Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

1,1-dichloroethylene; vinylidene chloride

EC Number: 200-864-0
CAS Number: 75-35-4

CLH-O-0000007324-77-01/F

Adopted

8 June 2023

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON 1,1-DICHLOROETHYLENE; VINYLIDENE CHLORIDE

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: 1,1-dichloroethylene; vinylidene chloride
EC number: 200-864-0
CAS number: 75-35-4
Dossier submitter: France

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
05.08.2022	France	<confidential>	Industry or trade association	1
Comment received				
<confidential>, as Lead registrant for the substance submits the comments on the classification proposal for VDC (CAS 75-35-4) on behalf of <confidential> and <confidential> (member of the joint submission). Giving the time frame for the comments submission, only majors points have been discussed.				
Dossier Submitter's Response				
Noted				
RAC's response				
RAC appreciates the comments submitted by the Industry within the time frame set by the legislation for public consultation.				

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
09.08.2022	Germany		MemberState	2
Comment received				
The classification of 1,1-dichloroethylene as Carc. 1B, H351 is supported. The well-conducted NTP studies (2015) in mice and rats are particularly relevant for the assessment of classification. As part of these studies, 1,1-dichloroethylene induced various benign and malig-nant tumours via the inhalation route. Relevant tumours were found in both sexes in rats and mice in the absence of excessive toxicity. Moreover, some tumours showed reduced tumour latency. 1,1-dichloroethylene is metabolised to mutagenic compounds (e.g. epoxides) and there is no evi-dence that this pathway is not				

relevant to humans. Overall, the criteria for category 1B are fulfilled.
Dossier Submitter's Response
Thank you for your support
RAC's response
RAC appreciates the support and the reasoning provided.

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
09.08.2022	Germany		MemberState	3
Comment received				
The proposed classification of 1,1-dichloroethylene as Muta 2, H341 is justified based on positive findings in an in vivo genotoxicity assay (comet assay) on somatic cells (Anonymous, 2016). These findings are supported by positive results after metabolic activation from in vitro mutagenicity as-says, including one MLA (Mc Gregor D. et al, 1991) as well as reverse bacterial mutation tests (e.g. Oesch et al., 1983).				
Dossier Submitter's Response				
Thank you for your support				
RAC's response				
RAC appreciates the support and the reasoning provided.				

Date	Country	Organisation	Type of Organisation	Comment number
05.08.2022	France	<confidential>	Industry or trade association	4
Comment received				
Based on the available in vivo data investigating genotoxicity to the germ cells, the classification for mutagenicity is not warranted. In a high number of in vitro data, VDC is genotoxic in vitro (gene mutation and clastogenic effects), and especially in the presence of metabolic activation. The in vivo micronucleus tests on bone marrow or circulating erythrocytes conducted in mice (Sawada et al., 1987; National Toxicology Program, 2015) or the chromosomal aberration test on rat bone marrow cells (Quast et al., 1986) did not show any evidence of chromosome aberrations. All 4 studies are negative. While the exposure of the target cells to VDC cannot be demonstrated in 3 studies, cytotoxicity was reported in the bone marrow micronucleus test conducted in mice and reported by Sawada (1987). A slight decrease in the PCE/NCE ratio was observed at the highest tested dose levels. At 200 mg/kg after one single administration, the decrease was 23% compared to the vehicle control group, and at 100 mg/kg after 4 administrations, the decrease was 8.3%. The decrease of 23% in the ratio PCE/NCE reported at 200 mg/kg demonstrates that the bone marrow was exposed to VDC. In addition, a positive control group giving a clear positive response was concurrently tested. This test is sufficiently reliable and can be used to prove that VDC reached the target cells and did not induce genotoxic effects in the in vivo micronucleus test. The in vivo Comet assay (Anonymous, 2016) showed significant and/or biologically relevant DNA damage without adverse histopathological findings in lung, liver and kidney cells. It has been concluded that VDC induces DNA damage in somatic cells, and probably gene mutations as negative results were observed in the in vivo micronucleus test				

(Sawada et al., 1987).

