Committee for Risk Assessment

RAC

Annex

Records of the targeted consultation following the submission of new information on acute inhalation toxicity of 2-butoxyethanol; ethylene glycol monobutyl ether (EGBE)

EC Number: 203-905-0
CAS Number: 111-76-2

A77-O-0000006933-67-01/F

Adopted

10 December 2020

ANNEX - RECORDS OF THE TARGETED CONSULTATION FOLLOWING THE SUBMISSION OF NEW INFORMATION ON ACUTE INHALATION TOXICITY OF 2-BUTOXYETHANOL; ETHYLENE GLYCOL MONOBUTYL ETHER (EGBE)

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

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

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

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Substance name: 2-butoxyethanol; ethylene glycol monobutyl ether
EC number: 203-905-0
CAS number: 111-76-2

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
03.09.2020	Belgium	Oxygenated Solvents Producer Association	Industry or trade association	1
Comment received				
For details, we refer to the attached document. ECHA note – An attachment was submitted with the comment above. Refer to public attachment Comments from OSPA on the consultation regarding the harmonised classification (final).pdf				
RAC's response				
RAC disagrees with the conclusion for no classification of 2-butoxyethanol (EGBE) for acute inhalation toxicity as well as with the justification provided for this by the Industry or trade association. There are no reasons to completely disregard data on acute inhalation toxicity of 2-butoxyethanol (EGBE) gathered in acute inhalation toxicity studies on rats, mice and rabbits. There is some uncertainty regarding reliability of the newly submitted study in Guinea pigs. For details see the revised RAC opinion prepared in response to the European Commission mandate.				

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
07.09.2020	Germany		MemberState	2
Comment received				
The newly submitted information on the acute inhalation toxicity of 2-butoxyethanol (EGBE) does not contradict the assessment of EGBE carried out by RAC, which is still supported. The DE-CA already commented on the present study from 2019 in December 2019 as a				

follow up to the 32nd CARACAL meeting and came to the conclusion that, based on this study, no new assessment by RAC would be necessary. You may find our statement from the 32nd CARACAL repeated below.

In the study by Dow (1974) it was shown again that guinea pigs and dogs are significantly less sensitive to a single exposure to EGBE than rabbits. This study had already been taken into account in the original assessment by the RAC in 2018 (see section "Comments received during public consultation" in the RAC oppinion). The results of the study therefore do not contradict a classification of EGBE as Acute Tox. 3, H331, since in the overall consideration of all available studies a classification as acutely toxic after inhalation in category 3 is justified.

The study by Bushy Run (1994) had also been available to RAC in 2018 and had already been included in the assessment (In the RAC opinion or CLH dossier referred to as "Gingell et al. 1998"). The results of this study do not contradict the conclusion of RAC. Taken together, the three studies presented do not contradict the conclusion of RAC of 2018, which is why its proposal to classify EGBE as Acute Tox. 3 (H331) is still supported.

DE-CA Comment on CA/90/2019 and CA/08/2019 from 3rd December 2019:
In 2018, RAC concluded on the harmonised classification of 2-butoxyethanol with regard to acute inhalation toxicity:

"The LC 50 values of 2-buthoxyethanol in several acute inhalation toxicity studies in rats were in the range of 2.21 - 4.92 mg/L/4 h, in 1 study in mice 4.12 mg/L and in one study in Guinea pigs 7.65 mg/L/4 h; thus, they were all within the classification criteria of 2-10 mg/L for Acute Tox. 3. It is noted that due to low volatility and low vapour pressure 2 butoxyethanol the Guinea pigs could have been exposed not to pure vapour but to a mixture of vapour and mist of 2-butoxyethanol, since the saturated vapour concentration at 20 °C is 4.4 mg/L. Hence, the data on Guinea pigs alone are borderline between classification and no classification for acute inhalation toxicity. However, due to this situation RAC took into account all available studies in rats, mice and Guinea pigs, and is of the opinion that 2-butoxyethanol warrants classification as Acute Tox. 3; H331 (Toxic if inhaled), with an ATE of 3.0 mg/L (Table 3.1.2 of Regulation (EC) No 1272/2008)."

Recently, a new acute inhalation toxicity study in Dunkin Hartley Guinea pigs was provided by the industry. Six males and six females were exposed to butoxyethanol (snout-only) once for 4 h at a concentration of 2.25 mg/L (75 % of the target concentration [3.0 mg/L]). This concentration was claimed to be the highest achievable concentration for pure vapour. No mortality was reported at this concentration. One male had to be sacrificed during the study period due to welfare reasons, but this incident was considered not treatment-related by the study author. No test-item related effects on haematology and urinalysis parameters were reported. The study author concluded that the LC-50 was >2.25 mg/L/4 h.

