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# WEIGHT OF EVIDENCE ASSESSMENT FOR THE SKIN SENSITISATION POTENTIAL OF 4-ISOPROPYLANILINE (CUMIDINE, CAS 99-88-7)

**Series on Testing and Assessment**
**No. 199**

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## FOREWORD

This Weight of Evidence case study has been prepared by experts from the Netherlands and Denmark, with support from the OECD Secretariat.

A hazard assessment of the industrial chemical 4-isopropylaniline (CAS 99-88-7) prepared by the Japanese authorities was discussed by OECD member countries at an OECD Cooperative Chemicals Assessment Meeting (CoCAM) on 16-18 October 2012.

Skin sensitisation is not a mandatory OECD SIDS endpoint, which means that there are no formal requirements for evaluation or generation of test data to conclude on this endpoint in a chemical hazard assessment of the OECD. However, if any data are available for this endpoint, it should be included in the assessment.

No experimental test data on skin sensitisation were available for 4-isopropylaniline. However, the chemical structure of this substance is similar to substances known to be potent skin sensitisers, including some well-known hair colouring agents such as *p*-phenylenediamine, *p*-toluenediamine and *p*-aminophenol. Therefore, it was decided that a case study with non-test information on skin sensitisation of 4-isopropylaniline would be prepared.

This case study aims to provide all available and relevant (non-testing) evidence on the skin sensitisation potential for 4-isopropylaniline, and subsequently uses a Weight of Evidence (WoE) approach to arrive at a conclusion. Although some evidence on its own may be considered insufficient (e.g. a QSAR prediction that has an out-of-applicability domain warning) to reach a conclusion, this information can still be taken into account in a WoE approach, especially if the information confirms other (equally or more reliable) sources of information. The WoE assessment presents a hypothesis on skin metabolism and mechanism through which the substance of interest can cause skin sensitisation. Five structural analogues for which experimental skin sensitisation data are available were selected based on hypothesised mechanism and the other selection criteria that are detailed in the document. Furthermore, positive predictions for the substance of interest from five independent QSAR models are presented, and the QSAR predictions for the selected structural analogues by these same QSAR models show the ability of the QSARs to (correctly) predict skin sensitisation potential for this type of substance. All this information points to the same conclusion: 4-isopropylaniline would very likely be a skin sensitiser.

This document is published under the responsibility of the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology.

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## Substance Identity

4-Isopropylaniline is a *para*-substituted aromatic amine. The identity and structure of 4-isopropylaniline are given below in figure 1. Its hazard profile was assessed within the OECD Cooperative Chemicals Assessment Programme in October 2012; the SIDS assessment was agreed and is publicly available (OECD 2013). The physico-chemical properties of 4-isopropylaniline are available in Annex 1 to this report.

CAS Number: 99-88-7

IUPAC Name: 4-Isopropylaniline

Molecular Formula: $C_9H_{13}N$

Structural Formula: 


Molecular Weight: 135.21

Synonyms: Aniline, 4-(1-methylethyl)-
p-Isopropylaniline
4-Aminocumene
4-(1-Methylethyl)benzenamine
4-Cumidine

**Figure 1. Structure and identity of 4-isopropylaniline (see Annex 1 for physico-chemical properties).**

## Substance Profile and Proposed Mechanism

4-Isopropylaniline does not contain any substructures that alert for potential protein binding according to the OECD QSAR Toolbox (v 3.0). However, it is believed that the sensitizing potential of *aromatic amines* depends on their biotransformation into reactive species. These metabolites can be formed via enzymatic transformations in the skin. One of these possible routes is the oxidation of amines to *N*-hydroxylamines which are then oxidized to *nitroso compounds* that can react with proteins through nucleophilic addition reactions (Estrada *et al*, 2004).

