Committee for Risk Assessment

RAC

Annex 1

Background document

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

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

30 November 2018

CLH report

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

Substance Name: 2,4-Dinitrophenol

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

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

Index Number: **609-041-00-4**

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Version number: 1.0 **Date: November 2017**

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	2,4-dinitrophenol
EC number:	200-087-7
CAS number:	51-28-5
Annex VI Index number:	609-041-00-4
Degree of purity:	Ca.85 %
Impurities:	Considered confidential, please refer to the confidential annex.

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation	**SCL, M-Factor, ATE**
Current entry in Annex VI, CLP Regulation	Acute Tox. 3*, H301, Acute Tox. 3*, H311, Acute Tox. 3*, H331, STOT RE 2*, H373**, Aquatic Acute 1, H400	
Current proposal for consideration by RAC	Acute Tox. 2, H300, Acute Tox. 3, H311, STOT RE 2, H373	ATE = oral 35 mg/kg bw ATE = dermal 600 mg/kg bw
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Acute Tox. 2, H300, Acute Tox. 3, H311, Acute Tox. 3*, H331, STOT RE 2, H373, Aquatic Acute 1, H400	ATE = oral 35 mg/kg bw ATE = dermal 600 mg/kg bw

1.3 Proposed harmonised classification and labelling based on CLP Regulation

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives				Data lacking
2.2.	Flammable gases				Data lacking
2.3.	Flammable aerosols				Data lacking
2.4.	Oxidising gases				Data lacking
2.5.	Gases under pressure				Data lacking
2.6.	Flammable liquids				Data lacking
2.7.	Flammable solids				Data lacking
2.8.	Self-reactive substances and mixtures				Data lacking
2.9.	Pyrophoric liquids				Data lacking
2.10.	Pyrophoric solids				Data lacking
2.11.	Self-heating substances and mixtures				Data lacking
2.12.	Substances and mixtures which in contact with water emit flammable gases				Data lacking
2.13.	Oxidising liquids				Data lacking
2.14.	Oxidising solids				Data lacking
2.15.	Organic peroxides				Data lacking
2.16.	Substance and mixtures corrosive to metals				Data lacking
3.1.	Acute toxicity - oral	Acute Tox. 2	ATE = 35 mg/kg bw	Acute Tox. 3*	
	Acute toxicity - dermal	Acute Tox. 3	ATE = 600 mg/kg bw	Acute Tox. 3*	
	Acute toxicity - inhalation			Acute Tox. 3*	
3.2.	Skin corrosion / irritation				Not assessed in this dossier
3.3.	Serious eye damage / eye irritation				Not assessed in this dossier
3.4.	Respiratory sensitisation				Not assessed in this dossier
3.4.	Skin sensitisation				Not assessed in this dossier
3.5.	Germ cell mutagenicity				Not assessed in this dossier
3.6.	Carcinogenicity				Not assessed in this dossier
3.7.	Reproductive toxicity				Not assessed in this dossier
3.8.	Specific target organ toxicity –single exposure				Not assessed in this dossier
3.9.	Specific target organ toxicity – repeated exposure	STOT RE2		STOT RE2*	

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
3.10.	Aspiration hazard				Not assessed in this dossier
4.1.	Hazardous to the aquatic environment			Aquatic Acute 1	Not assessed in this dossier
5.1.	Hazardous to the ozone layer				Not assessed in this dossier

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling:

Signal word: Danger

Pictogram:
GHS09
GHS08
GHS06

Hazard statements:
H300 "Fatal if swallowed"
H311 "Toxic in contact with skin"
H331 "Toxic if inhaled"
H373 "May cause damage to organs through prolonged or repeated Exposure"
H400 "Very toxic to aquatic life"

Proposed notes assigned to an entry: none

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

The current harmonised classification of dinitrophenol for acute toxicity and STOT RE 2 is the translation of the DSD classification with T; R23/24/25.

2.2 Short summary of the scientific justification for the CLH proposal

It is one of the intentions of this proposal to remove the asterisks of the minimum classification for acute toxicity for the oral and the dermal route. Additionally, the asterisk for specific target organ toxicity – repeated exposure and the two asterisks for H373 shall be removed.

This proposal is based on information available in the REACH-registration (accessed March 2017) and other information available in the scientific literature.

The proposed classification for acute oral toxicity with Acute Tox. 2; H300 is based on the LD_{50} range of 30 – 40 mg/kg bw in a key study on rats. This LD_{50} value fulfils the requirement for Acute Tox. 2, H300. Other studies and reports yielded LD_{50} values in similar ranges for other species such as mouse, rabbit and guinea pig. Several case reports from human poisonings suggested fatal doses of < 50 mg/kg bw.

Only one study is available for acute dermal toxicity and this study confirmed the existing classification of Acute Tox. 3, H311.

No information was available for acute inhalative toxicity and therefor no proposal for a change has been submitted.

The classification for STOT RE 2 has been re-evaluated and confirmed on the basis of the CLP regulation.

2.3 Current harmonised classification and labelling

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

Acute Tox. 3* (H301), Acute Tox. 3* (H311), Acute Tox. 3* (H331), STOT RE 2* (H373**), Aquatic Acute 1 (H400).

2.4 Current self-classification and labelling

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

Table 4: Self-classification by the registrant (on March, 22nd, 2017)

Harzard Class	Statement Code	# of notifiers
Expl. 1.1	H201	4
Flam Sol. 1	H228	1
Acute Tox. 2	H300	5
Acute Tox. 3	H301	43
Acute Tox. 1	H310	1
Acute Tox. 3	H311	47

Harzard Class	Statement Code	# of notifiers
Acute Tox. 3	H331	47
STOT RE 2	H373	47
Aquatic Acute 1	H400	48
Skin Irrit. 2	H315	1
Muta. 2	H341	1
Repr. 2	H361	1
STOT SE 1	H370	1
STOT RE 1	H372	1
Aquatic Chronic 1	H410	1

Total number of notifiers: 48, Number of aggregated notifications: 7.

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.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

2,4-dinitrophenol is currently classified in Annex VI of the CLP regulation with a minimum classification for the acute toxicity hazard classes and STOT RE 2. Pursuant to section 4.1.1 and 4.2. of the guidance on the preparation of dossiers for harmonised classification and labelling, a refinement of such classifications warrants action at the EU level.

Current classification of dinitrophenol for acute oral toxicity, Acute Tox. 3* is not adequate for this compound. Studies in several species and analysis of fatal human poisonings showed the necessity to correct the classification according to the LD_{50} values which are < 50 mg/kg bw. According to these values a classification of Acute Tox. 2, H300, seems to be appropriate for 2,4-dinitrophenol.

During recent years several fatal poisonings with dinitrophenol happened in the United Kingdom (Bartlett et al. 2010, Siegmueller & Narasimhaiah 2010, Kamour et al. 2015) and Germany (BfR, 2015 (http://www.bfr.bund.de/cm/343/nahrungsergaenzungsmittel-die-dinitrophenol-dnp-enthalten-koennen-zu-schweren-vergiftungen-bis-hin-zu-todesfaellen-fuehren.pdf)). Dinitrophenol is advertised as diet aid (‘fat burner’) and distributed via internet based mail-order platforms. The effect is contributed to the suppression of the ATP (energy) production by uncoupling the oxidatative phosphorylation of adenosine diphosphate in mitochondria. 2,4-DNP has a high acute toxicity causing fatal hyperthermia before death, other effects are increased basal metabolic rate, nausea, vomiting, sweating, dizziness, headache, loss of weight and cataracts. While its use in diet pills in the US were stopped in the 1930s, an increasing number of incidences of fatal overdoses including cases of intended suicide or intended weight/fat loss (of interest for people with body mass indices > 30 and bodybuilders) were observed after the year 2000. The classification as Acute Tox. 2, H300, would impose restrictions on placing on the market and distance sales in Germany for the substance on the basis of the German Prohibited Chemicals Act (Chemikalienverbotsverordnung).

Part B.