It is noted that this result is in line with the previous results taken into account by RAC in their opinion document (2018). Moreover, we agree with ECHAs response statement to the written comments to paper CA/08/2018 (received after CARACAL-30), indicating that the results of the new study in Guinea pigs do not allow to derive a category or ATE value.

As stated above, in its opinion of 2018 RAC was well aware that the LC-50 concentration for Guinea pigs in the existing key study might not have consisted of pure vapour, as the highest tested concentration was well above the saturated vapour concentration of

butoxyethanol at 20 °C of 4.4 mg/L (test concentration: 7.65 mg/L/4 h). RAC was also aware that the older data on Guinea pigs alone are, thus, borderline between classification and no classification for acute inhalation toxicity. Although the saturated vapour concentration of butoxyethanol is in fact higher, the newly presented study claims that the tested concentration of 2.25 mg/L/4 h was the highest achievable concentration for pure vapour and no aerosol droplets were observed during the exposure. On the contrary, in one of the older acute inhalation toxicity study with butoxyethanol in Guinea pigs the test atmosphere was also checked to ensure the absence of aerosol particles and concentrations of up to 691 ppm (3.4 mg/L) were tested. This indicates that the testing of higher pure vapour concentrations than 2.25 mg/L might be very well feasible.

RAC further noted in 2018 that there are considerable interspecies differences in sensitivity to this toxic action between animal species and humans. As reported in the EU RAR (2006), Guinea pigs and humans are relatively resistant, rodents are very sensitive (rats are 30-times more sensitive than humans), while rabbits are less sensitive than rodents, but more sensitive than humans and Guinea pigs. It is noted that the newly presented data do not provide new insights regarding differences in species sensitivity towards toxicity of butoxyethanol.

In its evaluation, RAC considered all available inhalation toxicity data in weight of evidence (eight studies in rats, mice and Guinea pigs) and further considered the specific situation with respect to species differences in sensitivity and potential limitations regarding butoxyethanol vapour generation. Overall, RAC concluded that 2-butoxyethanol warrants classification as Acute Tox. 3; H331 (Toxic if inhaled), with an ATE of 3.0 mg/L". This conclusion is considered appropriate based on the available data, including the new acute inhalation toxicity study.

RAC used the default ATE of 3 mg/L from Table 3.1.2 of the CLP Regulation, which refers to vapours. It is noted that this ATE value might be rather conservative; however, due to the lack of more specific and consistent data, the implementation of the default value is considered appropriate.

As the new study results do not contradict the conclusion made by RAC and further do not allow for setting a different ATE value as the one currently in place, it is considered – in line with ECHAs conclusion – that "RAC would not be in a position to reclassify the substance" based on the new data provided by the industry.

Rather, conducted an additional acute inhalation toxicity study is considered inappropriate in light of animal welfare and the 3R strategy to have, since a large data set on this endpoint has already been available.

RAC's response

The thourough and extensive analysis of data is noted and the position taken by the MS Germany, including the justification provided, is fully supported by RAC. It is reflected in the revised opinion of 2-butoxyethanol in response to European Commission mandate.

Date	Country	Organisation	Type of Organisation	Comment number
03.09.2020	Belgium	Oxygenated Solvents Producer Association	Industry or trade association	3

Comment received

For details, we refer to the attached document.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Comments from OSPA on the consultation regarding the harmonised classification (final).pdf
RAC's response
See response to comment no. 1

Date	Country	Organisation	Type of Organisation	Comment number
07.09.2020	France		MemberState	4
Comment received				
Even if the recent study provided is of good quality, FR is of the opinion to stick to the conclusions of the CLH report for acute inhalation toxicity (Acute Tox. 3 H331: Toxic if inhaled), based on the lowest ATE value available i.e. the lowest ATE in the most sensitive appropriate species tested, as recommended in the guidance. The similarity of sensitivity between human and specifically guinea pigs regarding haemolytic effects to justify the choice of the species is not substantiated. Moreover, a high interindividual variation in human regarding toxicokinetics has to be taken into account.				
RAC's response				
RAC fully agrees with the position taken by the MS France. Thank you for pointing out the high interindividual variation in humans regarding toxicokinetics.				

PUBLIC ATTACHMENTS
1. Comments from OSPA on the consultation regarding the harmonised classification (final).pdf [Please refer to comment No. 1, 3]

Comments from the Oxygenated Solvents Producers Association on the consultation regarding the harmonised classification and labelling of 2-butoxyethanol (EC 203-905-0, CAS 111-76-2)

End point: Acute toxicity by the inhalation route

Industry welcomes the opportunity to submit comments in response to the public consultation on the mandate to the ECHA RAC to review the opinion of 14/9/18 in relation to the classification for acute inhalation toxicity of 2-butoxyethanol. Industry supports the review of the decision in the light of new data and existing data that may not have been fully taken into account and the context of a substance that is of relatively low volatility. These comments have been structured in a similar format to an annex VI CLH report to provide detail in support of answers to the questions raised in the mandate.

