oral toxicity for 2,4-dinitrophenol the value 30 mg/kg bw was set, which is a dose lower than the dose that had induced the general failure of the organism, leading to death after repeated exposure (80 mg/kg bw/day). However, the results from the acute oral toxicity studies in rats had indicated that the range of LD_{50} was between 30 and 71 mg/kg bw and the results of acute oral toxicity in other species (mouse, rabbit, guinea pig, dog and cat) had indicated that the LD_{50} is between 20 and 81 mg/kg bw.

Human information

For specific target organ toxicity after repeated exposure, no human data has been submitted in the CLH report.

During the public consultation one MS pointed to the uncertainties in the key rat study. Taking into account higher sensitivity observed in humans than in rats and based on data in Table 13 of the CLH report, the MS was of the opinion that the reliability of the human cases should be carefully assessed, potentially leading to a STOT RE 1 classification based on human data.

2,4-dinitrophenol was used extensively in diet pills. It is estimated that 4500 patients in California were treated during the period from July 1934 till July 1935, and that 100000 people in this State had used this drug since its introduction as a remedy for obesity (Rodin FH, 1936). However, the total number of users is unknown.

There was information on human data in the acute toxicity part of the CLH report that referred to the ATSDR evaluation of 2,4-dinitrophenol from 1995, without presenting any further details quoted as follows:

> "*Little information is available regarding death in humans after acute oral exposure to 2,4-DNP. A case report details the death of an 80-kg man who took ≈46 mg 2,4-DNP/kg as the sodium salt, followed by another 46 mg/kg dose 1 week later (Tainter and Wood 1934). The first dose produced a high fever; the second dose resulted in admission to the hospital 6.5 hours later because of hyperpnoea and chest pain. The rectal temperature was 105 ° F, and pulse was rapid (as high as 146 beats per minute). Despite the administration of aspirin, the temperature rose to 105.7 ° F by 10.5 hours following ingestion of the drug. Death occurred 0.5 hours later, with rigor mortis setting in 10 minutes after death and the temperature rising to ≈115 ° F by 20 minutes after death. The clinical signs and the autopsy and histological findings were considered by the authors to be similar to those seen in heat stroke. A woman who took 7 mg/kg/day 2,4-DNP as the sodium salt for 5 days was admitted to the hospital in a comatose condition and subsequently died (Poole and Haining 1934). She had complained of headache, backache, weakness, dizziness, shortness of breath, and excessive perspiration. Her temperature was at least 101.8 °F, pulse 140 beats per minute and respiratory rate 56 per minute. Upon autopsy and histological examination, hyperemic and hemorrhagic lungs, degeneration of renal tubules and liver cells, segmentation and fragmentation of cardiac muscles, and hemorrhagic spleen, stomach mucosa, spinal cord, pons, and medulla were found. Slight ganglion cell degeneration was found in the pons. In another case, a psychiatric patient was given sodium 2,4-DNP in an experimental study to determine whether 2,4-DNP would be beneficial in treating depression (Masserman and Goldsmith, 1934). Over the course of 14 days, she had been given 2.66 mg/kg/day 2,4-DNP. She died after her pulse increased to 148 beats per minute and respirations to 48 per minute, her temperature rose to 102 ° F, she became comatose, and blood pressure fell to 36/0. Because autopsy was delayed for 4 days, no conclusions regarding histopathological lesions could be made. There were no deaths, however, in a number of clinical and experimental studies in which obese or normal subjects were given 2,4-DNP or its sodium salt at oral dosages of 1.2-4.3 mg/kg/day 2,4-DNP for ≤14 days (Castor and Beierwaltes 1956; Cutting et al. 1934; Cutting and Tainter 1933; MacBryde and Taussig 1935; Stockton and Cutting 1934; Tainter et al. 1935b).* "

> "*In studies of intermediate-duration oral exposure to 2,4-DNP, cases of death from agranulocytosis have been attributed to 2,4-DNP. These cases occurred during the usual dosing regimens for weight loss, employing increasing doses in one case from 2.9 to 4.3 mg/kg/day of 2,4-DNP for 6 weeks (Dameshek and Gargill 1934); a dose of 1.03 mg/kg/day 2,4-DNP for 46 days in another case (Goldman and Haber 1936); and in another, from 0.62 to 3.8 mg/kg/day 2,4-DNP as sodium 2,4-DNP for 41 days (Silver*

1934). In all cases, the patients were under medical supervision. Several clinical studies regarding the effects of 2,4-DNP or its sodium salt in obese and non-obese humans taking the drug for an intermediate duration at doses of 3.5-5.27 mg/kg/day 2,4-DNP have reported no deaths from this treatment (Cutting et al. 1934; Grant and Schube 1934; Looney and Hoskins 1934; MacBryde and Taussig 1935; Simkins 1937a, 1937b; Tainter et al. 1934a, 1935b). A woman who took 3-5 tablets a day of 2,4-DNP for several months, discontinued its use for 3 months, and then resumed taking 5 tablets a day for 1 week, became ill only after resumption of dosing and subsequently died (Lattimore 1934). The data reported were insufficient to determine a dose in this case. It is not known why this woman tolerated the treatment for several months without developing any signs of illness, then subsequently became ill and died within 1 week after resumption of the same dose."