SCIENTIFIC EVALUATION OF THE DATA

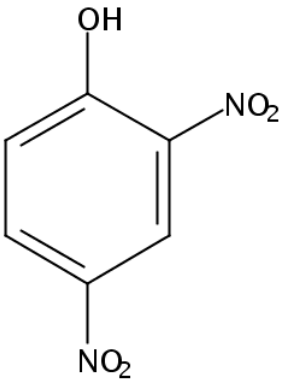
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	200-087-7
EC name:	2,4-dinitrophenol
CAS number (EC inventory):	51-28-5
CAS number:	51-28-5
CAS name:	Phenol, 2,4-dinitro-
IUPAC name:	2,4-dinitrophenol
CLP Annex VI Index number:	609-041-00-4
Molecular formula:	$C_6H_4N_2O_5$
Molecular weight range:	184.11 g/mol

Structural formula:



1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
2,4-dinitrophenol	ca 85% (w/w)	>80% - <90 % (w/w)	

Table 7: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks

Considered confidential, please refer to the confidential annex.

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
none				

1.2.1 Composition of test material

1.3 Physico-chemical properties

Table 9: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	Solid (Manufacture as platelets, leaflets or crystal)		Visual inspection
Melting/freezing point	112-114 °C	Secondary source that mentions: CRC Handbook of Chemistry and Physics 88TH Edition 2007-2008. CRC Press, Taylor & Francis, Boca Raton Secondary source[1]	
Boiling point	Not applicable, the substance sublimes	O'Neil, M.J. (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. Whitehouse Station, NJ: Merck and Co., Inc., 2006., p. 556.	
Density	1.683 g/mL at 24 °C	Secondary source that mentions: CRC Handbook of Chemistry and Physics 88TH Edition 2007-	

Property	Value	Reference	Comment (e.g. measured or estimated)
		2008. CRC Press, Taylor & Francis, Boca Raton Secondary source[1]	
Vapour pressure	1.49 x 10-5 mmHg at 18 °C	Secondary source that mentions Mabey WR, Smith JH, Podoll RT, et al. 1981. Secondary source[1]	
Surface tension	-		Substance is a solid.
Water solubility	5.600 mg/L at 18 °C _____ 2790 mg/L at 20 °C	Secondary source that mentions: Harvey DG., J Pharm Pharmacol 11:462-474., 1959; Secondary source[1] _____ secondary source EPISUITE 4.1 that mentions Schwarzenbach et al. 1988.	
Partition coefficient n-octanol/water	logPow 1.54	Secondary source that mentions: Hansch C, Leo AJ., Claremont, CA: Pomona College. 1985; Secondary source[1]	
Flash point	-		The study does not need to be conducted for explosives.
Flammability	-		The study does not need to be conducted for explosives.
Explosive properties	Explosive or desensitized explosive	UN 0076 or UN 1320, see United Nations Recommendations on the Transport of Dangerous Goods, Chapter 3.2, Rev. 19, 2015.[1]	No experimental data available

[1] Dinitrophenol in dry form has explosive properties. Because of its explosive properties, the compound is used in the form of a water paste.

Regulations on the Transport of Dangerous Goods: In the United Nations Recommendations on the Transport of Dangerous Goods is an entry for Dinitrophenol (subsumed all isomers) which is assigned to be explosives and falls within Class 1 of those recommendations. The substance Dinitrophenol is specifically listed by name in the Dangerous Goods List in Chapter 3.2, and is assigned to two UN numbers:

UN 0076: DINITROPHENOL, dry or wetted with less than 15% water, by mass; Class 1: Explosives - Division 1.1D: Substances and articles which have a mass explosion hazard

UN 1320: DINITROPHENOL, WETTED with not less than 15% water, by mass; Class 4.1, UN packing group I: Solid desensitized explosives

The class of desensitized explosives comprises amongst others:

Property	Value	Reference	Comment (e.g. measured or estimated)
Self-ignition temperature	-		The study does not need to be conducted for explosives.
Oxidising properties	-		The study does not need to be conducted for explosives.
Solubility in organic solvents	2,4-dinitrophenol has solubility at 15 °C (g/100 g solution): 15.55 in ethyl acetate; 35.90 in acetone; 5.39 in chloroform; 20.05 in pyridine; 0.423 in carbon tetra-chloride; 6.36 in toluene; soluble in alcohol and benzene.	Secondary source that mentions: The Merk Index, 1989; Secondary source[2]	
Dissociation constant	pKa: 4.09	Secondary source that mentions: Pearce PJ, Simkins RJJ., Can J Chem 46:241-248., 1968 Secondary source: HSDB - Hazardous Substances Data Bank	
Viscosity	-		Substance is a solid.

(a) Solid desensitized explosives: explosive substances or mixtures which are wetted with water or alcohols or are diluted with other substances, to form a homogeneous solid mixture to suppress their explosive properties.

To classify desensitized explosives, data for the explosive potential and the corrected burning rate should be determined as described in Part V of the UN Recommendations on the Transport of Dangerous Goods, Manual of Tests and Criteria.

However, the correct classification for "Desensitized explosives" in categories 1 to 4 could not be established because sufficient data are not available for the application of the classification criteria in GHS/CLP-Regulation.

The correct classification shall be confirmed by testing. Furthermore, the substance has to comply with the requirements of Directive 2014/28/EU.

[2] secondary source: M. Olivia Harris, M.A; James J. Corcoran, Ph.D. (peer rewied by Dr. martin Alexander; Dr.Leon Koller; Dr.Norman Trieff), U.S Department of Health and Human Services, Public Health Service Agency for Toxic Substances and Disease Registry

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

Absorption

Quotation from ATSDR (1995 and 2011):

Oral absorption

"Limited additional information is provided by animal studies. The half-time for absorption of 2,4-DNP following gavage administration of a single 22.5 mg/kg dose to mice was 0.5 hours (Robert and Hagardom 1983). A highly specific capillary gas chromatography (GC)-mass spectrometry (MS) technique was used to measure serum concentrations of 2,4-DNP at 1, 3, 6, 12, and 24 hours after dosing. The data were best represented by a two-compartment open model, and the analysis was performed accordingly. Fractional absorption was not determined. In an additional study employing the same analytical methods, 2,4-DNP (and its metabolites 2-amino-4-nitrophenol and 4-amino-2-nitrophenol) were monitored in plasma for 0.5, 1, 2, 4, 6, 9, 12, 24, 48, and 96 hours following a single gavage dose of 22.5 mg/kg (Robert and Hagardom 1985). Maximum values for the plasma concentration of 2,4-DNP were seen at 0.5 and 1.0 hours after dosing, giving additional evidence of rapid absorption."

"The time course of plasma concentrations of 2,4-DNP following oral administration to dogs (1 per dose) at 5, 12.5, 25, or 125 mg/kg indicated substantial absorption by the first sampling period, 0.5 hours (Kaiser 1964). Peak plasma levels were attained in 0.5-4 hours after dosing. In general, plasma levels tended to be higher in dogs that received higher doses. The 24-hour urine collections were analyzed for 2,4-DNP but not for metabolites; results were highly variable, raising the suspicion that collection may have been incomplete in some instances. Hence, the excretion data do not provide a reliable estimate of the fraction of the dose absorbed."

Inhalative exposure

"No studies were located regarding absorption in animals after inhalation exposure to 2,4-DNP."

Dermal exposure

"No studies were located regarding the rate or extent of absorption in animals after dermal exposure to 2,4-DNP. However, the death of 1 of 5 guinea pigs dermally exposed to 300 mg/kg (Spencer et al. 1948) suggests that dermal absorption occurred."

Metabolism

Quotation from ATSDR (1995 and 2011):

"Additional information regarding metabolism of 2,4-DNP is available from studies in animals. In a study from the older literature, 2,4-diaminophenol was identified in the urine of rabbits treated orally with 2,4-DNP, and was concluded to be a metabolite of 2,4-DNP (Ogino and Yasukura 1957). The total dose given (probably to all the rabbits combined) was 30 grams (30,000 mg), and the total amount of 2,4-diaminophenol isolated from 10 liters of urine (presumably collected from all the dosed rabbits throughout the study) was 50 mg from 20 liters of urine. Hence, the yield of this

urinary metabolite was 0.17% of the dose. The metabolite was extracted from the urine, purified, and identified by its properties, including melting point, nitrogen analysis, absorption curve, and various color reactions. Limitations of the study include the lack of adequate reporting of dose, route, and number of animals, relative lack of specificity in the identification methods available at the time, and the lack of experiments to quantitate losses of metabolite during the extraction and purification processes. Accordingly, only tentative conclusions regarding the identity of the metabolite as 2,4-diaminophenol and the percentage of the dose metabolized to this metabolite can be drawn from this study."