1 HISTORY AND BACKGROUND OF THE PREVIOUS CLASSIFICATION AND LABELLING

In August 2017 a CLH dossier was submitted by the Federal Institute for Occupational Safety and Health (BAuA – Germany) with a proposal for the classification of 2-butoxyethanol to be amended. Classification proposals were made for the skin and eye irritation end points, for repeat dose toxicity (STOT) and for acute toxicity by all routes of exposure. The Risk Assessment Committee (RAC) considered the CLH dossier along with comments received during the public consultation and the critique of these by the appointed rapporteur (ECHA, 2018a). They concluded that the existing classifications for skin and eye irritancy should be retained unchanged and rejected the proposal to classify for STOT effects. For acute toxicity, based on the data presented and discussed, the RAC concluded the following:

- Oral route: Existing category 4 classification retained with the assignment of an ATE based on guinea pig data and therefore higher than the default
- Dermal route: Not classified (deletion of existing classification based on guinea pig data)
- Inhalation route. Category 3 classification in the absence of reliable guinea pig data and therefore based on rat data

It has been widely established that the key toxic effect of 2-butoxyethanol (or more specifically its main metabolite, 2-butoxyacetic acid) is that it rapidly causes haemolysis following acute exposure. More specifically, this effect varies significantly between species: rats, mice and rabbits are notably sensitive to the effect whereas humans and guinea pigs are very resistant. This species difference in sensitivity has been widely acknowledged by regulatory authorities, including in the EU as documented in the risk assessment for 2-butoxyethanol (EU, 2006), and taken into account in determining the extrapolation of hazard data from animal studies to humans. It was also taken into account by RAC in that they concluded it was preferable to use guinea pig data to determine the appropriate hazard classification for acute toxicity. As recorded in the minutes and opinion (ECHA, 2018a, 2018b), reliable guinea pig data was used to determine the ATEs and classification for the oral and the non-classification for the dermal route.

The meeting minutes of the RAC indicate that only two guinea pig studies were considered for the inhalation route of exposure. One of these references documented a LC50(7hr) of 6.4mg/L. However, if true, this would be well above the saturated vapour concentration. Because exposure conditions were unclear (the original (1943) study is no longer available), this study

was considered unreliable for further consideration. The one other study in guinea pigs was reliable and showed no adverse effects at the maximum achievable vapour concentration (~3mg/L) but only used an exposure of 1 hour. RAC noted that although it is preferred to use the same species for all routes when allocating ATE values (and therefore classification categories), due to these two guinea pig studies being considered unreliable, it was decided to revert to the rat data and use an ATE value of 3 mg/L and hence derive a classification of category 3.

2 PHYSICOCHEMICAL PROPERTIES

In order to interpret the available data on inhalation toxicity and put it into context, it is necessary to understand the volatility of the substance. The vapour pressure of 2-butoxyethanol is shown in the table below:

Property	Value	Reference
Vapour pressure	80 Pa at 20° C	IUCLID dossier

Like all substances, 2-butoxyethanol is subject to the laws of thermodynamics and to compliance with the gas law, PV = nRT , where P is the pressure, V is the volume, n is the number of moles, T is the temperature in degrees Kelvin and R is the universal gas constant. Moles is a measure of the amount of a substance. The universal gas constant is 0.0821 atm.liter.K-1.mol-1. The saturated vapour pressure can be derived from the vapour pressure at a given temperature. The vapour pressure of 2-butoxyethanol at 20° C (293K) is 80Pa or 80/101325 = 0.00079atm (where 101325Pa is 1 atmosphere), therefore the saturated vapour concentration in moles/litre at 293K= 0.00079 / (0.0821*293) = 0.000033. By multiplying by the molecular weight in mg/mol (118000) this comes to 3.9 mg/L. This is the theoretical maximum concentration of vapour that can be reached at 20° C. Any reported exposure concentration above this must also involve exposure to 2-butoxyethanol in aerosol form. Note that under dynamic testing conditions, it is not possible to attain this – see later section 5.1.1. on the CLH criteria.

3 ACUTE TOXICITY DATA BY THE INHALATION ROUTE

Given the context that the RAC acknowledged that the guinea pig appears to have a sensitivity to the leading toxic effect (haemolysis mediated through the metabolite butoxy acetic acid) which is similar to that of humans, and therefore used guinea pig data to assess the acute toxicity hazard by the oral and dermal routes,studies on rats and mice are not included. Only the available guinea pig data needs to be considered to determine if it is now sufficiently reliable to base a decision on. The limited data available for the dog is also included, as this species is not sensitive to haemolysis caused by exposure to the metabolite 2-butoxyacetic acid and should help to confirm if a haemolysis resistant species is appropriate to use to assess acute toxicity in humans.