The nitroso group is strongly electron withdrawing and similar to the carbonyl group (C=O). There is polarisation of the N=O bond and it behaves as a weak C=O. It undergoes addition of nucleophiles and condensation with primary amines. Oxygen is more electronegative than nitrogen, and the polarized N=O bond gives the nitrogen atom some degree of positive charge that attracts negatively charged nucleophiles and makes reaction with body proteins (Pr-SH, protein thiols) possible (Estrada *et al*, 2004; Eyer *et al*, 1994; Hopkins *et al*, 2005 cited from the OECD QSAR Toolbox (v3.0)). The structural alert acting through the mechanism of nucleophilic addition is illustrated below (figure 2).

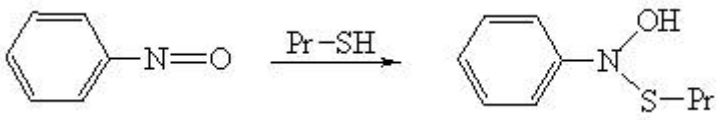



**Figure 2. Structural alert acting through nucleophilic addition at the nitroso group.**

The skin metabolism simulator of the OECD QSAR Toolbox (v3.0) indeed generates a metabolite of 4-isopropylaniline with a nitroso group (see figure 3 and table 1) which, according to the Oasis profiler for protein binding, binds to protein via nucleophilic addition at the polarized *N*-functional double bond (likelihood of generation and amount not stated; also see table 1).

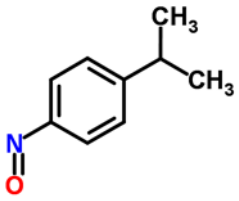



**Figure 3. 4-Isopropyl nitrosobenzene, skin metabolite of 4-isopropylaniline predicted by the skin metabolism simulator of the OECD QSAR Toolbox (SMILES: c1(C(C)C)ccc(N=O)cc1)**

The mechanism proposed by the DEREK knowledge database also proposes that nitroso formation of anilines by skin cytochrome p-450 enzymes leads to skin sensitisation potential of (parent) substances that have a primary or secondary substituted aromatic amino-group (alert nr. 427 in the skin sensitisation module of DEREK).

Experimental evidence, although limited to one substance, that aromatic nitroso compounds are indeed skin sensitizers comes from reports on the contact allergenic effect in humans for *N*,*N*-dimethyl-p-nitroso-aniline (CAS-RN 138-89-6) (Kayser & Schlede, 2001).

Nitroso formation is not the only plausible pathway that could result in a reactive species with sensitisation potential for anilines that are suitably *para* or *ortho* substituted. Roberts et al (2007) postulated that aromatic amines can form quinone methide imines, and indeed TIMES-SS and the skin metabolism simulator (OECD QSAR Toolbox, v. 3.1) predict the potential formation of a quinone methide imine for three analogue substances discussed below (p-methyl- and 4-pentylaniline, and *N*,*N*-dimethyl-p-benzenediamine). Such structures would be capable of undergoing Michael additions and are predicted to be strong sensitisers by TIMES-SS. However, the analogous quinone methide imine is not predicted for the target compound itself by either software package. This means that it is not possible to draw any conclusions about the formation of this metabolite and its relevance for the target chemical's mechanism.

## Analogue Approach

When the focus of the assessment is on filling data gaps for one specific chemical, empirical data from one or more similar chemical(s) ("the analogue(s)") or "source" chemical can be used to predict the same endpoint for the "target" chemical, which is considered to be "similar." This analogue approach is useful when the target and source chemicals share a known common mode (and/or mechanism) of action, and the adverse effect(s) driven by this mode (and/or mechanism) of action is evaluated.

### *Read-across hypothesis*

The read-across hypothesis that supports the analogue approach for anilines is based on two primary considerations (mechanistic and metabolic):

1. The skin sensitisation potential of the aniline derivatives can be based on the reactivity of the amino group which depends on the mesomeric interaction with the aromatic system. Factors that may influence this mesomeric interaction include steric factors and further substituents on the aromatic group (IARC 2010). Thus, reactivity of the amino group of the analogues needs to be comparable to the reactivity of the amino group of 4-isopropylaniline.