"2,4-DNP and two of its metabolites, 2-amino-4-nitrophenol and 4-amino-2-nitrophenol, were monitored in plasma of mice at 0.5, 1, 2, 4, 6, 9, 12, 24, 48, and 96 hours following a single gavage dose of 22.5 mg/kg using a highly specific capillary GC/MS technique (Robert and Hagardom 1985). The authors concluded that the amount of 2-amino-4-nitrophenol formed was 7.9 times the amount of 4-amino-2-nitrophenol, and that 50% of 2,4-DNP elimination involved direct conversion to these two compounds. Plasma concentrations of these two metabolites reached their highest levels within the first half hour after dosing, indicating rapid metabolism. The results demonstrate that 2-amino-4-nitrophenol is the major circulating metabolite of 2,4-DNP and that 4-amino-2-nitrophenol is also a significant circulating metabolite. The authors mentioned that they could not analyze for 2,4diaminophenol because of its reactivity with oxygen to form reactive quinones that could not be extracted and chromatographed by their method."

"Analysis of urine for 2,4-DNP and its aminonitrophenol metabolites following a single subcutaneous injection of 20 mg/kg 2,4-DNP into rats revealed only parent compound and 2-amino-4-nitrophenol (Parker 1952). 4-Amino-2-nitrophenol was not detected. The urine was collected over the 24-hour period following dosing."

"An in vitro study of 2,4-DNP metabolism using rat liver homogenates identified 2-amino-4-nitrophenol and 4-amino-2-nitrophenol as metabolic products, with the 4-amino-2-nitrophenol present in greater abundance (Parker 1952). An additional ether-insoluble metabolite was tentatively identified as 2,4-diaminophenol. When 2-amino-6-nitrophenol or 4-amino-2-nitrophenol was incubated with rat liver homogenates, the 2-amino-4-nitrophenol was slowly metabolized to the ether-insoluble compound, while 4-amino-2-nitrophenol rapidly disappeared but with very little accumulation of the ether-insoluble compound. The reduction of the aminonitrophenols to the ether-insoluble compound appeared to be catalyzed by the same nitroreductase that reduces 2,4-DNP to the aminonitrophenols. A comparison of the activity of homogenates of various tissues in the rat and rabbit revealed that liver homogenate metabolized 2,4-DNP at a higher rate than did other tissue homogenates. In the rat, enzyme activities (mg 2,4-DNP metabolized per 100 grams wet weight of tissue) of other tissue homogenates relative to that of the liver homogenate (100%) were 60% in kidney, 59% in spleen, 47% in intrascapular fat, 29% in heart, 16% in muscle, and 3% in brain homogenates. In the rabbit, kidney homogenate activity was 41%, and heart homogenate activity was 3%, relative to liver homogenate activity. The rabbit spleen homogenate had no activity. The other rabbit tissues (intrascapular fat, muscle, and brain) were not analyzed. No activity was found in the blood of rats or rabbits."

"A more extensive investigation of the in vitro metabolism of 2,4-DNP by rat liver homogenates found that, under optimal pH and cofactor levels, 81% of the 2,4-DNP was metabolized. 2-Amino-4-nitrophenol accounted for 75%, 4-amino-2-nitrophenol for 23%, and 2,4-diaminophenol for ≈1% of the total amine metabolites produced (Eiseman et al. 1972). Even under suboptimal conditions, 2-amino-4nitrophenol was the predominant metabolite."

"The distribution of enzyme activity was analyzed in subcellular fractions: nucleic, mitochondrial, microsomal, and cytosol (Eiseman et al. 1972). The maximum activity was found in the cytosol, which is the site of other nitroreductases, although nitroreductases can also be located in microsomes (Fouts and Brodie 1957; Juchau et al. 1970; Kamm and Gillette 1963; Kato et al. 1969; Parker 1952).

The properties of nitroreductases have been extensively studied for the reduction of p-nitrobenzoic acid (Kato et al. 1969). Two separate enzyme systems are involved, one located in the cytosol, and the other in the microsomes. Both forms require the presence of reduced nicotinamide adenine dinucleotides (NADH or NADPH) (Kato et al. 1969). The cytosolic reducing activity for 2,4-DNP required NADPH, since the activity in both the whole homogenate and in the cytosol was enhanced by adding glucose-6-phosphatase and NADP (Eiseman et al. 1972). The fact that the washed microsomal fraction contained no appreciable activity with 2,4-DNP could be due to the absence of soluble NADPH-generating enzymes, such as a glucose-6-phosphate dehydrogenase. Oxygen partially inhibited the formation of the aminonitrophenols. This inhibition is consistent with a reoxidation of cofactors FADH2 or NADPH in the presence of oxygen (Kamm and Gillette 1963). Reduction of [^{14}C]2,4-DNP to 2-amino-4-nitrophenol and 4-amino-2-nitrophenol by rat liver homogenates was not affected by the addition of p-nitrobenzoic acid, suggesting that different nitroreductases are involved (Eiseman et al. 1974). However, p-nitrophenol, o-nitrophenol, and 2,4-dinitro-6-sec-butylphenol inhibited the reduction of 2,4-DNP. The reduction was competitively inhibited by o-nitrophenol and noncompetitively inhibited by p-nitrophenol and 2,4-dinitro-6-sec-butylphenol. These results indicate separate metabolic pathways for 2,4-DNP and p-nitrobenzoic acid. The competitive inhibition by o-nitrophenol, however, suggests that 2,4-DNP and o-nitrophenol compete for the same active site on the nitroreductase, while the noncompetitive inhibition by the other two nitro compounds suggests binding at different sites on the enzyme."

"Limited information indicates that 2,4-DNP may also be conjugated to glucuronic acid or sulfate in the liver and then be excreted in the urine (NRC 1982)."

"No studies were located regarding possible fecal metabolites of 2,4-DNP."

Distribution

Quotation from ATSDR (1995 and 2011):

Oral exposure

"Limited information is available regarding distribution in animals after oral exposure to 2,4-DNP. Pharmacokinetic analysis indicated that a two-compartment open model best characterized the disposition of 2,4-DNP in the serum, liver, and kidney of mice given a gavage dose of 22.5 mg/kg of 2,4-DNP (Robert and Hagardorn 1983). Serum and tissue levels of the parent compound were quantitated by a highly specific capillary GC-MS method at l-24 hours postdosing. Although concentrations of 2,4-DNP were much lower in liver and kidney than in serum, similar half-times for absorption (t1/2 = 0.50-0.62 hours) and for the fast (alpha) (t1/2 = 1.00-1.20 hours) phase of biphasic elimination in all 3 tissues were determined. However, terminal (beta) elimination phase from kidney was very slow (t1/2 = 76.2 hours) compared with the beta phase in liver (t1/2 = 8.7 hours) and in serum (t1/2 = 7.7 hours). The similar half-times for absorption and biphasic elimination in all three tissues (except terminal elimination phase in kidney) indicated that rapid exchange of 2,4-DNP occurred among these sites. The authors suggested that the apparent persistence of 2,4-DNP in the kidney could be related to tissue binding of the compound."

"The time course of plasma concentrations of 2,4-DNP following oral administration to dogs (1 per dose) at 5, 12.5, or 25 mg/kg gave no evidence of a trend towards higher plasma levels with continued daily dosing (Kaiser 1964). Hence, 2,4-DNP did not appear to accumulate."

Inhalation exposure

"No studies were located regarding distribution in animals after inhalation exposure to 2,4-DNP".

Dermal exposure

"No studies were located regarding distribution in animals after dermal exposure to 2,4-DNP."

Elimination

Quotation from ATSDR (1995 and 2011):

Oral exposure

"Pharmacokinetic analysis indicated that a two-compartment open model best characterized the disposition of 2,4-DNP in the serum, liver, and kidney of mice given a gavage dose of 22.5 mg/kg of 2,4-DNP (Robert and Hagardorn 1983). Serum and tissue levels of parent compound were quantitated by a highly specific capillary GC-MS method at l-24 hours postdosing. Half-times for the slow terminal elimination phases were 7.7 hours for serum, 8.7 hours for liver, and 76.2 hours for kidney. The authors suggested that the apparent persistence of 2,4-DNP in the kidney could be related to tissue binding of the compound."