Method, guideline, deviations if any Reliability	**Species, strain, sex, no/group/ exposure**	**Test substance, form and particle size (MMAD)**	**Dose levels, duration of exposure**	**Result**	**Reference (* shows studies where full reports included in public consultation)**
LC_{50}-Test, according to OECD TG 433. Additional haematology and urine collection Klimisch 1 (GLP)	**Guinea pig,** Dunkin Hartley strain (42-56 days of age; ~400-530 g), 6/sex	2-butoxyethanol (CAS: 111-76-2), purity 99.6 %, vapour	Target 3 mg/L. Measured 2.25 mg/L (maximum attainable stable vapour only concentration) for 4 h, nose only; 14 day observation	One male sacrificed for welfare reasons on day 5. Animal showing adverse clinical signs and body weight loss. Group mean weight loss on day 2 but recovered by day 4. Effects seen in this sacrificed male not considered related to substance exposure. No other animal showed adverse effects. No adverse change to haematology or urine parameters measured. LC50>2.25 mg/L (maximum attainable under study conditions	Covance (2019)*
LC_{50}-Test, according to CFR title 49, section 173.132; similar to OECD TG 403; deviation in exposure time, only 1h was used	**Guinea pig,** Hartley strain (5 wk of age; 400-500 g), 5/sex	2-butoxyethanol (CAS: 111-76-2), purity 99.87 %, vapour	633±14.2 ppm (males) and 691±37.6 ppm (females) for 1h, whole body; 14 day observation	No mortalities, No clinical signs, no adverse body weight changes. LC0>= 633 ppm (males; 3.11 mg/L) LC0>= 691 ppm (females; 3.4 mg/L)	Gingell et al. (1998) Dow Chemical Company (1994)*

Method, guideline, deviations if any Reliability	**Species, strain, sex, no/group/ exposure**	**Test substance, form and particle size (MMAD)**	**Dose levels, duration of exposure**	**Result**	**Reference (* shows studies where full reports included in public consultation)**
Klimisch 1 (GLP)					
LC_{50}-Test, no guideline followed Klimisch 2 (not GLP). Second exposure and audible startle response measurements included	**Guinea pig,** strain unspecified, 8M	2-butoxyethanol (CAS: 111-76-2), described as purified. Source Shell UK	411ppm ±28 for 7 h, whole body; total 14 day observation	No mortalities, No clinical signs, no adverse body weight changes. LC0>= 411 ppm (7 hrs) (corresponding to 495 ppm/4h = 2.43 mg/L/4h)*	Dow Chemical Company (1974)*
LC_{50}-Test, no guideline followed Klimisch 2 (not GLP). Second exposure and audible startle response measurements included	**Guinea pig,** strain unspecified, 8M	2-butoxyethanol (CAS: 111-76-2), described as purified. Source Shell US	409 ppm ±28 for 7 h, whole body; total 14 day observation	No mortalities, No clinical signs, no adverse body weight changes. LC0>= 409 ppm (7 hrs) (corresponding to 492 ppm/4h = 2.42 mg/L/4h)*	Dow Chemical Company (1974)*
LC_{50}-Test, no guideline followed Klimisch 2 (not GLP). Second exposure and audible startle response measurements included	**Guinea pig,** strain unspecified, 8M	2-butoxyethanol (CAS: 111-76-2), described as purified. Source Dow US	408ppm ±6 for 7 h, whole body; total 14 day observation	No mortalities, No clinical signs, no adverse body weight changes. LC0>= 408 ppm (7 hrs) (corresponding to 491 ppm/4 h = 2.41mg/L/4 h	Dow Chemical Company (1974)*
LT_{50}-Test, no guideline followed Klimisch 4	**Guinea pig,** strain unspecified, adult	2-butoxyethanol (CAS: 111-76-2), "Substantially saturated vapour" Recirculating exposure system	1300 ppm for 7 h, whole body exposure, 14 d post exposure period No further information	1 death at 4 hours LC50=1300 ppm, 7 h (corresponding to 1565 ppm/4h = 7.65 mg/L/4h) *	Mellon Institute of Industrial Research (1943) cited in Tyler (1984) and Mellon Institute of Industrial Research (1952)
LC_{50}-Test, no guideline followed	**Dog,** Beagle strain: unspecified, 2M	2-butoxyethanol (CAS: 111-76-2), described as	411 ppm ±28 for 7 h, whole body; total 14 day observation	No mortalities, No clinical signs, no adverse body weight changes.	Dow Chemical Company (1974)*