Three in vivo tests assessing mutagenicity to germ cells are available.
Two dominant lethal (DL) assays show negative results. According to the OECD guideline 478, the "purpose of the DL test is to investigate whether chemicals produce mutations resulting from chromosomal aberrations in germ cells". In addition, the guideline states that "DLs generally are the result of gross chromosomal aberrations (structural and numerical abnormalities), but gene mutations cannot be excluded".
The reliability of the test reported by Short et al., (1977) is limited. Only one dose was tested (220 mg/m3) and no positive control was added in the study. But the second DL assay in mice (Anderson et al., 1977) was conducted similarly to the OECD guideline 478. The reliability is acceptable for a mutagenicity assessment. Animals were exposed for 5 days at 3 concentrations (10, 30 and 50 ppm) and a positive control group was present in the assay. At 50 ppm, pregnancy frequency was significantly different at weeks 0-6. This effect was probably due to infertility of the males and was representative of a toxic effect. No evidence of mutagenic effects was reported.
A Sex-Linked Recessive Lethal Mutation assay in Drosophilia melanogaster was reported by Foureman et al. (1994). This test, equivalent to the OECD guideline 477 (deleted in 2014), addresses lethal mutations in germ cells. This study was well described, was reviewed and used by NTP. It can be considered reliable for genotoxicity assessment. Adult male Drosophila melanogaster were exposed to VDC via feeding (20 000 or 25 000 ppm) for 3 days. As the test was negative, retest by injection (5 000ppm) was conducted. The concentrations were selected at a level inducing 30% mortality after 72 hours of feeding or 24 hours after injection. No increase in sex-linked recessive lethal mutations was seen. This demonstrated that VDC did not induce mutations in germ cells of Adult male Drosophila melanogaster.
On the basis of the in vivo dataset about genotoxicity to germ cells (two Dominant Lethal assays and one Sex-Linked Recessive Lethal Mutation assay), VDC is not expected to induce heritable genetic damage (chromosome aberrations or gene mutations). Therefore the classification is not warranted in accordance with the EU regulation 1272/2008 (CLP regulation).

Dossier Submitter's Response

Thank you for your comment.

All the studies mentioned in your comment have been taken into account in the report.

If we understand correctly, according to your reasoning, as tests (of questionable quality, at least for one of them) on germ cells are negative, VDC should not be classified, despite the positive in vitro dataset and the positive comet assay.

Regarding the DL assay in mice (Anderson et al. 1977), we agree that this study does not show any mutagenic effect and is negative. This is the reason why we did not intend to classify VDC as muta. 1B. However, as mentioned in the CLP guidance, the criteria for muta. 2 are as follow : "*It is also warranted that where there is evidence of* ***only somatic cell genotoxicity, substances are classified as suspected germ cell mutagens****. Classification as a suspected germ cell mutagen may also have implications for potential carcinogenicity classification. This holds true especially for those genotoxicants which are incapable of causing heritable mutations because they cannot reach the germ cells (e.g. genotoxicants only acting locally, 'site of contact' genotoxicants).* [which is the case for VDC where the genotoxic effects *in vivo* are seen locally and on detoxification organs, being consistant with the formation of mutagen epoxy metabolites]*. This means that* ***if positive results in vitro are supported by at least one positive local in vivo,***

somatic cell test, such an effect should be considered as enough evidence to lead to classification in Category 2.". This indicate then that the dataset would be sufficient to classified VDC as Muta. 2.

Moreover, as indicated in the CLH report, it has to be kept in mind that the negative studies *in vivo*, without mentioning their quality, do not investigate the same endpoints and the same organs that the comet assay, which could explain the results observed. A comet assay is the only assay allowing the identification of site of contact genotoxicants.

In summary, based on the overall weight of evidence based on in vitro and in vivo studies and the strict application of CLP criteria, our conclusion regarding mutagenicity endpoint differs from yours. According to our assessment, Muta. 2 is required for VDC.