"In an additional study employing the same analytical methods, 2,4-DNP and its metabolites, 2-amino-4-nitrophenol and 4-amino-2-nitrophenol, were monitored in plasma for 0.5-96 hours following a single gavage dose of 22.5 mg/kg in mice (Robert and Hagardorn 1985). Pharmacokinetic analysis indicated that two-compartment open models best characterized the disposition of 2,4-DNP and 2-amino-4-nitrophenol from plasma, whereas a three-compartment open model best characterized the disposition of 4-amino-2-nitrophenol from plasma. The elimination half-lives (t1/2) for the terminal phase were estimated at 10.3 hours for 2,4-DNP, 46.2 hours for 2-amino-4-nitrophenol, and 25.7 hours for 4-amino-2-nitrophenol."

"In dogs (1 per dose) that received 1, 12.5, or 25 mg/kg/day 2,4-DNP, 24-hour excretion of parent compound in the urine at 1, 3, and 6 days of treatment was erratic (Kaiser 1964), raising the suspicion that collection may have been incomplete in some instances. Greater amounts of 2,4-DNP were excreted after the first than after subsequent doses. Excretion of metabolites was not investigated."

"Species differences in elimination of 2,4-DNP do not appear to be large (less than two-fold) on the basis of a single limited study. The elimination rate constants for 2,4-DNP from blood of rats, rabbits, guinea pigs, and mice following unspecified single oral doses of 2,4-DNP were 0.062, 0.10, 0.12, and 0.098 $hours^{-1}$, respectively (Lawford et al. 1954). "

Inhalation exposure

"No studies were located regarding excretion in animals after inhalation exposure to 2,4-DNP."

Dermal exposure

"No studies were located regarding excretion in animals after dermal exposure to 2,4-DNP."

4.1.2 Human information

Absorption

Quotation from the ATSDR (1995 and 2011):

Oral exposure

"The data regarding absorption in humans after oral exposure are limited. Evidence of substantial 2-4-DNP absorption was obtained from the case of an 80-kg man who ingested two 4.5-g doses of the sodium salt of 2,4-DNP (each equivalent to 46 mg 2,4-DNP/kg) 1 week apart and died 11 hours after the second dose (Tainter and Wood 1934). Analysis of a blood sample for 2,4-dinitrophenol and estimation of the total body burden, assuming the drug was evenly distributed between blood and tissues, gave a body burden of ≈2.72 grams 2,4-DNP, which corresponds to 3.31 grams of the sodium salt of 2,4-dinitrophenol at the time of death. Since some of the drug would have been metabolized, and some excretion of parent compound and metabolites probably would have occurred during the interval between ingestion and death, this value is not inconsistent with complete absorption of the second dose. 2,4-DNP and 2-amino-4-nitrophenol were detected in the urine of a woman who had taken sodium 2,4-DNP at 3.5 mg/kg/day 2,4-DNP for 20 days (Davidson and Shapiro 1934), indicating that absorption had occurred. Quantitative data were not reported. Indirect evidence of rapid absorption is provided by the maximal increases in basal metabolic rate that occurred within 1 hour of ingestion of 2-5 mg/kg 2,4-DNP from 2,4-DNP (Cutting et al. 1933) or sodium 2,4-DNP (Dunlop 1934) by patients in clinical studies."

Inhalative exposure

"No quantitative data were located regarding absorption in humans after inhalation exposure to DNPs. A metabolite of 2,4-DNP, 2-amino-4-nitrophenol, was commonly detected by the Derrien test in the urine of workmen (women were generally not employed in dangerous processes) exposed via inhalation to vapor and airborne dust of 2,4-DNP and by direct contact of the skin with the solid chemical in the munitions industry in France (Perkins 1919). Exposure may have occurred by the dermal and possibly oral routes, as well as by inhalation. In addition, examination of the blood, unspecified organs, and urine of workmen in this industry who died from exposure to 2,4-DNP revealed the presence of 2,4-DNP and its metabolites; quantitative data were not provided (Perkins 1919). Despite its limitations, the study provides some evidence of absorption from inhalation exposure."

"In a case of fatal occupational 2,4-DNP poisoning from exposure to mists and airborne dust of 2,4-DNP in the U.S. chemical industry, the urine contained 2.08 g/L of 2,4-DNP and 50 mg/L of 2-amino-4-nitrophenol (Gisclard and Woodward 1946). Workroom air levels of 2,4-DNP, determined subsequent to the death, were "normally" ≥40 mg/m³. This may underestimate breathing-zone levels, and significant dermal absorption was thought to have occurred following deposition of the chemical on the skin. Hence, no conclusions regarding fraction absorbed can be drawn from this study, but it does provide additional evidence of absorption from the inhalation route."

Dermal exposure

"The 1917 and 1918 records from two French munitions factories show that the percentage of positive Derrien tests (for the metabolite 2-amino-4-nitrophenol) tended to rise during the warm months of the year (Perkins 1919). One of these factories also provided records of the percentage of clinical cases, which, although much lower than the percentage of positive Derriens, roughly paralleled the Derrien results. The increases in percentages of positive Derrien tests and clinical cases during the warmer months provide support for the idea that dermal absorption may be an important route of entry to the body, since greater exposure of the skin would be expected during the warmer months. Alternatively, the higher ambient temperatures may have caused greater loss of body water through sweating, resulting in more concentrated urine, which could result in a greater percentage of positive Derrien tests. The higher ambient temperatures would also tend to exacerbate the effects of 2,4-DNP."

"Similarly, the details of two fatal cases of 2,4-DNP in the U.S. chemical industry suggest that dermal absorption may have been a contributing factor. Two workers exposed to mists and dust of 2,4-DNP for a few months developed signs of toxicity; following treatment and rest, and then a return to the job during warmer weather, they collapsed and died (Gisclard and Woodward 1946). The warmer weather during the second period of exposure (duration not specified) was thought to be a contributing

factor because of the greater skin exposure and potential for increased dermal absorption; it may also have exacerbated the effects by contributing to hyperpyrexia and increased pulse produced by 2,4-DNP."

Metabolism

Quotation from ATSDR (1995 and 2011):

"Some information regarding metabolism of 2,4-DNP in humans is available from cases of occupational 2,4-DNP poisoning and from a case involving ingestion of the chemical as a diet pill. The limitations of these studies include the relative lack of specificity of the methods employed to detect or quantify 2,4-DNP and its metabolites. Examination of the blood and organs of workmen who died from exposure to 2,4-DNP in the French munitions industry revealed, the presence of 2,4-DNP and its reduced metabolites (not further specified) (Perkins 1919). The compounds found in urine were 2,4-DNP, 2-amino-4-nitrophenol, 4-amino-2-nitrophenol, 2,4-diaminophenol, and other unidentified nitrogen compounds, which may have been glucuronide conjugation products (NRC 1982). In cases of serious 2,4-DNP poisoning, the presence of large amounts of 2-amino-4-nitrophenol in the urine were found and were the basis of a-particular test called the Derrien test, used as an indicator of exposure to 2,4-DNP. This test is a color reaction for aminonitrophenols, with the formation of a characteristic red wine to violet color for 2-amino-4-nitrophenol and a more or less yellow-orange color for 4-amino2-nitrophenol constituting a positive test. Quantitation apparently was limited to visual inspection for color intensity. The test is not highly specific, as picramic acid also yields a red wine to violet color. The test cannot detect diaminophenols because the ability to produce the colored reaction depends on the NO2 group in aminonitrophenols. Mild cases of intoxication produced positive Derrien tests; when the urine gave a positive Derrien that increased in intensity day by day or remained fairly high, acute intoxication frequently developed. The percentage of positive Derriens did not appear to correlate with race ("white, yellow, black") (Perkins 1919)."