Method, guideline, deviations if any Reliability	Species, strain, sex, no/group/ exposure	Test substance, form and particle size (MMAD)	Dose levels, duration of exposure	Result	Reference (* shows studies where full reports included in public consultation)
Klimisch 2 (not GLP). Second exposure added		purified. Source Shell UK		LC0>= 411 ppm (7 hrs) (corresponding to 495 ppm/4 h = 2.43 mg/L/4 h)*	
LC_{50}-Test, no guideline followed Klimisch 2 (not GLP). Second exposure added	**Dog,** Beagle strain: unspecified, 2M	2-butoxyethanol (CAS: 111-76-2), described as purified. Source Shell US	409 ppm ±28 for 7 h, whole body; total 14 day observation	No mortalities, No clinical signs, no adverse body weight changes. LC0>= 409 ppm (7 hrs) (corresponding to 492 ppm/4 h = 2.42 mg/L/4 h)*	Dow Chemical Company (1974)*
LC_{50}-Test, no guideline followed Klimisch 2 (not GLP). Second exposure added	**Dog,** Beagle strain: unspecified, 2M	2-butoxyethanol (CAS: 111-76-2), described as purified. Source Dow US	408 ppm ±6 for 7 h, whole body; total 14 day observation	No mortalities, No clinical signs, no adverse body weight changes. LC0>= 408 ppm (7 hrs) (corresponding to 491 ppm/4 h = 2.41 mg/L/4 h	Dow Chemical Company (1974)*

* For direct comparison with the classification criteria, LC50 values need to be adjusted to a 4-hour equivalent using Haber's law ($C^n*t=k$). The value of n, which is specific to individual substances, should be chosen using expert judgement. If an appropriate value of n is not available in the literature, the Guidance on IR/CSA, Section R.7.4.4.1 recommends to set n = 3 for extrapolation to shorter duration and to set n = 1 for extrapolation to longer duration. Such a factor is used here to extrapolate the 7hr figure to a 4hr predicted figure.

4 SUMMARY OF HUMAN DATA ON ACUTE INHALATION TOXICITY

There is limited data available on human exposure to 2-butoxyethanol by the inhalation route. This is included as supportive of the conclusion of the guinea pig animal data.

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Volunteer study	2-butoxy-ethanol (CAS: 111-76-2), purity	Exp. 1: Exposure of 2 men to 113 ppm (0.55 mg/L) for 4 h, and one year later exposure of the	Clinical signs: Irritation to the eyes (probably due to direct contact with the vapours), nose and throat, a disturbance of taste, a slight	Carpenter et al. (1956)

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
	commercial grade, vapour	same 2 men and one woman to 195 ppm (0.95 mg/L) for two 4 h periods separated by a 30-min interval Exp. 2: Exposure of 2 men and 2 woman to 98 ppm (0.48 mg/L) for 8h	increase in nasal mucous discharge and headache; women appeared to be more sensitive to the induction of these effects than the men No evidence of changes from pre-exposure values in erythrocyte fragility, blood pressure, pulse rate or urinary levels of glucose or albumin; urinary excretion of BAA (100-200 mg) with the next 24h with considerable individual variation Haematology: No adverse effects seen at either exposure concentration.	
Determination of pharmacokinetic data	2-butoxyethanol (CAS: 111-76-2), purity commercial grade, vapour	Exposure of 4 male volunteers to 50 ppm (0.24 mg/L) for 2 h in an open-system exposure chamber	50 ppm: No consistent effects on the lungs (ventilation or breathing rate) or the heart (electrocardiogram readings or heart rate)	Johanson (1986)
Determination of the respiratory uptake	2-butoxyethanol (CAS: 111-76-2), purity commercial grade, vapour	Exposure of 7 male volunteers (age range 23-36, bw 75-80 kg, body length 178-187 cm) to 50 ppm (0.24 mg/L) for 2h vapour inhalation (through the mouth alone)	50 ppm: No overt signs of toxicity	Johanson and Boman (1991)

5 SUMMARY AND OVERALL RELEVANCE OF THE PROVIDED INFORMATION ON ACUTE INHALATION TOXICITY

Animal data

According to the previous discussions at RAC (ECHA, 2018a) the guinea pig is the preferred species to determine the appropriate hazard classification for acute toxicity. This is because, similar to humans, the guinea pig is resistant to the haemolytic properties of 2-butoxyethanol in contrast to rats and mice that are particularly sensitive to this effect. Data from rats and mice will therefore overestimate the potential toxic effects to humans. Guinea pigs are the most appropriate species to model the toxicity to humans. For this reason, RAC used reliable guinea pig data to conclude on the acute toxicity hazard classification by the oral and the non-classification for the dermal route, but concluded there was guinea pig data with insufficient reliability by the inhalation route. This is no longer the case and arguably was not at the time if all the available data had been considered. There is now sufficient reliable data available from guinea pigs to assess the acute toxicity hazard classification.

A recent GLP guideline study is available in guinea pigs and this showed no test substance related deaths from exposures to the maximum practical vapour concentration achievable in nose only exposure system for a 4-hour exposure of 2.25 mg/L. There were no adverse effects noted beyond a transient pause in body weight gain. There were no adverse changes to the haematology or urine parameters measured. As a point of note, the target concentration was 3 mg/L but only 2.25 mg/L (measured) was achievable without the detection of aerosol particulate in the atmosphere. (Note that other studies at higher concentrations do not indicate if aerosol was measured).