RAC's response

As explained in the Guidance on the Application of the CLP criteria, v5, 2017 "Classification in Category 2 may be based on positive results of at least one *in vivo* valid mammalian somatic cell mutagenicity test, indicating mutagenic effects in somatic cells. A Category 2 mutagen classification may also be based on positive results of a least one *in vivo* valid mammalian somatic cell genotoxicity test, supported by positive in vitro mutagenicity results."

In the case of VDC, *in vivo*, there is a recent well-performed comet assay of high reliability, conducted according to OECD TG 489 which demonstrates that VDC induces DNA damage in the lungs, liver and kidneys of male rats, after inhalation.
Histopathological lesions (from minimal to severe in severity) were also observed in these organs (at the highest dose in kidney, the two highest doses in lung, and the three highest doses in liver), but, in accordance with the OECD guideline, this does not confound the relevance of the DNA damages observed, taking also into account the fact that the observed DNA damages occurred also at concentrations below to those inducing histopathological findings. DNA damages observed in liver, kidney and lung are consistent with the fact that VDC is extensively metabolised into genotoxic metabolites in these tissues. In contrast, no DNA damages were seen in bone marrow. However, under these experimental conditions bone marrow exposure to VDC is not confirmed by the study authors.
In addition, there are several available *in vitro* studies which were positive in the presence of exogenous metabolic activation and provide evidence for the mutagenic properties of VDC. VDC induced gene mutations in bacteria systems, yeast models and in mouse lymphoma cells, was positive in a UDS test in rat hepatocytes, induced chromosomal aberrations and sister chromatid exchanges in Chinese hamster lung cells and in a single study in *Saccharomyces cerevisiae* induced aneuploidy in the presence and absence of metabolic activation. The *in vitro* results support the positive findings observed in the *in vivo* comet assay, especially since the liver, the kidney and the lung express several enzymes involved in the metabolism of VDC to mutagenic metabolites, such as epoxides, as explained in the Toxicokinetics section.
In conclusion, VDC is found to be mutagenic in an *in vivo* somatic cell genotoxicity test and in various in vitro mutagenicity assays. Therefore, the criteria for a classification in Category 2 are fulfilled.

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
09.08.2022	Germany		MemberState	5

Comment received

Acute Toxicity – inhalation route
The proposed classification of 1,1-dichloroethylene as Acute Tox. 1, H330 as well as the setting of an ATE of 0.5 mg/l are supported.

Acute Toxicity – oral route
It is questioned why the LD50 value of 365 mg/kg bw (female mice) derived from the NTP study (1982) by the DS is not used for the purpose of classification. Even if no LD50 could be calculated for male mice, the value should be between 100 (0 % mortality) and 500 (100 % mortality) mg/kg bw indicating a similar range as for females. Therefore, a classification in category 4 with an ATE of 365 mg/kg bw seems more appropriate. Furthermore, classification should depend solely on hazard properties based on reliable data and not on the precautionary principle.

The rationale for the ATE setting is not completely supported because the study by Jones et al. (1978a) is considered less appropriate for this purpose. In the study of Ban et al. (1995) only one dose was tested at a post-observation time of 8 h only. However, its results indicating an LD50 > 200 mg/kg bw would support the choice of an ATE of ≥ 200 mg/kg bw. It is agreed that the proposed value, rather than the cATpE of 100 mg/kg bw, seems more appropriate for establishment of the ATE. The study by Jones et al. (1978a) supports an ATE of this but should not be chosen as the main argument. An ATE of 200 mg/kg bw is further supported by a 50 % mortality in male mice at 200 mg/Kg bw reported in the micronucleus test in mice (Sawada et al, 1987).

Dossier Submitter's Response

Thank you for your supporting the proposal for Acute Toxicity by inhalation route.