2. The aniline derivatives are metabolically activated by *N*-oxidation in the skin in order to exert skin sensitisation properties (Payne & Walsh 1994). Only analogues that follow this metabolic pathway are suitable to use for read-across to 4-isopropylaniline.

### *Identification and selection of analogues*

A relatively large range of aromatic amines have been tested for skin sensitisation. The following selection criteria that reflect the read-across hypothesis were applied in order to identify the most suitable analogues.

| Selection criteria for analogues | Reasoning |
|---|---|
| **1. Must be an aniline** | The target chemical is an aniline. |
| **2. Must not be substituted in the *ortho* position** | Due to potential steric interactions that may result in chemical reactivity that differs from the target chemical. |
| **3. If present, substituents on the aromatic ring must have weak electron donating properties** | The isopropyl substituent on the target chemical has weak electron donating properties. This property is known to affect the chemical reactivity of the amino group (Gross & Seybold 2000; Argese *et al*. 2002). Substituents on suitable analogues should therefore have similar electron donating properties. |
| **4. Must be able to form a protein reactive functional group by *N*-oxidation in the skin.** | The amino group in itself has no structural alerts for skin sensitisation. It is well known from other aromatic amines that this group needs to undergo *N*-oxidation before exerting sensitizing properties. |
| **5. Must have *in vivo* test data for skin sensitisation** | This data is needed for read across. |

Analogues that fulfill the selection criteria were identified in the following way:

- Analogue identification in the OECD QSAR Toolbox
- Search on the ECHA disseminated website for REACH registered substances containing "aniline" in their names
- Identification of analogues used in the training set of QSAR models (Danish QSAR Database and VEGA)

Five analogues were selected (see table 1). None of the methods mentioned above proved alone to be sufficient to identify all five analogues. However, all methods gave varying degrees of overlap, meaning that the same analogues were detected with at least two of the three methods.

Selection criterion 3 was applied by deselecting anilines containing substituents that are known to have different electron withdrawing/donating properties compared to the alkyl substituent in 4-isopropylaniline. Therefore, only chemicals containing alkyl or alkyl-like substituents were selected. This also means that extreme sensitizers like *p*-phenylenediamine (PPD) and *p*-toluene diamine (PTD) were excluded from the group. In addition, the $E_{LUMO}$ (lowest unoccupied molecular orbital) was calculated in the OECD QSAR Toolbox for 4-isopropylaniline and its analogues. $E_{LUMO}$ is a descriptor for hydrogen bonding capacity and has a good correlation with reactivity of aniline derivatives (R = 0.86) (Argese *et al*, 2002).

Selection criterion 4 was first applied by use of the skin metabolism simulator in the OECD QSAR Toolbox (v3.0). However, the 5 selected analogues were checked for additional information on metabolism in the following sources: REACH registrations on the ECHA disseminated website, the EU RAR for aniline and the OECD SIDS for *p*-Toluidine, *m*-Toluidine and 4-isopropylaniline. In addition, mutagenicity of anilines is also believed to be dependent on *N*-oxidation of the amino group. Therefore, it was checked whether or not positive responses were recorded in *in vitro* assays with S9. A positive result in such an assay would be an indication of *N*-oxidation. Finally, a mutagenic response *in vitro* can also be used in its own right as an indicator for skin sensitisation potential since there is a positive correlation between these two effect types (e.g. Hilton *et al*, 1993; Mekenyan *et al*, 2010; Patlewicz *et al*, 2010; Rosenkranz *et al*, 1999).

***Results for the analogues***

The five structural analogues are presented in Table 1 (split between Table 1a, suitable analogues, and Table 1b, more distant analogues) below. A more detailed description of test results for the analogues and an assessment of their suitability for read-across is presented in Annex 2.

