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ORIGINAL ARTICLE

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Skin sensitization data for methyl esters of sulphonic acids—Corrections to published databases required

David W. Roberts

School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Liverpool, UK

Correspondence
David W. Roberts, School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Byrom Street, Liverpool L3 3AF, UK.
Email: d.w.roberts@ljmu.ac.uk

Abstract

Background: Several methyl esters of sulphonic acids are listed in murine local lymph node assay (LLNA) databases, with dose–response data and EC3 values. However, some of these entries are questionable—in one case the chemical tested is not the chemical named in the databases and in others the EC3 value has been derived by extrapolation from data that do not meet the applicability criteria for the approved extrapolation method.

Objectives: To consider how LLNA data came to be attributed to the wrong chemical and to address the inappropriate extrapolated EC3 values.

Methods: Dose–response data for methyl hexadec-3-enesulphonate (wrongly named as methyl hexadec-1-enesulphonate), two other methyl sulphonates and hexadec-1-ene-1,3-sultone are re-evaluated using the single dose probit extrapolation method (SDPEM). The different reaction chemistry profiles of methyl hexadec-3-enesulphonate and methyl hexadec-1-enesulphonate are discussed.

Results: Extrapolated EC3 values for hexadec-1-ene-1,3-sultone are the same by both methods but for the methyl sulphonates the differences are substantial.

Conclusions: Current databases should be corrected and further analysed to identify other cases where EC3 values are likely to be unreliable due to inappropriate estimation by extrapolation.

KEYWORDS
methyl sulphonates, misattribution of LLNA data, potency by extrapolation

1 | INTRODUCTION

The large curated skin sensitization LLNA databases that are now available are highly useful in several respects, such as: providing reference skin sensitization data for quantitative risk assessment (QRA); providing in vivo data for assessing predictive performance of in vitro, *in chemico* and *in silico* approaches; providing read-across reference data that can be used to predict potency of new chemicals; providing data from which expert systems, quantitative structure–activity relationships (QSARs) and quantitative mechanistic models (QMMs) can be derived. The curation exercises underlying these databases largely eliminate misleading results that are attributable to deficiencies in how the assays were carried out but may not always have detected errors in the recording and publishing of the data. One such example is methyl isothiazolinone (MI) which is found in some databases with a listed EC3 value of 1.9%; this value in fact corresponds to the 19.7% solution that was tested and the correct potency value on a 100% basis is 0.4%. This was pointed out in 2013[1] as an error in the 2005 database of Gerberick et al.,[2] but the error has reappeared in the 2018 database of Hoffman et al.[3] This short paper discusses another error that occurs in current databases, relating to "methyl hexadecenesulphonate" and reassesses the interpretation of dose–response data leading to listed EC3 values for this and other methyl sulphonates.

2 | METHYL HEXADECENESULPHONATES

Methyl hexadecenesulphonates are the methyl esters of hexadecenesulphonic acids which, usually together with their C14 homologues, are found as their sodium salts in alpha-olefin sulphonates (AOS) which are used as surfactants in consumer products. AOS is made by sulphonation of olefins with sulphur trioxide, producing a mixture of sulphonic acids and sultones which is neutralised and hydrolysed to give a mixture of sodium alkenesulphonates and sodium hydroxyalkanesulphonates.[4] The major alkenesulphonates in AOS are the C14 and C16 alk-1-enesulphonates, alk-2-enesulphonates and alk-3-enesulphonates. The corresponding methyl esters, shown in Figure 1, are not present in AOS but can be synthesised for use as analytical standards.

As shown in Figure 1, methyl hexadec-3-enesulphonate is the only methyl hexadecenesulphonate that has published LLNA data. Although LLNA data are listed in databases for methylhexadec-1-enesulphonate, in reality these data correspond to an assay done on the 3-isomer. How did this situation arise?

The sequence of event leading to this incorrect entry appears to be as follows (see also Figure 2). The current databases available online as Excel documents refer to the Gerberick et al. (2005) database[2] for the data attributed to methyl hexadec-1-enesulphonate. In that database the source of the data is given as "Ashby et al. (1995)."