Regarding the setting up of the ATE, and concerning oral route: Here are the reasons explaining the classification proposal in category 3 despite the calculated LD50 of 365 mg/kg in female mice :

- It has to be keep in mind that the NTP study, despite being the highest quality study of the database does however not meet current guidelines as it was not conducted to determine a LD50
- The LD50 value for females was only calculated for information purpose as we do not have access to NTP study details, and has therefore to be used with caution
- It cannot be excluded that males are slightly more sensitive than females in the NTP study
- The value of 365 mg/kg, in addition to the uncertainties associated to it, is closed to the threshold value of 300 mg/kg between the categories 3 and 4.
- Finally, a category 3 is in line with the results of the other studies available (even if less reliable).

RAC's response

RAC appreciates the support provided for classification as Acute Tox. 1 by inhalation, ATE=0.5 mg/L.
Regarding classification by oral route, RAC uses the results of the NTP 1982 study on mice for classification purposes, with the Jones et al., 1978a study as supporting evidence. The value of LD_{50} 365 mg/kg bw for female mice, as estimated by the DS using

the maximum likelihood method, warrants classification in Category 4, but the value is close to the threshold value of ATE 300 mg/kg for Category 3. The Jones *et al*. (1978a) study, supports a Category 3 with an LD_{50}=194 mg/kg bw for female mice. These findings would lead to a borderline case between Category 3 (Jones *et al*., 1978a) and 4 (NTP 1982). Other available studies discussed in the CLH report also provide evidence for a borderline case. In a weight of evidence approach, classification as Acute Tox. 3 could be justified.
RAC finds the converted acute toxicity point estimate (cATpE) for category 3 (100 mg/kg bw), to be not realistic and the ATE proposed by the DS of 200 mg/kg bw too conservative, as explained above. Therefore, the upper ATE limit of Category 3, an ATE of 300 mg/kg bw, is proposed.

Date	Country	Organisation	Type of Organisation	Comment number
05.08.2022	France	<confidential>	Industry or trade association	6

Comment received

The toxicity and the toxicokinetics of 1,1-dichloroethylene or vinylidene chloride (VDC) have been reviewed by several groups over the past years, including EPA, WHO, ATDSR, Health Canada, NTP and IARC (EPA, 2002; WHO, 2003; ATSDR, 2009; Health Canada, 2015; NTP, 2015 and IARC, 2019). The discussion below is mainly based on discussions and references described in the most recent documents from NTP (2015) and IARC (2019); more data and references are available in these documents.

On the basis of the metabolism differences between the species, the toxicity observed in rats is the most representative of the toxicity expected in humans, and the acute classification should be defined on the LD50 from rat studies.

At first, when exposed at equivalent vapour concentrations, the systemic exposure to VDC is expected to be lower in humans than in rats and mice. It is generally recognized that exposure by inhalation should result in higher systemic doses of volatile organic chemicals (VOCs) in rodents than in humans because of the higher alveolar ventilation rate, blood/air partition coefficient, cardiac output, and metabolic rate of rodents (NAS, 2009).

Secondly, the metabolization of VDC and therefore the formation of toxic metabolic products are expected to be lower in humans than in rats and mice.
The toxicity of VDC is largely dependent on metabolism which leads to the formation of toxic metabolites. In mammals, VDC is mainly metabolized by CYP2E1 to at least three reactive metabolites: vinylidene chloride epoxide, 2-chloroacetyl chloride, and 2,2-dichloroacetaldehyde. Vinylidene chloride epoxide is the major and likely the most cytotoxic metabolite (associated with covalent bindings to proteins and nucleic acids). Thereafter, these metabolites undergo secondary reactions including mainly glutathione (GHS) conjugation and hydrolysis, and finally, in the kidney, a potential re-activation by the β-lyase is suspected. It was also demonstrated that CYP2F2 could bioactivate VDC in murine lung. Variance in levels and expressions of CYP2E1 and CYP2F2, as well as GSH and epoxide hydrolase, are therefore important factors in the extent of toxicity.