Another GLP study in guinea pigs showed no deaths for exposures at the maximum practical vapour concentration achievable in a whole body exposure system for a 1 hour exposure of 633 ± 14.2 ppm in males and 691 ± 37.6 ppm in females (around 3.2 mg/L). This exposure time was only for one hour and extrapolation using the Haber equation would be equivalent to a 4 h exposure of around 0.8 mg/L.

In another reliable study, guinea pigs were exposed for 7 hours to a nominal vapour concentration of 2 mg/L. No mortalities, no adverse clinical signs and no adverse body weight changes were observed. The study was performed in triplicate using 2-butoxyethanol samples sourced from three different commercial suppliers. The same results were obtained in each case. These results can be extrapolated using the Haber equation to a 4 h LC0 value of around 2.4 mg/L. In these studies, half the animals were exposed again after 7 days along with four fresh animals to the same nominal concentration for a further 7 hours. Again, no adverse effects were reported. (After a further 7 days, the original 4 animals plus four fresh animals were exposed again for a further additional 7 hours per day for five consecutive days. Again, no adverse effects were noted. At the end of each phase, the four animals that were not further used were subject to gross pathology with no adverse changes noted.)

The protocol used with the guinea pig study reported in the previous paragraph was also used to expose pairs of Beagle dogs. In all three replicate studies, the only notable observation was salivation during the 7-hour exposure to a nominal concentration of 400 ppm (2mg/L) with no other adverse observations.

One old study with guinea pigs derived a LT50 value (time for 50% mortality) of 7 hours exposure at a reported exposure of 1300 ppm, with 25 % mortality at 4 hours. This can be interpreted as a LC50 of 1300 ppm (extrapolated to a 4-hour value of 1566 ppm (= 7.65 mg/L) using the Haber equation). However, it should be noted that the reported exposure concentration of 1300ppm (6.4 mg/L) is well above the saturated vapour concentration and, if correct, must therefore have exposed the animals to aerosol as well. The only available data on this study is brief information from secondary sources – the original study is no longer available. This study cannot therefore be regarded as reliable to use for a decision on classification, which is a conclusion that was also reached by the RAC.

Human data

Acute human toxicity data were reported from volunteers for determination of toxicokinetic data. The symptoms reported by the volunteers were signs of irritation (throat and ocular) and headache. These symptoms did not seem to be dose related. No overt signs of systemic toxicity were noted after exposure twice (30 minutes apart) at 195 ppm (0.95 mg/L) for 4 hours, including no adverse haematology.

5.1.1 Comparison with the CLP criteria

Acute inhalation toxicity means those adverse effects occurring following an exposure by inhalation over 4 hours to a single concentration of a substance or a mixture. Acute toxicity

relates to effects occurring after a single or relative brief exposure to a substance or mixture. Acute toxicity classification is generally assigned based on evident lethality. The evidence for acute inhalation toxicity of 2-butoxyethanol is primarily obtained from animal testing. There is some human data on acute inhalation toxicity of 2-butoxyethanol that is relevant for classification. Substances can be allocated to one of four toxicity categories based on acute toxicity by inhalation according to the criteria shown in the Table 3.1.1 of Annex I, Part 3, Table 3.1.1 of CLP.

The following applies for the classification as:

'Acute inhalation toxicity - Category 3 (vapour): 2.0 < ATE ≤ 10.0 mg/L'
'Acute inhalation toxicity - Category 4 (vapour): 10.0 < ATE ≤ 20.0 mg/L.'
'Acute inhalation toxicity - Category 4 (mist/aerosol): 1.0 < ATE ≤ 5.0 mg/L.'

The available data needs to take into account the fact that the calculated maximum theoretical vapour concentration for 2-butoxyethanol at room temperature under static equilibrium conditions is 3.8 mg/L. Exposures above this must be to aerosol. It is not possible to achieve the saturated vapour concentration (SVC) in a dynamic environment, such as a test chamber for any period. As the concentration exceeds 50 %, there is increasingly the risk of aerosol formation and condensation in equipment and lines as well as deposition on the test animals. This risk of aerosol formation will further increase if longer exposure times are used.