"In a case of fatal occupational 2,4-DNP poisoning from exposure to mists and airborne dust of 2,4-DNP in the U.S. chemical industry, the urine contained 2.08 g/L 2,4-DNP and 50 mg/L of 2-amino-4-nitrophenol (Gisclard and Woodward 1946). "

"A woman who ingested sodium 2,4-DNP at 3.5 mg/kg/day 2,4-DNP for 20 days tested positive for the presence of 2-amino-4-nitrophenol (Derrien test) and 2,4-DNP ("indicator test" not further described) in the urine (Davidson and Shapiro 1934)."

Distribution

Quotation from ATSDR (1995 and 2011):

Oral exposure

"No studies were located regarding distribution in humans after oral exposure to 2,4-DNP."

Inhalation exposure and dermal exposure

"Examination of the blood and unspecified organs of workmen who died from exposure to 2,4-DNP in the munitions industry in France revealed the presence of 2,4-DNP and its metabolites (Perkins 1919), indicating distribution to the tissues. Analysis of the organs (not specified) of two workmen who died following exposure to 2,4-DNP in the U.S. chemical industry, however, did not demonstrate the presence of the chemical or metabolites, although 2,4-DNP and 2-amino-4-nitrophenol were detected in the urine of one worker (urine was not tested in the other case) (Gisclard and Woodward

1946). Because one report (Perkins 1919) did not provide details of extraction and analytical methods, reasons for the discrepancy in results cannot be determined. In both studies, exposure may have occurred by the dermal as well as the inhalation routes."

Elimination

Quotation from ATSDR (1995 and 2011):

Oral exposure

"Both 2,4-DNP and its metabolite, 2-amino-4-nitrophenol, were detected in the urine of a woman who had taken the sodium salt of 2,4-DNP at 3.5 mg/kg/day 2,4-DNP for 20 days and was admitted to the hospital 5 days after cessation of DNP treatment because of severe illness (agranulocytosis) (Davidson and Shapiro 1934). Detection of parent compound (method not described) and 2-amino-4-nitrophenol (Derrien test) occurred on the second day of hospitalization and of parent compound on the third. The bromsulphalein test for liver function showed evidence of impaired function, which may have accounted for the persistence of 2,4-DNP and 2-amino-4-nitrophenol in the body 7-8 days after cessation of intake."

Inhalation exposure and dermal exposure

"In humans exposed to 2,4-DNP by inhalation, both the parent compound and metabolites appear to be excreted in the urine. 2,4-DNP and its metabolites have been detected in the urine of workmen who died from exposure to 2,4-DNP in the munitions industry in France; quantitative exposure or urinary data were not provided (Perkins 1919). In addition, a yellow staining of the skin was observed after workers perspired profusely, indicating that 2,4-DNP was excreted in the sweat. As discussed in Section 2.3.1.1, a metabolite of 2,4-DNP, 2-amino-6nitropheno1, was commonly detected in the urine of such workmen; quantitative data were not provided (Perkins 1919). In a case of fatal occupational 2,4-DNP poisoning in the U.S., the urine contained 2.08 g/L of 2,4-DNP and 50 mg/L of 2-amino-4-nitrophenol (Gisclard and Woodward 1946). In both occupational studies, exposure may have occurred by the dermal as well as inhalation routes."

4.1.3 Summary and discussion on toxicokinetics

Quotation from the ATSDR (1995 and 2011):

"The toxicokinetics of 2,4-DNP in humans and animals have not been studied systematically. The available data from human case reports and experimental animal studies indicate that 2,4-DNP is readily absorbed by the oral and inhalation routes, and possibly by the dermal route. Some evidence about distribution is available suggesting that a portion of the 2,4-DNP in the blood is bound to serum proteins and that the unbound fraction enters organs such as the eye. 2,4-DNP is rapidly metabolized via reduction of the nitro groups; the parent compound and metabolites are excreted in the urine."

4.2 Acute toxicity

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

Table 10: Summary table of relevant acute oral toxicity studies

Method	Results	Remarks	Reference
Rat oral (gavage) in olive oil containing 5 -10 % gum Arabic solution. white rats, young mature, both sexes (9 to 40 animals per dose group). Following doses were applied: 10, 20, 23, 25, 27, 30, 40, 50, 60, 70, 80 and 100 mg/kg b.w.	No mortality occurred at doses of 10 – 27 mg/kg bw 37 % (11/30) mortality occurred at 30 mg/kg bw 90 % (18/20) mortality occurred at 40 mg/kg bw 100 % (20/20) mortality occurred at 100 mg/kg bw	2 (reliable with restrictions) Test material: 2,4-dinitrophenol	Spencer et al. (1948)
Rat Oral Weanling male rats (Sherman-strain) no information on doses and numbers of animals	LD_{50} 71 mg/kg bw (95 %) confindence limits: 57-89	4 (not assignable) Test material: 2,4-dinitrophenol	Kaiser, 1964
Rat Oral: no further details	LD_{50}: 30 mg/kg b.w.	4 (not assignable) Test material: 2,4-dinitrophenol	Schafer (1972)
Rat Oral: gavage White rats, no further details	LD_{0}: 20 mg/kg bw LD_{100}: 60 mg/kg bw	4 (not assignable) Test material: 2,4-dinitrophenol	Dow chemicals (1940) cited according to ATSDR (1995)
Rat Oral: gavage White rats, no further details	LD_{50}: 30 mg/kg bw	4 (not assignable) Test material: 2,4-dinitrophenol	Dow chemicals (1950) cited according to ATSDR (1995)
Rat Oral: no further details	LD_{50}: 30 mg/kg bw	4 (not assignable) Test material: 2,4-dinitrophenol	According to HSDB database
Mouse Oral Weanling male mice (white C.F.1-strain) no information on doses and numbers of animals	LD_{50} 72 mg/kg b.w. (95 % confindence limits: 62-84 mg/kg b.w.)	4 (not assignable) Test material: 2,4-dinitrophenol	Kaiser, 1964
Mouse Oral: No further details	LD_{50}: 45 mg/kg bw	4 (not assignable) Test material: 2,4-dinitrophenol	according to RTECS-Website
Rabbit Oral: No further details	LD_{50}: 30 mg/kg bw	4 (not assignable) Test material: 2,4-dinitrophenol	according to RTECS-Website
Rabbit Oral: No further details	LD_{50}: 30 mg/kg bw	4 (not assignable) Test material: 2,4-dinitrophenol	according to HSDB database
Guinea pig Oral: no further details	LD_{50}: 81 mg/kg bw	4 (not assignable) Test material: 2,4-dinitrophenol	according to RTECS-Website
Dog Oral: No further details	LD_{Lo}: 30 mg/kg bw	4 (not assignable) Test material: 2,4-dinitrophenol	according to RTECS-Website

Method	Results	Remarks	Reference
Dog Oral: No further details	LD_{50}: 20 -30 mg/kg bw	4 (not assignable) Test material: 2,4-dinitrophenol	according to RTECS-Website
Cat Oral: No further details	LD_{50}: 75 mg/kg bw	4 (not assignable) Test material: 2,4-dinitrophenol	according to RTECS-Website

Reference: Spencer et al., 1948

Study design:

The oral toxicity of 2,4-dinitrophenol was investigated in young mature white rats of both sexes (no further information on strain and age of the animals was given, furthermore no details were given on the distribution of male and female rats). Dinitrophenol was applied in olive oil containing a 5 – 10 % gum Arabic solution via gavage. The volume of oil given to each rat was less than 3 ml and was usually of the order of 1 ml.

Results:

All of the rats that survived were observed until it was certain that they had fully recovered (usually about two weeks). Deaths that resulted from the treatment with dinitrophenol were attributed to be due mainly to their pyretic effect; as a rule, death occurred an hour or two after the treatment or not at all.

A total number of 239 animals were treated with dinitrophenol. The results are presented in table 12. It was concluded, that dinitrophenol was a rapidly acting agent. A survival dose of 27 mg/kg bw and a lethal dose of 100 mg/kg bw were defined.