In humans, CYP2E1 is the main enzyme in liver (Hakkola et al., 1994), lung, and kidney responsible for the metabolism of VDC. Although the formation of vinylidene chloride epoxide and 2,2-dichloroacetaldehyde was demonstrated in lung and liver microsomes (Dowsley et al., 1999), CYP2E1 activity is low in human lungs (Shimada et al., 1996), and

is low or non-de¬tectable in human kidneys microsomal samples (Amet et al., 1997; Caro & Cederbaum, 2004; Sasso et al., 2013).
In rodents, high levels of CYP2E1 are present in three preferential target organs of VDC (liver, kidney, and lung). The VDC cyto¬toxicity is higher in murine cells, and this finding is correlated with the highest CYP2E1 content compared to human cells (namely centrilobular hepatocytes, bronchiolar Clara cells and renal proximal tubular cells (Speerschneider & Dekant, 1995; Forkert, 2001). Other studies demonstrated that biotransformation of VDC is about six times higher in liver micro¬somes from mice compared with those from rats (Dowsley et al., 1995), and the expression of CYP2F in the lung is much higher in mice than in humans (Chen et al., 2002).

D'Souza & Andersen (1988) developed physiologically based pharmacokinetic (PBPK) models for VDC in the rat for both oral and inhalation exposure. No validated model is available for humans. D'Souza & Andersen (1988) used allometric scaling to estimate comparative amounts of epoxide formed (mg/kg body weight) in rats and humans. Cardiac output and pulmonary ventilation were scaled by (body weight) 0.7, Vmax was scaled by (body weight) 0.74, and body fat was estimated at 7% in the 200-g rat and 20% in the 70-kg human. When the oral exposure was less than 5 mg/kg body weight, the estimated amount of epoxide formed was about the same in rats and humans. When the inhalation exposure was less than 400 mg/m3, the estimated amount of epoxide formed was 5-fold lower in humans than in rats. (WHO, 2003).

All these data show the metabolism differences between species. Mice is likely the most sensible specie. It can be reasonable assumed that the toxicity of the VDC is not higher in humans as compared to the rat at equivalent oral or inhalation concentrations. Therefore, rats is the most appropriate specie to estimate the toxicity in humans.

For acute oral toxicity, 4 studies conducted on rats are available. The lowest LD50 value observed in rats is 1500 mg/kg in female rats (Ponomarkov et al. (1980). Based on this value, VDC should be classified as category 4, H302: harmful if swallowed according to EU regulation 1272/2008.
For acute inhalation toxicity, 6 studies conducted on rats are available. The lowest LC50 reported for rats was 28 350 mg/m3 / 4 h (from Zeller and Klimish, 1979). This value does not require any classification according to EU regulation 1272/2008. Nevertheless, a harmonised classification exists for VDC in Annex VI of EU regulation 1272/2008 stating that it should be classified as acute toxicity cat. 4, H332 Harmful if inhaled. This classification is maintained by a conservative approach.

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vinylidene chloride. Toxicology and Applied Pharmacology, 95:230–240.
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Dossier Submitter's Response

Thank you for your comment.

You cited numerous publications to strengthen your commentary. As you mentioned, your rationale is mainly based on IARC and NTP assessments; it is also the case in the CLH report (see section 9 on toxicokinetics). As mentioned in the CLH report, we agree with you concerning the fact that mice seems to be the most sensitive rodent species, compared to hamster and rat.

However, it can also be reasonably assumed that the toxicity of the VDC could be lower in the rat compared to human at equivalent oral or inhalation concentrations, which would preclude the use of rat data to determine the acute toxicity of VDC. Therefore, in the absence of robust data, the CLP guidance recommends to use the most sensitive species, which is the reason why data on mice were used.

RAC's response

In the Guidance on the Application of the CLP criteria, v5, 2017, classification for acute toxicity "is based on the lowest ATE value available i.e. the lowest ATE in the most sensitive appropriate species tested. However, expert judgement may allow another ATE value to be used in preference, provided this can be supported by a robust justification. 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".
In the case of acute toxicity studies for VDC, mice is certainly found to be the most sensitive rodent species.
Evidence from metabolism/toxicokinetics suggest that the same metabolic pathways are applicable to humans as for mice and rats.
Therefore, there is no evidence to support the fact that rat is a more relevant species than mice. As a result, classification is based on mice, which is the most sensitive species.