The following statement was provided by Covance Laboratories in relation to the 2019 guinea pig study regarding the maximum achievable test concentration:

> *There are technical challenges to vaporise any liquid with a relatively low vapour pressure. Data within the literature is often variable and often to only a nominal level of accuracy.*
>
> *The methodology used for this study was more complex than other inhalation exposure systems used for this approach. This includes the introduction of a water bath and more importantly filtration units prior to the animal exposure chamber.*
>
> *The water bath is included to aid vapour formation and the filtration units were included to remove any residual droplet formation from entering the inhalation chamber and ensuring that all of the aerosol was vapour.*
>
> *Therefore, it is considered that the achieved vapour concentration was the maximum practical.*

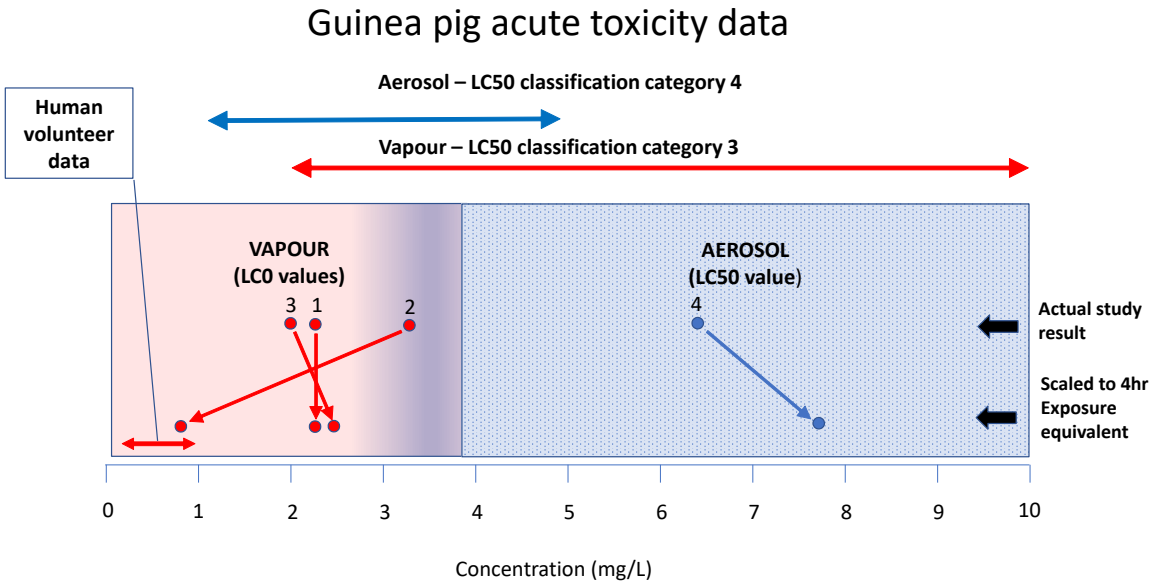
The following is a generic statement from Dow, Midland, USA test laboratory on the practicalities of testing at the maximum attainable concentration:

> *When performing inhalation toxicology studies, it is seldom possible to achieve the theoretical maximum saturated vapor concentration (SVC). The SVC is estimated by using the vapor pressure of the material [SVC in ppm = (vapor pressure in mmHg x 10^6/760 mm Hg)] (1). When performing toxicology testing and the limit concentration is not possible to achieve, the maximum attainable concentration (MAC) is used. As indicated in the glossary of terms of the OECD Guidance Document on Acute Inhalation Toxicity Testing (GD 39) (2), the MAC 'For vapour atmospheres, this concentration depends on the vapour saturation concentration of the test article under test conditions.' The key part is 'under test conditions.' The MAC is dependent on the physical properties of the material, the temperature of the exposure atmosphere and the equipment used to generate the vapor. There are several factors that can influence the failure to reach the SVC, including temperature changes between the vapor generation system and exposure chambers, loss/deposition of test material within the system and formation of condensation aerosols. As indicated in the book Inhalation Toxicology (1), 'As a matter of practicality, it is often technically difficult to generate concentrations equivalent to the SVC under the experimental circumstances used in inhalation toxicology studies.'*

(1) *Cope, Rhian B., Nance, Patricia and Dourson, Mike. "Human Health Risk Assessment of Inhaled Materials" in Inhalation Toxicology. 3rd ed., edited by Harry Salem and Sidney A. Katz, CRC Press, 2015.*

(2) *OECD (2009). Guidance Document on Acute Inhalation Toxicity Testing. Environmental, Health and Safety Publications Series on Testing and Assessment No. 39.*

The relationship of the guinea pig toxicity data to the saturated vapour concentration which defines whether a specific concentration is a vapour or aerosol is shown below. The headline results from the studies are shown along with extrapolation to an equivalent 4-hour exposure result using the Haber equation. For comparison, the range of exposures to which there is 4-hour human volunteer data is also shown.



The figure shows the cross over point between vapour and aerosol exposure at the saturated vapour concentration limit of 3.8 mg/L at 20C.

Key. 1: Covance (2019), 2: Dow (1994), 3: Dow (1974 – 3 studies with identical results), 4: Tyler (1984). Note that studies 1-3 are all LC0 values so cannot be compared directly to the classification criteria. They cannot be used to derive ATE values.