In the acute toxicity section of the CLH report, the DS also presented the evaluation that had been performed in Germany in 2015 for fatal poisonings by dinitrophenol (Table 13 and Table 14). There were 14 cases of fatal oral poisonings with the information about doses applied presented in Table 13 of the CLH report. The majority of the fatal cases (11 cases) occurred after short duration of exposure of the substance (from 1 to 14 days), and 3 fatal cases were after intermediate duration of exposure (from 15 to 364 days). In Table 13 of the CLH report indicates that in humans, deaths after exposure to dinitrophenol occurred at much lower doses than 80 mg/kg bw/day, suggesting that humans might be more sensitive than rats. A summary of the available data on fatal oral poisonings with the information on doses applied and the duration of exposure is provided in the Table below.

Additional fatal oral poisonings without information on the doses applied are presented in the Table 14 of the CLH report. There are 10 cases presented (6 females and 4 males), with information that is too limited to deduce a duration of an exposure. These data supports evidence for acute toxicity, but are not relevant for specific target organ toxicity –repeated exposure.

Generally, the case reports are of limited value for hazard identification for specific target organ toxicity, especially if the exposure represents a single exposure, abuse or misuse of the substance.

***Table**: Summary of the fatal oral poisoning in human after acute and repeated exposure (compiled from data available in the CLH report)*

Case	Exposure duration (days)	Dose (mg/kg bw/day)	Remarks	Ref.
1	1	40 (suicidal)	M, 46 yrs, 70 kg est. sodium DNP Effects before death: hypotension, tachycardia (170bpm), increased body temp. (37.8°C), diarrhoea, vomiting, sweating, dehydrated	Bartlett *et al.*, 2010
2	1	40 (suicidal)	M, 70 kg est. Effects before death : hyperkalaemia, increased body temp (39.5°C)	Siegmueller&Narasimhaiah 2010
3	1	40	M, 70kg est.	Kamour *et al.,* 2015
4	1	43	M, 70 kg est.	Kamour *et al*., 2015
5	1	46	M, 70 kg est.	Kamour *et al*., 2015
6	1	57 (suicidal)	M, 70 kg est.	BfR 2015
7	1	57 (suicidal)	M, 70 kg est.	BfR 2015
8	2 doses within 7 days	46	M, 80 kg	Tainter and Wood 1934
9	4	6.2	M, 22-yrs, 97 kg	McFee *et al,* 2004

			Effects before death: change in mental status 16 hrs after last dose, increased body temp. (38.9°C), bradycardia, deteriorated cardiac status, asystol developed	
10	5	7	F, 27 yr sodium DNP Effects before death: headache, backache, weakness, dizziness, shortness of breath, excessive perspiration, increased body temp. (38.7°C), increased pulse (140 beat/min), increased respiration rate Histology : hyperaemic and haemorrhagic lungs, degeneration of renal tubules and liver cells, segmentation and fragmentation of cardiac muscles, and haemorrhagic spleen, stomach mucosa, spinal cord, pons, and medulla	Poole and Haining 1934 ; ATSDR 1995
11	14	2.66	F, psychiatric patient sodium DNP experimental study – DNP for treating depression: died after her pulse increased to 148 beats/minute, respiration to 48 /min, Body temp: 38.8°C, blood pressUre: 36/0 Autopsy delayed 4 days - No conlusion on histopathol. lesions.	Masserman and Goldsmith 1934 ATSDR 1995
12	**41**	**0.62 to 3.8**	Patient under medical supervision, dosing regimens for weight loss sodium 2,4-DNP Death – agranulocytosis	ATSDR 1995 (Silver 1934)
13	**42**	**2.9 to 4.3**	Patient under medical supervision dosing regimens for weight loss 2,4-DNP Death – agranulocytosis	Dameshek and Gargill 1934 ATSDR 1995
14	**46**	**1.03**	Patient under the medical supervision dosing regimens – weight loss 2,4-DNP Death – agranulocytosis	Goldman and Haber 1936 ATSDR 1995

For specific target organ toxicity after repeated exposure information from studies with intermediate duration could be relevant. As can be seen in the Table above, deaths after exposure to dinitrophenol occurred at doses between 1.03 – 4.3 mg/kg bw/day. However, there were only 4 fatal cases reported in the CLH report. In three cases patients were under medical supervision and one was a psychiatric patient. Based on data available it is not known why these patients were under medical supervision, whether due to deterioration of the patient's health or other reasons. It can also not be excluded, that the patients took other medicines that could have interfered with 2,4-dinitrophenol activity and could therefore have potentially resulted in a lower fatal dose.