**Table 1a: Target Chemical and Structural Analogues that are Suitable for Read Across**

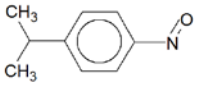
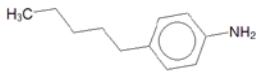
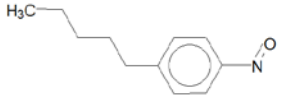
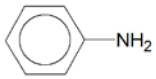
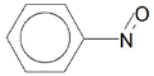
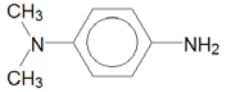
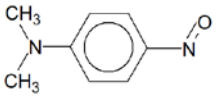
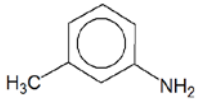
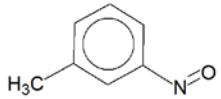
| Chemical | Predicted metabolite using skin metabolism simulator (OECD QSAR Toolbox, v. 3.1) | Protein binding profile for skin metabolite (OASIS, v.1.1) | $E_{LUMO\ (eV}$ for parent compound / skin metabolite) | Selection criteria assessment | *In vitro* genotoxicity | Experimental data on *in vivo* skin sensitisation[1] |
|---|---|---|---|---|---|---|
| 4-Isopropylaniline (CAS 99-88-7)  |  SMILES: c1(C(C)C)ccc(N=O)cc1 | Nucleophilic addition reaction at polarized *N*-functional double bond | 0.636 / -0.563 | Target chemical | Gene mutation with S9 | Target chemical No test data available |
| *p*-Toluidine (CAS 106-49-0)  |  SMILES: c1(N=O)ccc(C)cc1 | Nucleophilic addition reaction at polarized *N*-functional double bond | 0.617 / -0.552 | Very close structural analogue | Clastogenicity with S9 | Positive in GPMT (Klimisch code 2) Kleniewska and Maibach, 1980; OECD, 2005 |
| 4-Pentylaniline (CAS 33228-44-3)  |  SMILES: c1(N=O)ccc(CCCCC)cc1 | Nucleophilic addition reaction at polarized *N*-functional double bond | 0.622 / -0.767 | Very close structural analogue | No information available | Positive in LLNA Roberts et al, 2007 |
| Aniline (CAS 62-53-3)  |  SMILES: c1(N=O)ccccc1 | Nucleophilic addition reaction at polarized *N*-functional double bond | 0.640 / -0.408 | Close structural analogue | Clastogenicity and possible gene mutation with S9 | Positive in 2 of 3 GPMTs (Studies judged reliable for use in EU Existing Substances regulation) EU, 2004. |

**Table 1b: More Distant Structural Analogues**

| Chemical | Predicted metabolite using skin metabolism simulator (OECD QSAR Toolbox, v. 3.1) | Protein binding profile for skin metabolite (OASIS, v.1.1) | $E_{LUMO\ (eV}$ for parent compound / skin metabolite) | Selection criteria assessment | *In vitro* genotoxicity | Experimental data on *in vivo* skin sensitisation[1] |
|---|---|---|---|---|---|---|
| *N*,*N*-Dimethyl-*p*-benzenediamine (CAS 99-98-9)  |  SMILES: c1(N=O)ccc(N(C)C)cc1 | Nucleophilic addition reaction at polarized *N*-functional double bond | 0.600 / -0.691 | Outlier in the category; read across should be performed with caution | Gene mutation with S9 | Positive in GPMT NICEATM/ICCVAM, 1999 Skin sensitiser US National Library of Medicine, 2012 [2] |
| *m*-Toluidine (CAS 108-44-1)  |  SMILES: c1(N=O)cc(C)ccc1 | Nucleophilic addition reaction at polarized *N*-functional double bond | 0.605 / -0.759 | Outlier in the category; read across should be performed with caution | Not genotoxic | Negative in LLNA (Klimisch code 1) ECHA, 2013 |

[1] More details on experimental results can be found in Annex 2. No assessment of data quality has been performed. Reliabilities, when available, have been taken from relevant assessment frameworks.