In Ashby et al.,[5] LLNA data for 105 chemicals are discussed. Dose–response data are given but not EC3 values. The correct structure corresponding to methyl hexadec-3-ene sulphonate is given in a figure, but in the table giving dose–response data it is simply named methyl hexadecenesulphonate. References for the individual chemicals are not given, it being stated that "… results were derived using a standard protocol and have been reported elsewhere" (referencing a list of sources including "unpublished data"). When the Ashby et al. paper[5] was written no LLNA data for methyl hexadecenesulphonate had been published, but its synthesis and guinea pig test results had been published,[6,7] making quite clear that it was the 3-isomer, as correctly shown by Ashby et al.[5] So at the time that the Ashby et al. paper[5] was written the LLNA information for methyl hexadec-3-enesulphonate was still unpublished. From this point the most plausible explanation is that in the course of compiling the Gerberick et al. database[2] the name and dose–response data were taken from the Ashby et al. paper[5] and methyl hexadecenesulphonate was looked up and the CAS number and structure of methyl hexadec-1-enesulphonate were found. These were entered in the database and an EC3 value was calculated, using the not yet published extrapolation method of Ryan et al.,[8] from the dose–response data that in reality were for methyl hexadec-3-enesulphonate.

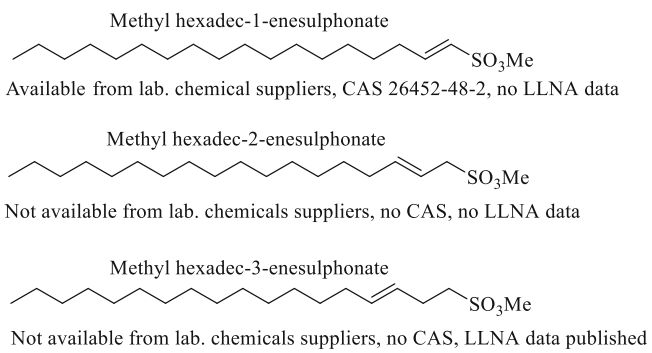


FIGURE 1 Methyl hexadecenesulphonates corresponding to sodium C16 alkenesulphonates present in AOS.

3 | DOES THE POSITION OF THE DOUBLE BOND MATTER?

Methyl esters of sulphonic acids are electrophiles, chemically reactive as S_N2 methyl transfer agents. This applies to methyl esters of aliphatic, aromatic, saturated, and unsaturated sulphonic acids. Little if any difference in S_N2 reactivity would be expected between the methyl esters of alkanesulphonic acids and alkenesulphonic acids with the double bond in any position. On that basis, since skin sensitization potency for S_N2 electrophiles is dependent on a combination of reactivity and hydrophobicity[9,10] the skin sensitization potency of methyl hexadecenesulphonates would not be expected to vary significantly according to the position of the double bond. However, when the double bond is in the 1-position it is activated toward nucleophilic addition, i.e., methyl hexadec-1-enesulphonate has the potential to react not only as an S_N2 methyl group transfer agent but also as a Michael acceptor. Although no experimental studies on the reaction chemistry of methyl hexadec-1-enesulphonate appear to have been published, the shorter chain homologue methyl vinyl sulphonate is known to be highly reactive both by nucleophilic addition and by S_N2 methylation.[11] Consequently, although it can be predicted with confidence that methyl hexadec-1-enesulphonate is unlikely to be significantly less potent as a skin sensitizer than the methyl hexadec-3-enesulphonate that has been tested in the LLNA, it cannot be confidently assumed that it would not be significantly more potent.

4 | DERIVATION OF POTENCY (EC3 VALUE) FROM DOSE–RESPONSE DATA

The dose–response data given in the 2005 Gerberick et al. database[2] and in subsequent databases for methyl hexadec-3-enesulphonate (incorrectly designated methyl hexadec-1-enesulphonate) are: 5%, SI = 26.7; 10%, SI = 35.5, 25%, SI = 32.7. The EC3 value given is 0.8% (estimated) and it corresponds to the value calculated by the extrapolation method of Ryan et al.[8] In the Ryan et al. method,[8] devised for use when the SI value is above 3 at all concentrations tested, the EC3 value is estimated by log-linear extrapolation between the SI values at the two lowest concentrations tested, using the expression:

$$EC3\,(\text{extrapolated}) = 10\text{^}(\log C + ((3 - D)/(B - D))(\log A - \log C))$$

where A and C are the higher and lower of the two concentrations and B and D are the SI values at those concentrations. Logs are to the base 10.