The CLP Regulation (EC) 1272/2008 and the guidance for its application state:

- Recital 28 states *"The results of animal studies should be weighed against the results of data from humans and expert judgement should be used to ensure the best protection of human health when evaluating both the animal and human data."*
- Annex 1, paragraph 1.1.1 states that in relation to the role and application of expert judgement and weight of evidence determination *"Where the criteria cannot be applied directly to available identified information, or where only the information referred to in Article 6(5) is available, the weight of evidence determination using expert judgment shall be applied in accordance with Article 9(3) or 9(4) respectively."*
- Annex 1, paragraph 3.1.2.2.1 states *"The preferred test species for evaluation of acute toxicity by the oral and inhalation routes is the rat, while the rat or rabbit are preferred for evaluation of acute dermal toxicity. When experimental data for acute toxicity are available in several animal*

species, scientific judgement shall be used in selecting the most appropriate LD 50 value from among valid, well-performed tests."

- Section 3.1.2.3.2 of the ECHA guidance on application of the CLP criteria states *"If there is information available to inform on species relevance, then the studies conducted in the species most relevant for humans should normally be given precedence over the studies in other species".*
- On this basis, there appears to be ample justification to use data from the guinea pig in preference to classify for the acute inhalation toxicity to humans. The main mode of action of EGBE is not in doubt. The EU risk assessment (2006) states *"The main mechanism of systemic toxicity of 2-buthoxyethanol is haemolysis of erythrocytes caused by its metabolite butoxy acetic acid (BAA). There are considerable interspecies differences in sensitivity to this toxic action between animal species and humans. As reported in the EU RAR (2006), guinea pigs and humans are relatively resistant, rodents are very sensitive (rats are 30 times more sensitive than humans), while rabbits are less sensitive than rodents, but more sensitive than humans and guinea pigs."* RAC have already applied this logic for the oral and dermal routes since it is stated in the opinion "*to ensure relevance for human hazard assessment, RAC is of the opinion that the lowest oral LD_{50} of 1200 mg/kg bw for guinea pigs, a species reportedly having similar sensitivity as humans to the haemolytic effect of 2-buthoxyethanol, should be chosen as the oral ATE value*." Since systemic effects are independent of route of exposure, for consistency this conclusion should apply not only to oral exposure but also to inhalation as well as to dermal exposure routes if reliable data is available to permit it.

There are three reliable guinea pig studies available. Two of these (Covance, 2019; Dow, 1974) demonstrate that exposure for 4 hours to the maximum practically attained vapour concentration (2.25 – 2.5 mg/L, 60-65 % of the saturated vapour concentration) consistently produces no substance related adverse effects in the exposed animals. The Dow 1974 study was actually three repeat studies using different samples of 2-butoxyethanol[1]. These results suggest that the acute toxicity hazard to guinea pigs is low, that the LC50 cannot be reached with vapour exposure only and that classification for acute inhalation toxicity is therefore not warranted.

The other available data is consistent with this conclusion. The Dow study (1994), whilst only exposing the animals for 1 hour, demonstrated no adverse effects at the maximum attainable vapour concentration in this particular laboratory of 3.1-3.4 mg/L (80-90% of the SVC). Tyler (1984) did report an LC50 for guinea pigs equivalent to a 4 hour figure of 7.65 mg/L. However, as this is well above the SVC, it must have been exposure to aerosol. While this study is considered to be of insufficient reliability and was not used for classification decisions, it should be noted that if such a figure would have been reliable, it would not meet the requirement for classification. The Dow study (1974) also exposed dogs, another species resistant to the haemolytic effects of 2-butoxyethanol, in triplicate to the equivalent of 4 hours at ~2.5 mg/L 2-butoxyethanol without adverse effects. The human data is also consistent in demonstrating that 2-butoxyethanol presents a low acute toxicity hazard to species that are not sensitive to haemolysis. A volunteer study showed no significant adverse effects from exposure to 0.95 mg/L for four hours. No changes to blood parameters were seen.

The human data should be taken into account as supporting evidence. Following the same logic used for the other routes and the criteria of the regulation and guidance on its interpretation, it

[1] This study was carried out to repeat earlier work carried out by Shell which claimed to see CNS effects in guinea pigs when exposed to EGBE vapours. This newer Dow study repeated the earlier work using purified samples of Shell product along with Dow product but was unable to repeat the effects seen. Subsequent analysis of the Shell product used in the original study found it to be impure. The uncertain nature of the product used in these earlier tests therefore renders this earlier work unreliable. The Shell study reports are also no longer available.

seems inconceivable that the data on 2-butoxyethanol would warrant classification as 'toxic by inhalation'.

The weight of evidence resulting from the guinea pig and the human data does not support the classification for the acute inhalation toxicity endpoint according to CLP criteria. No lethality is seen or even significant adverse effects up to the maximum attainable vapour concentration.

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