The data presented in the Table above are limited, but indicate effects consistent with 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.

Conclusion

The DS proposed classification of 2,4-dinitrophenol with STOT RE 2 on the basis of the mortality that had been observed in rats at 80 mg/kg bw/day in one subacute oral toxicity study (Koizumi *et al.,* 2001).
Even though the 28-day oral toxicity study in rats was evaluated by the DS as reliable without restriction, there are some uncertainties that lower the reliability of the study for a decision on classification for STOT RE for this substance.

The data available in the CLH dossier indicated that humans might be more sensitive than rats and are considered more important than the available data from the animal study for this case. The information on repeated exposure on humans included in the Table above demonstrated that the observed lethality cannot be attributed to acute toxicity alone.

Although the adverse effects observed in humans before death occurred and available histopathology findings indicated obvious consequences of the uncoupling of oxidative phosphorylation, based on data available it is not possible to confirm if co-exposure and other co-morbidities could be excluded, which would affect the size of the fatal dose. The original studies are not available for the assessment of reliability for the purpose of harmonised classification for STOT RE. Based on the information from ATSDR (1995) quoted in the CLH report, no deaths have been reported in several clinical studies on the effects of 2,4-DNP or its sodium salt at doses of 3.5-5.27 mg/kg bw/day 2,4-DNP in obese and non-obese humans for an intermediate duration of exposure, suggesting that large variations in sensitivity to 2,4-dinitrophenol may exist among humans.

The mechanism of the action of 2,4-dinitrophenol –suppression of adenosine triphosphate (ATP) production by uncoupling the oxidative phosphorylation of adenosine diphosphate (ADP) in mitochondria is very well documented and known. All energy-dependent biochemical processes are likely to be affected, resulting in toxicity to any exposed organs. Moreover, local metabolic poisoning may exacerbate other pre-existing diseases. The most sensitive indicators are increased basal metabolic rate, increased body temperature and increased pulse. This mode of action indicated that the substance does not target a specific organ but induces failure of several organs of the organism, leading to death.

According to the CLP criteria the substance is classified in Category 1 for specific target organ toxicity (repeated exposure (STOT RE1) on the basis of reliable and good quality evidence from human cases or epidemiological studies; or observation from appropriate studies in experimental animals in which significant and/or severe toxic effects of relevance to human health were produced at generally low exposure concentrations.

2,4-dinitrophenol was used extensively in diet pills in the US and several cases of intoxication were reported in connection with this use. There is an extensive database on the effects of dinitrophenols in humans which indicates that humans could be more sensitive than rodents (ATSDR, 1995).

Based on the data presented in the ATSDR evaluation (Table 2-1 and Figure 2-1 of the ATSDR report) it appears that in humans who ingested 2,4 dinitrophenol for intermediate and chronic durations, respiratory, cardiovascular, gastrointestinal, haematological, musculoskeletal, hepatic, renal, dermal, ocular, body weight, metabolic, immunological/lymphoreticular and neurological effects were observed. The effects in humans appear to occur with the same intensity at the same oral dose levels during an intermediate-duration exposure as during an acute-duration exposure. The lungs, cardiovascular system, gastrointestinal tract, haematological system (agranulocytosis), musculoskeletal system, liver, kidney and eyes were identified as target organs of the effects of the substance following exposure for an intermediate duration. The effects on these organs and systems observed after intermediate duration oral exposure was comparable

to those observed after acute oral exposure. In a few people, oral ingestion of the substance at above 1.2 mg/kg bw/day for longer periods of time led to serious toxic effects including cataracts, agranulocytosis, peripheral neuritis and serious dermatological conditions. The LOAELs for serious health effects in humans upon intermediate and chronic dosing (<10 mg/kg bw/day) seem to be lower than those in experimental animals (ATSDR, 1995). This data shows that classification as STOT RE 1 is more appropriate than STOT RE 2.

Overall, considering the mode of action and the significant toxic effects in several organ/systems due to an ATP depletion possibly leading to the fatal consequences seen in humans, RAC concludes that **classification of 2,4-dinitrophenol as STOT RE 1; H372 (Causes damage to organs through prolonged or repeated exposure) is warranted**. The justification is the failure of several organs that can lead to mortality.

Additional references

Rodin FH (1936):Rodin FH, West. Med. 1936 Apr;44(4):276-9

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).