Table 11: Acute oral toxicity of dinitrophenol (Spencer et al., 1948)

Dose [in mg/kg bw]	No of animals treated	No died	Percentage
10	9	0	0
20	20	0	0
23	10	0	0
25	10	0	0
27	10	0	0
30	30	11	37
40	20	18	90
50	20	17	85
60	20	19	95
70	30	15	50
80	40	18	45
100	20	20	100

Conclusion:

It can be concluded that dinitrophenol has a very steep dose-response and that the LD_{50} can be estimated for a dose between 30 and 40 mg/kg bw. The study has been performed before the establishment of guidelines and good laboratory practice. Some information is lacking such as the precise age

of the animals and the ratio of male and female rats in each dose group. However, these information gaps are considered as minor important and the total conclusion is Klimisch score 2 (reliable with restrictions).

Reference: Kaiser, 1964

Study design:

The oral toxicity of 2,4-dinitrophenol was investigated in weanling male rats of the Sherman strain weighing 40 – 55 g when received. Male weanling C.F. 1 white mice weighing 15-18 g were also used. The animals were deprived of solid food for approximately 18 hours prior to dosing. Solid food was again given to the animals 30 minutes after dosing and was available for the duration of the period of observation, which lasted at least 7 days after treatment. A suspension of dinitrophenol was prepared using a 1 % aqueous starch solution.

Results:

The following signs of toxicity were observed in rats and mice: tremors, prostration, increase in respiratory rate, tonic convulsions and rigidity of limbs just prior to or immediately after signs of death. The survivors experienced a temporary increase in respiration. Their weight gain was similar to that of the controls.

LD_{50} values of 71 mg/kg bw were presented for rats and for mice the LD_{50} values were presented with 72 mg/kg bw.

Conclusion:

LD_{50} values were almost identical in rats and mice. The study has been performed before the establishment of guidelines and good laboratory practice. However, important information is lacking such as the number of animals investigated and doses of dinitrophenol applied. Due to these important deficiencies the conclusion is that the Klimisch score is 4 (not assignable). Nevertheless, the LD_{50} values will be used as supporting information.

Further publications and information mentioned in Table 11

The publication of Schafer (1972) summarizes the acute oral toxicity of 369 compounds in different species. Table 1 of this publication referred to a LD_{50} of 30 mg/kg bw in rats without any further information.

A study report from Dow Chemicals (1940) was cited by the ATSDR (1995) with a 100 % survival at the dose of 20 mg/kg bw and a 100 % lethal dose of 60 mg/kg bw in white rats treated once by gavage. No further information was available.

Another study report from Dow Chemicals (1950) was cited by the ATSDR (1995) with a LD_{50} value of 30 mg/ kg bw for white rats treated once by gavage. No further information was available. It is possible, that the Schafer review (1972) referred to this study report since it mentioned an identical LD_{50} value. However, without any further information it cannot be investigated any further.

On the RTECS website information was available for five species: mouse (LD_{50}: 45 mg/kg bw), rabbit (LD_{50}: 30 mg/kg bw), Guinea pig (LD_{50}: 81 mg/kg bw), dog (LD_{Lo}: 30 mg/kg bw) and cat (LD_{50}: 75 mg/kg bw). No further information was accessible.

In the HSDB database information was available for three species: rat (LD_{50}: 30 mg/kg bw), rabbit (LD_{50}: 30 mg/kg bw) and dog (LD_{50}: 20 - 30 mg/kg bw). No further information was accessible.

Due to significant deficiencies in the description these publications and information were allocated a Klimisch score of 4 (not assignable). However, it will be used as supporting information.

4.2.1.2 Acute toxicity: inhalation

No data were available

4.2.1.3 Acute toxicity: dermal

Table 12: Summary table of relevant acute dermal toxicity studies

Method	Results	Remarks	Reference
Guinea pig Dermal, alcoholic solution of 2,4-dinitrophenol was applied to the clipped abdomen. Guinea pig, both sexes (5 animals per dose group). Following doses were applied: 100, 200, 300, 400, 500, 700 and 1000 mg/kg bw	No mortality occurred at doses of 100 and 200 mg/kg bw 20 % (1/5) mortality occurred at 300 and 400 mg/kg bw 40 % (2/5) mortality occurred at 500 mg/kg bw 100 % (5/55) mortality occurred at 700 and 1000 mg/kg bw	2 (reliable with restrictions) Test material: 2,4-dinitrophenol	Spencer et al. (1948)

Reference: Spencer et al., 1948

Study design:

Guinea pigs of both sexes (heterogenous stock from a commercial supplier) were treated with a single dose of dinitrophenol in alcoholic solution on the clipped abdomen. Each guinea pig was restrained on an animal board in such a manner that the treated area could be kept wet with ethanol during the four-hour period following the application of the test material in order to facilitate its absorption. At the end of this period, the surviving animals were removed from the boards, bandaged so as to prevent oral ingestion, caged, and observed until it was certain that they fully recovered.

Results:

A total number of 35 animals were treated dermally with dinitrophenol. No mortality occurred at 100 and 200 mg/kg bw. 20 % (1/5) mortality occurred at 300 and 400 mg/kg bw, at 500 mg/kg bw the mortality increased to 40 % (2/5). A mortality of 100 % occurred at 700 and 1000 mg/kg bw. A survival dose of 200 mg/kg bw and a lethal dose of 700 mg/kg bw were defined.

Conclusion:

It can be concluded that dinitrophenol has a steep dose-response and that the LD_{50} can be estimated for a dose between 500 and 700 mg/kg bw. The study has been performed before the establishment of guide lines and good laboratory practice. Some information is lacking such as the precise age of the animals and the ratio of male and female guinea pigs in each dose group. However, these information gaps are considered as minor important and the total conclusion is Klimisch score 2 (reliable with restrictions).

4.2.1.4 Acute toxicity: other routes

No data were available.

4.2.2 Human information

Dinitrophenol has been used historically in dyes, wool preservatives, herbicides, and explosives since the early 20th century. In the 1930s it has been also used as an agent to lose weight (Yen & Ewald, 2012). Several cases of dinitrophenol poisonings have been published in the 1930s. The evaluation of the ATSDR (1995) is 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 hyperpnea 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."

More recent reports were described in the following part.

McFee et al., (2004) reported the case of a 22-year-old man with a change in mental status 16 hours after his last dose of dinitrophenol. The patient admitted he had taken 600 mg dinitrophenol each day for the last four days. The patient was obese and showed a body temperature of 38.9 °C. The patient received mechanical cooling and i.v. treatment with midazolam. He experienced progressive bradycardia, the cardiac status deteriorated; he developed asystole and finally died. Calculating with the last of four doses of 600 mg each and assuming a body weight of 97 kg (corresponding to a body height of 1.8 m and a body-mass-index of 30), the dinitrophenol dose would be 6.2 mg/kg bw.

Suozi et al. (2005) reported the case of a 24-year-old man who collapsed during workout. Paramedics received a bottle of capsules filled with yellow powder and a page titled "DNP and weight loss". In the hospital the patients' temperature rose to 40.8 °C despite cooling measures and rigorous fluid hydration. The patient developed pulmonary edema, suffered multiple organ damage, including renal failure, went into cardia arrest and subsequently died.

Tewari et al. (2009) reported the case of 27-year-old woman with a BMI of 33, complaining of fatigue, nausea and excessive sweating. She admitted to starting a new diet tablet (bought over the internet) a week before her admission. She had doubled the recommended doses for faster results. Heart rate was 140 bpm, blood pressure 122/86 and the temperature 38 °C. The patient received saline and diazepam. Seven hours after admission she desaturated to < 90 % and became asystolic. Despite resuscitation measure the patient died.

Bartlett et al. (2010) published a case report on a 46-year-old white man who ingested 14 capsules containing 200 mg dinitrophenol each (total dose 2.8 g) with suicidal intent. Fourteen hours after ingestion he was brought to the emergency department. The patient was tachycardic (170 bpm), normotensive (140/80 mm Hg), sweaty, flushed and agitated but oriented. The body temperature was 37.8 °C. Supportive management included fluid resuscitation, active cooling measures (including paracetamol and dantrolene) and diazepam for agitation. However, the situation continued to deteriorate with worsening hypotension and the patient lost cardiac output. He died 7 h after admission and 21 h after ingestion of 2.8 g Dinitrophenol (Bartlett et al., 2010). Assuming a body weight of 70 kg, the dinitrophenol dose would be 40 mg/kg bw.