OTHER HAZARDS AND ENDPOINTS – Eye Hazard

Date	Country	Organisation	Type of Organisation	Comment number
09.08.2022	Germany		MemberState	7
Comment received				
Since the in vitro irritancy score (IVIS) of a BCOP test (Anonymous, 2010) performed with 1,1-dichloroethylene falls within the range between 3<IVIS≤55, no stand-alone prediction can be made according to the guideline. As no further in vitro tests are available, it is agreed that no classification can be proposed due to insufficient data.				
Dossier Submitter's Response				
Thank you for your support				
RAC's response				
RAC appreciates the support and the reasoning provided.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
09.08.2022	Germany		MemberState	8
Comment received				
The assessment of classification is solely based on the available 14- and 90-day NTP studies (2015). It is agreed that after inhalation exposure of 1,1-dichloroethylene for 14 and 90 days in rats and mice related findings in liver, kidney and nose/respiratory tract, justify a classification as STOT RE in category 1. Supportive information is shown by the studies after oral administration of 1,1-dichloroethylene in rats and mice, which also identify the same target organs, albeit these effects are within the criteria for category 2 (NTP, 1982). As part of the other available studies, which are of lower quality, liver and kidney were also identi-fied as target organs in the other studies supporting the findings of the NTP studies. Overall, the proposed classification of 1,1-dichloroethylene as STOT RE 1 is warranted.				
Dossier Submitter's Response				
Thank you for your support				
RAC's response				
RAC appreciates the support and the reasoning provided.				

Date	Country	Organisation	Type of Organisation	Comment number
05.08.2022	France	<confidential>	Industry or trade association	9
Comment received				
According to the section 3.9.1.1 of the EU regulation 1272/2008 (CLP regulation) related to the STOT RE criteria, "other specific toxic effects that are specifically addressed in sections 3.1 to 3.8 are not included here". In the "Guidance on the application of the CLP criteria", it is clearly explained that the classification STOT-RE should be only assigned where the observed toxicity is not covered by another hazard class (for example, reproduction or carcinogenicity). Therefore, the STOT RE classification for VDC is not required for the organs identified as target organs for carcinogenicity.				

Dossier Submitter's Response
Thank you for your comment. The CLP guidance also stated that "*For example **specific effects like tumours** or effects on the reproductive organs should be used for classification for carcinogenicity or reproductive toxicity, respectively, but not for STOT-RE*". Observation of tumours are not in any cases taken in consideration in the criteria for STOT RE classification. Moreover, the effects taken into consideration for classification purpose will not necessarily conduct to carcinogenicity of VDC in the same organs, such as hepatic centrilobular alteration, olfactory epithelium necrosis or even nephropathy.
RAC's response
Classififcation in STOT RE 1 is based on histopathological findings reported in inhalation studies in rats (such as centrilobular cytoplasmic alteration, hepatocellular fatty change, focal, disseminated vacuolisation etc in the liver, olfactory epithelium atrophy, mineralisation, necrosis and turbinate atrophy in the nose, renal tubules casts) and mice (such as renal tubule necrosis, granular casts and renal tubule regeneration, mild inflammatory infiltrates of lymphocytes, macrophages, and neutrophils within the interstitium and subcapsular areas, along with occasional tubule mineralisation in the kidney, necrosis of the respiratory epithelium or respiratory epithelium squamous metaplasia in the nose, and liver necrosis) in the absence of systemic toxicity. These non-neoplastic lessions are responsible for functional disturbance of the respective target organ and do not necessarily progress to tumour formation, either benign or malignant. A similar, but not as clear a picture, appears for the oral studies. Therefore, the findings used for STOT RE classification are different from those used for classification in carcinogenicity and they are not necessarily mechanistically linked.