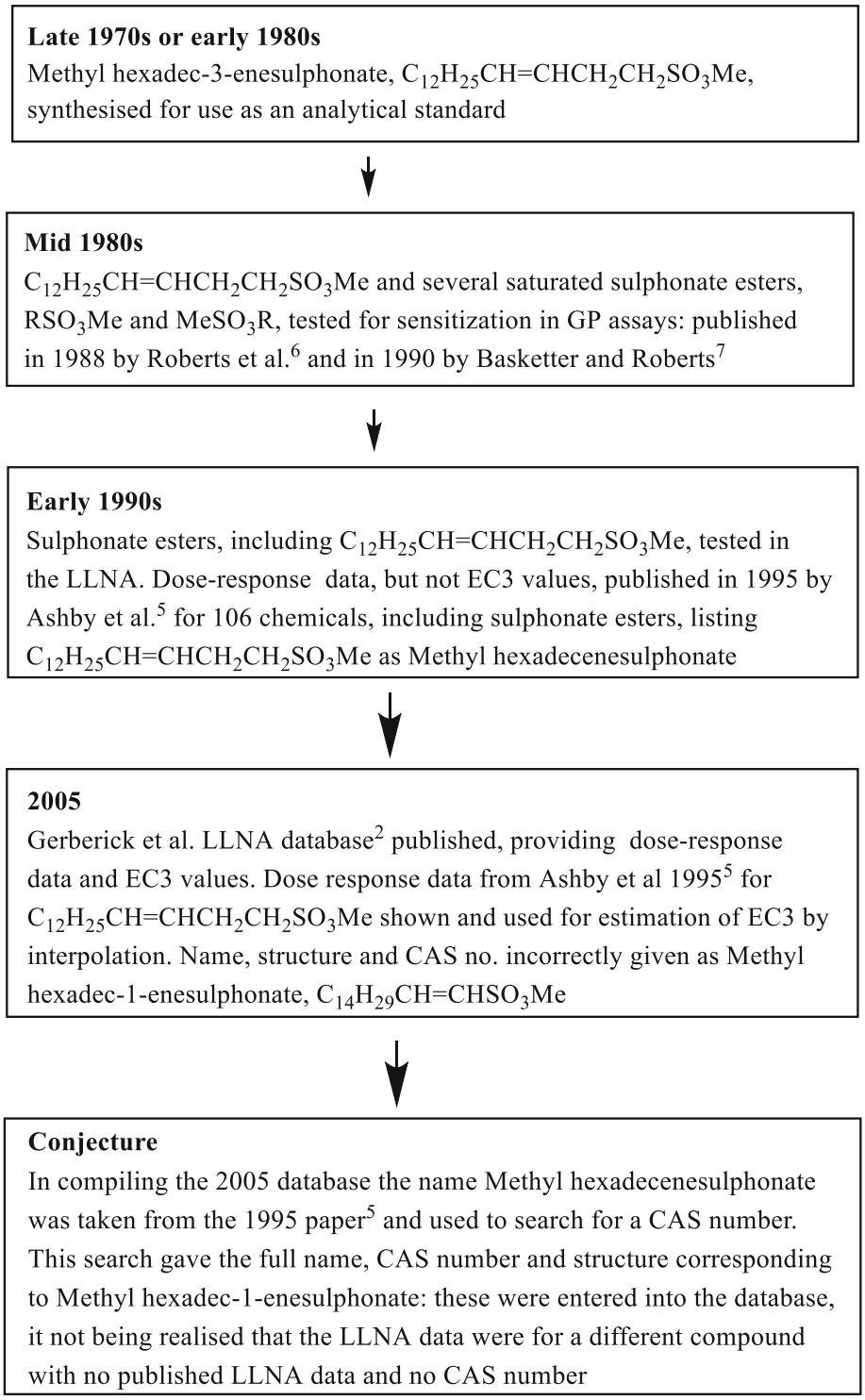
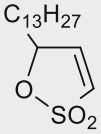


FIGURE 2 Sequence of events leading to incorrect name and structure in databases.

(The original publication[8] gives an expression based on logs to the base 2: EC3 (extrapolated) = 2^($\log_2$C +((3 − D)/(B − D))($\log_2$A − $\log_2$C)) and this expression has continued to appear in many subsequent publications and documents (e.g., a 2021 OECD document[12]), apparently without it being realized that the logarithmic base is irrelevant.)

It is recommended by Ryan et al.[8] that this method should only be used when the lowest SI value is not greatly different from 3 and when slopes between the first two points and between the second and third points are both positive and do not differ by a factor >2. For the first criterion Ryan et al.[8] do not specify a minimum SI value (<10 is often taken as being acceptable) but more recently it has been suggested that the lowest SI should not exceed 5.[12] There are good reasons for these applicability criteria: dose–response curves are usually sigmoid or bell-shaped and while treating them as linear is an adequate approximation for interpolation or short extrapolation, the EC3 value can be substantially underestimated or overestimated when

TABLE 1 Dose response-data and potency values for methyl sulphonates.