Siegmueller & Narasimhaiah (2010) published a case report of a man who ingested 14 tablets containing 200 mg dinitrophenol each (total dose 2.8 g) with suicidal intent. Twelve hours after ingestion he was brought to the emergency department. On admission the patient was conscious, complaining of dizziness, back and abdominal pain, and reporting frequent diarrhoea as well as vomiting. He was sweating profusely and appeared clinically dehydrated. His temperature was 38.4 °C, blood pressure 104/64 mm Hg and a heart rate of 150/min. The immediate management consisted of intravenous fluid resuscitation and treatment of the hyperkalaemia with calcium gluconate and an insulin/dextrose infusion. The condition of the patient deteriorated and body temperature rose to 39.5 °C. Following intubation dantrolene was administered; however, the patient suffered an asystolic cardiac arrest and died three hours after admission (Siegmueller & Narasimhaiah 2010). Assuming a body weight of 70 kg, the dinitrophenol dose would be 40 mg/kg bw.

Kamour et al. (2015) reviewed the inquiries to the National Poisons Information Service in the United Kingdom from 2007 to 2013. Five fatal poisonings were reported. For three of the five cases data on the ingested dose were presented (2800, 3200 mg and 2980 mg, respectively). All three casualties were male and assuming a body weight of 70 kg, the dinitrophenol doses would be 40, 46 and 43

mg/kg bw, respectively (see Table 13). No information was available whether the intake of dinitrophenol happened with suicidal intent.

In Germany, the Federal Institute for Risk Assessment (BfR) collects information on poisoning incidents in a poison information database. In 2015 an evaluation has been performed for fatal poisonings by dinitrophenol (BfR, 2015) (see Table 13 and Table 14). In two cases information was available on the dinitrophenol dose (4000 mg each). The casualties were male and assuming a body weight of 70 kg, the dinitrophenol dose would be 57 mg/kg b.w. (see Table 13).

Table 13: Fatal oral poisonings with information about doses applied

Reference	Dose [mg] Number of doses [N]	Body weight	Dose in mg/kg bw
ATSDR (1995)	3680 2	80 kg male patient, intake of two doses within one week	46 mg/kg bw
ATSDR (1995)	- 5	A woman took DNP for five dayss. No information was available on bw. However a dose per kg bw was presented.	7 mg/kg bw
ATSDR (1995)	- 14	A female patient received DNP for 14 days. No information was available on bw. However a dose per kg bw was presented.	2.66 mg/kg bw
ATSDR (1995)	- 42	A patient received DNP for 6 weeks. No information was available on bw. However a dose per kg bw was presented. The dose was increased from 2.9 to 4.3 mg/kg bw/day	2.9 to 4.3 mg/kg bw
ATSDR (1995)	- 46	A patient received DNP for 46 days. No information was available on bw. However a dose per kg bw was presented.	1.03 mg/kg bw
ATSDR (1995)	- 42	A patient received DNP for 6 weeks. No information was available on bw. However a dose per kg bw was presented. The dose was increased from 0.62 to 3.8 mg/kg bw/day	0.62 to 3.8 mg/kg bw
McFee et al. (2004)	600 4	The male patient was obese. Estimation of bw with 97 kg for 1.80 m height and body mass index of 30 (corresponding to obesity). The patient took DNP for four days.	600 mg / 97 kg = 6.2 mg/kg bw
Bartlett et al. (2010)	2800 mg with suicidal intent 1	Male, bw was estimated with 70 kg	2800 mg / 70 kg = 40 mg/kg bw
Siegmueller & Narasimhaiah (2010)	2800 mg with suicidal intent 1	Male, bw was estimated with 70 kg	2800 mg / 70 kg = 40 mg/kg bw
Kamour et al. (2015)	2800 mg 1	Male, bw was estimated with 70 kg	2800 mg / 70 kg = 40 mg/kg bw
Kamour et al. (2015)	2980 mg 1	Male, bw was estimated with 70 kg	2980 mg / 70 kg = 43 mg/kg bw

Reference	Dose [mg] Number of doses [N]	Body weight	Dose in mg/kg bw
Kamour et al. (2015)	3200 mg 1	Male, bw was estimated with 70 kg	3200 mg / 70 kg = 46 mg/kg bw
BfR (2015)	4000 mg 1	Male, bw was estimated with 70 kg	4000 mg / 70 kg = 57 mg/kg bw
BfR (2015)	4000 mg with suicidal intent 1	Male, bw was estimated with 70 kg	4000 mg / 70 kg = 57 mg/kg bw

Additionally, fatal poisonings without information on the dinitrophenol doses were compiled in Table 14 to demonstrate the multitude of fatalities.

Table 14: Fatal oral poisonings without information about doses applied

Reference	Basic information	More information
ATSDR (1995)	female, intake over several month	No further information
Suozzi et al. (2005)	male	Member of a health club
Miranda et al. (2006)	female	DNP was used for weight-loss
Miranda et al. (2006)	male	Use of bodybuilding supplements
Tewari et al. (2009)	female, BMI of 33	DNP was used to support a diet
Grundlingh et al. (2011)	female	Suicidal intent
Grundlingh et al. (2011)	male	Use of bodybuilding supplements
BfR, (2015)	female	No further information
Kamour et al. 2015	male	No further information
Kamour et al. 2015	female	No further information

Jiukun et al. (2010) reported two fatal cases of dinitrophenol poisoning caused by non-oral exposure. A 49-year-old woman and 41-year-old man were poisoned when they were recycling useless nylon bags that were used as packages of dinitrophenol. They wore no protective clothing and only wore ordinary masks after seeing a lot yellow powder. According to statements given by the victims and witnesses, oral exposure can be excluded. The skin of hands, feet, cnemis and forearms were heavily stained by dinitrophenol.

4.2.3 Summary and discussion of acute toxicity

There is long history of oral poisonings by dinitrophenol in humans. The ATSDR (1995) compiled several cases from the 1930s with doses in the range of less than 1 mg dinitrophenol/kg bw and 7 mg/kg bw leading to a fatal outcome. During recent years new case reports have been published with estimated doses of 6.2 mg/kg bw in one case and seven cases in a dose range of 40 to 57 mg/kg bw. It should be noted that three of the seven high-dose cases rely on suicidal intent. Furthermore, ten additional fatal poisonings (Table 14) demonstrate the serious problem in the acute toxicity of dinitrophenol.

Only very limited information is available on non-oral poisonings. However, the case report from China clearly shows that a dermal exposure towards dinitrophenol can lead to a fatal outcome.

For acute oral toxicity in animals, only one study can be considered for the evaluation. The rat study by Spencer et al. (1948) came close to fulfil the OECD TG requirements, although both sexes have been used in the study. All other reports indicated the LD_{50} values and the test species, but showed serious deficiencies, mostly all experimental details were lacking. The Spencer study yielded a range from 30 to 40 mg/kg bw for the LD_{50} value. Three reports referenced to a LD_{50} of 30 mg/kg bw in rats. It is not possible to investigate, if these values rely on the same study. Another LD_{50} value in rats was calculated with 71 mg/kg bw. Finally, a study from Dow Chemicals was cited in the ATSDR with a LD_0 of 20 mg/kg bw and a LD_{100} of 60 mg/kg bw, therefor the LD_{50} is somewhere between 20 and 60 mg/kg bw. Despite the deficiencies of most of the studies and reports presented here, the range of LD_{50} in rats is in the range between 30 and 71 mg/kg bw.

Additional information on oral toxicity in other animals resulted in two studies in mice with LD_{50} of 45 and 72 mg/kg bw, two study in rabbits with 30 mg/kg bw, two studies in dogs with a LD_{50} of 20 – 30 mg/kg bw and a LD_{Lo} of 30 mg/kg bw, one study in cats with a LD_{50} of 75 mg/kg bw and one study in Guinea pigs with 81 mg/kg bw. Since all studies have serious deficiencies they can only be used to confirm the range of acute oral toxicity of dinitrophenol (30 to 81 mg/kg bw).

Finally, only one acute dermal toxicity study was available (Spencer et al., 1948), showing a LD_{50} in the range of 500 to 700 mg/kg bw. As in the oral toxicity study, the use of both sexes was the major deficiency in the dermal toxicity study.

4.2.4 Comparison with criteria

oral

Spencer et al. (1948) is selected as key study. This study gave a range for acute oral LD_{50} in the rat from 30 to 40 mg/kg bw. An acute oral LD_{50} of 30 to 40 mg/kg bw fulfils the requirement for classification in Category 2 (LD_{50} ranging between 5 and 50 mg/kg bw). For the calculation of the ATE the average of the doses below and above the LD_{50} have been used and yielded a value of 35 mg/kg bw.

Furthermore, the human evidence will be considered: Twelve of 14 fatal poisonings with information on the dose taken showed doses < 50 mg/kg bw. Six of the 14 fatal poisonings presented with doses < 10 mg/kg bw. However, these victims repeatedly ingested doses of DNP as presented in Table 13. Ten additional fatal poisonings without detailed information on the intake highlight the relevance of the acute toxicity of DNP for humans.

dermal

Spencer et al. (1948) is selected as key study. This study gave a range for acute dermal LD_{50} in Guinea pigs from 500 to 700 mg/kg bw. An acute dermal LD_{50} of 500 to 700 mg fulfils the requirement for classification in Category 3 (LD_{50} ranging between 200 and 1000 mg/kg bw). For the calculation of the ATE the average of the doses below and above the LD_{50} have been used and yielded a value of 600 mg/kg bw.

4.2.5 Conclusions on classification and labelling

According to Regulation (EC) No 1272/2008 on Classification, Labelling and Packaging, dinitrophenol should be classified as Acute Tox. 2, H300, thereby replacing the current classification of Acute Tox. 3, H301. It is proposed to assign an ATE of 35 mg/kg bw for acute oral toxicity.

The available data do not warrant a change in the current classification for acute dermal toxicity (Acute Tox. 3, H311). It is proposed to assign an ATE of 600 mg/kg bw for acute dermal toxicity.

No data have been obtained for acute inhalation toxicity. Therefor no change is proposed in the current classification for acute inhalative toxicity (Acute Tox. 3, H331).

4.7 Repeated dose toxicity

Table 15: Summary table of relevant repeated dose toxicity studies

Method	Results	Remarks	Reference
Subacute oral toxicity testing on rats (according to test guideline of the Japanese Chemical Control Act). Test procedure is similar to OECD TG 407. Sprague-Dawley SPF rats [Crj:DC(SD)IGS] were treated orally by gastric intubation once a day for 28 days. Doses were 0, 3, 10, 30, 80 mg dinitrophenol/kg bw/day. Six animals per dose and sex were treated except for the 30 and 80 mg/kg b.w./day groups, where 12 animals per sex were used for a recovery protocol. 2,4-dinitrophenol (purity 85.2 %) was suspended in 1 w/v% methylcellulose solution.	In the highest dose group 6 of 12 females treated died and 2 of 12 males treated died. All animals in high dose group showed repeatedly symptoms of toxicity such as decrease in locomotor activity, prone position, ptosis, panting, crawling position and salivation.	1 (reliable without restrictions) Test material: 2,4-dinitrophenol	Koizumi et al. (2001)

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

A subacute oral toxicity study was performed on young Sprague-Dawley rats, aged 5 to 6 weeks (Koizumi et al., 2001). 2,4-dinitrophenol (purity 85.2 %) was suspended in a methycellulose solution and administered per gavage. Rats were treated with doses of 0, 3, 10, 30, 80 mg/kg bw/day for 28 days. Recovery groups (0, 30, 80 mg/kg bw/day) were maintained for 2 weeks without treatment and fully examined at 11 to 12 weeks of age. The number of animals for each sex/dose was 6 for both scheduled-sacrifice and recovery. Rats were examined for general behaviour, body weight, food consumption, urinalysis, hematology and blood biochemistry, necropsy finding, organ weights and histopathology finding in compliance the Test guideline of the Japanese Chemical Control Act (Koizumi et al., 2001).

There was no mortality in the control group and in the lower dose groups (3, 10, 30 mg/kg bw/day). Symptoms of toxicity such as decrease in locomotor activity and salivation were observed in the 30 mg/kg bw/day-group mostly after the first dosing only. No changes in histopathology or organ weights were observed at 30 mg/kg bw/day or lower (Koizumi et al., 2001).

In the highest dose group 6 of 12 females and 2 of 12 males died. All animals in the high dose group showed repeatedly symptoms of toxicity such as decrease in locomotor activity, prone position, ptosis, panting, crawling position, salivation. Tonic convulsion was observed in 2 of 12 males and 4 of 12 females. Rigidity was observed in 2 of 12 males and 5 of 12 females. The relative liver weights

were increased in both sexes of the scheduled-sacrifice group and this persisted through the recovery period. Relative organ weights for brain, kidneys and testes were increased only in males. On histo-pathological examination, mineralization of the corticomedullary junction in kidneys was observed in both sexes in the scheduled-sacrifice and recovery group, but the change was only statistically significant in males of the scheduled-sacrifice group. On haematological examination, increase in haemoglobin and haematocrit during the treatment, and decrease in red blood cell count, haemoglobin and haematocrit in the recovery group were observed, limited to males. (Koizumi et al., 2001).

It can be concluded that dinitrophenol shows a clear toxicity at 80 mg/kg bw/day with a mortality of 6 of 12 females and 2 of 12 males. In comparison to this clear toxicity the other effects on organ weight and on histopathology are irrelevant. The study has been performed according to the Japanese guideline and seems to be equivalent to the OECD TG 407. The study has been performed under GLP conditions and therefor the total conclusion is Klimisch score 1 (reliable without restrictions).

4.7.1.2 Repeated dose toxicity: inhalation

There is no information available.

4.7.1.3 Repeated dose toxicity: dermal

There is no information available.

4.7.1.4 Repeated dose toxicity: other routes

There is no information available.

4.7.1.5 Human information

There are several very old studies from the 1930s which are summarized in the ATSDR (1995).

4.7.1.6 Other relevant information

4.7.1.7 Summary and discussion of repeated dose toxicity

Dinitrophenol clearly shows toxicity in a repeated dose toxicity study on rats. In a 28-day oral toxicity study 6 of 12 female rats and 2 of 12 male died during treatment with 80 mg/kg bw/day. The study seems to be reliable without restrictions.

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.

<u>Human information</u>

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) that commented in PC supported the proposed classifications for acute oral and dermal toxicity. One of these MS did agree 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 sett an ATE because they involved repeated exposure or extreme dosing for a suicidal purpose, 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 underestimatedbut 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 a Acute Tox. 3* (H331)**.

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

For details see section 4.7.

4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

There is long history of oral poisonings by dinitrophenol in humans. The ATSDR (1995) compiled several studies from the 1930s with different doses. However, these studies are not necessary to confirm the toxicological profile of dinitrophenol.

The key study on the repeated dose toxicity of dinitrophenol in animals is a guideline study performed in rats. In a 28-day oral toxicity study 6 of 12 female rats and 2 of 12 male died during treatment with 80 mg/kg b.w./day. The study seems to be reliable without restrictions.

4.8.2 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

Kouizumi et al. (2001) was selected as key study. In a 28-day oral toxicity study on rats a dose of 80 mg/kg bw/day elicited a mortality of 6 out of 12 females and 2 out of 12 males. Mortality is the most severe endpoint in toxicity. The value of 80 mg/kg bw/day corresponds to the guidance values for category STOT RE2 (30 < C =< 300, for 28-day studies). It is also evident, that the effects are not sufficient to classify into STOT RE1 (C = < 30).

4.8.3 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

According to Regulation (EC) 1272/2008 on Classification, Labelling and Packaging, the classification of dinitrophenol as STOT RE2, H373 should be maintained.

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	0, 3, 10, 30, 80 mg/kg bw/day (Recovery group: 0, 30, 80 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**	Koizumi *et al.* (2001)

(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% methylcellulose solution	Guidance value for classification Category 1: **≤**30 mg/kg bw/day Category 2: **≤300** mg/kg bw/day	Observation: ↓ locomotor activity, prone position, ptosis, panting, crawling position and salivation repeatedly in all animals, - 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 Effects before death: change in mental status 16 hrs after last dose, increased body temp. (38.9°C), bradycardia, deteriorated cardiac status, asystol developed	McFee *et al,* 2004
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	Dameshek and Gargill 1934

			2,4-DNP Death – agranulocytosis	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.

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