Name and structure	LLNA doses D and SI values		EC3 (%) by Ryan method[8]	EC3 by SDPEM	Ryan et al. (2007) criteria[8] met?	
	D (%)	SI			Lowest SI not >>3	Slope ratio <2
Methyl dodecanesulphonate[2] $C_{12}H_{25}SO_3Me$	1	21.6		0.03		Yes, ratio 1.58
	2.5	39.9	0.39	0.02	No	
	5	48.6				
Methyl hexadec-3-enesulphonate[2] $C_{12}H_{25}CH{=}CHCH_2CH_2SO_3Me$	5	26.7		0.09		No, bell-shaped
	10	35.5	0.77	0.09	No	
	25	32.7				
Dimethyl sulphostearate[5] $C_{16}H_{33}CH(CO_2Me)SO_3Me$	2.5	5.1		1.05		No, ratio 2.2
	5	11.6	2.0	0.53	Marginal	
	10	25.6				
Hexadec-1-ene-1,3-sultone[16] 	0.25	7.4		0.06		Yes, ratio 1.1
	0.5	9.8	0.07	0.07	Marginal	
	1	15.1				

these criteria are not met. The mathematical basis is explained in more detail, with examples, in earlier papers.[13,14]

It is quite obvious from inspection of the data that the first of the Ryan et al. applicability criteria is not met in the case of the methyl hexadec-3-enesulphonate data The experimental SI values are all substantially larger than 10.

A more generally applicable method is to apply the single dose probit extrapolation method (SDPEM) which is derived mathematically from the probability functions on which sigmoid dose–response curves are based.[13] The SDPEM was originally developed for use in estimating potency from rLLNA data, where the chemical is tested at one concentration only, but it is also useful for interpreting conventional LLNA data when all SI values are above 3.[15] Here the approach taken is to apply the SDPEM to estimate EC3 from each of the lowest two doses. These two estimates should not differ substantially (if they do it suggests either an overload effect at the higher of the two doses or an anomalous result at the lower dose).

Table 1 compares the EC3 values estimated by the Ryan et al method[8] with those estimated by the SDPEM method for the three methyl sulphonates that have been tested in the LLNA: methyl hexadec-3-enesulphonate, methyl dodecanesulphonate and dimethyl sulphostearate. For comparison hexade-1-ene-1,3-sultone is also shown: this is also a sulphonate ester but it is cyclic and its reaction chemistry is different.[17] In the first two cases the dose–response data cannot realistically be regarded as meeting both of the criteria for applicability of the Ryan et al. method[8] as defined by Ryan et al.[8] In the third case the original criteria for applicability can be regarded as being almost met but the more restrictive OECD criteria[12] are not met. Hexade-1-ene-1,3-sultone meets the slope ratio criterion and would be regarded as meeting the lowest SI not >>3 criterion as discussed by Ryan et al.[8] but not as defined in the 2021 OECD document.[12]

For methyl hexadec-3-enesulphonate and methyl dodecanesulphonate the applicability criteria for the Ryan et al. method[8] are very clearly not met, and the EC3 values estimated by the two methods are substantially different. For both compounds the SDPEM gives very similar EC3 estimates at each of the two doses and on that basis together with the Ryan et al. criteria[8] not being met, the SDPEM-derived EC3 values should be considered more realistic. With dimethyl sulphostearate the estimates of the two methods differ less. Since the Ryan et al. criteria[8] are not met the SDPEM estimates should be considered to be the more reliable. With hexadec-1-ene-1,3-sultone the Ryan et al. criteria[8] are met (the more stringent OECD criteria[12] are close but not quite met) and the agreement between the two methods is very close.

5 | CONCLUSIONS

Cases like that of the methyl hexadecenesulphonates, where sensitization data for one chemical have incorrectly been attributed to another chemical are probably rare. Unless noticed by someone who was involved when the data were generated, as in the present case, errors of this type would be difficult to detect.

Cases where LLNA EC3 values have been estimated by inappropriate application of the Ryan et al. extrapolation method[8] are probably less rare, and as shown in Table 1 the errors can be large. Further curation of databases to address errors or this type would be desirable.

AUTHOR CONTRIBUTIONS

David W. Roberts: Conceptualization; investigation; writing – original draft; writing – review and editing.

CONFLICT OF INTEREST STATEMENT

The author declares no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from published scientific literature and are referenced in the present manuscript.

ORCID

David W. Roberts https://orcid.org/0000-0001-6112-5868

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