[2] Information from Haz-Map, which comes from textbooks, journal articles, the Documentation of the Threshold Limit Values (published by ACGIH), and electronic databases such as NLM's Hazardous Substances Data Bank (HSDB®).

***Interpretation of experimental results for the analogues***

The two closest analogues to 4-isopropylaniline (*p*-toluidine and 4-pentylaniline) both tested positive for skin sensitisation. The two analogues differ from the target chemical only by the number of carbon atoms and the degree of branching in the *p*-alkyl chain. Based on the descriptors that have been included in this assessment (structural features, electron withdrawing/donating properties of substituents, $E_{LUMO}$, *in vitro* genotoxicity, and metabolism), the two closest analogues are suitable to use for read-across for skin sensitisation. A conclusion on potency for the target chemical is not possible, but it could be envisaged that the sensitisation potency of 4-isopropylaniline (substituent with 3 carbon atoms) is in between that of *p*-toluidine (substituent with 1 carbon atom) and 4-pentylaniline (substituent with 5 carbon atoms).

Two of the five identified analogues were rated as outliers in the group after closer examination of the available information. *N*,*N*-Dimethyl-*p*-benzenediamine differs from 4-isopropylaniline in structural features; the substituent containing a tertiary amine, which are known to increase the electron withdrawing properties compared to an alkyl group. In addition, a second predicted skin metabolite for this chemical has different alerts for protein binding according to TIMES-SS and the OECD QSAR Toolbox (v3.0) (OASIS protein binding). This does not disqualify *N*,*N*-Dimethyl-*p*-benzenediamine as an analogue, but means that there is a higher degree of uncertainty involved in the read-across to this substance.

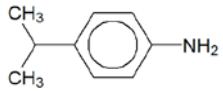
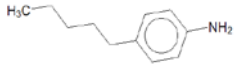
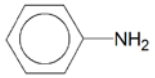
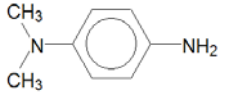
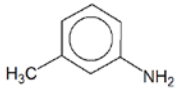
The second outlier, *m*-toluidine, differs from the rest of the group in its structural features (substituted in the *meta* position) and in the available test data (negative results for skin sensitisation and *in vitro* genotoxicity). It is not possible to explain the differences from the applied chemical reactivity descriptors since $E_{LUMO}$ is in the same range as for *para*-substituted anilines and since the electron donating tendency is very similar for methyl groups placed in the *para* and *meta* position (Gross & Seybold, 2000). It is therefore speculated that the differences are caused by differences in the metabolic pathway (in this context, it should be noted that monosubstituted anilines at the meta position cannot undergo transformation to quinone methide imines because of the relative positions of the substituents on the aromatic ring; only ortho and para-substituted anilines are capable of this transformation).

Further, the effects of *para*-substituted anilines compared to *meta*- or *ortho*-substituted ones have also been reported in human case studies. Positive reactions in patch tests have been reported in monitoring surveys and in studies with patients suffering from eczematous dermatitis. Here, the positive reactions are often associated with a group allergy to other aromatic amines which are substituted at the *para* position (*para*-group compound cross reactivity) (EU, 2004).

**QSAR Estimates for Skin Sensitisation**

All QSAR predictions are summarized in Table 2, both for 4-isopropylaniline and the five structural analogues for which experimental data on skin sensitisation are present.

**Table 2: Skin Sensitisation Potential Predictions from Five Independent QSAR Models for 4-Isopropylaniline and its Analogues**

| **Chemical** | **Danish QSAR Database (MC4PC)[1]** | **VEGA/ CAESAR, v.2.1.5[2]** | **DEREK Nexus v.1.5[3]** | **TOPKAT[4] Sensitizer vs. non-sensitizer** | **TOPKAT[5] Severe vs. moderate** | **TIMES-SS[6]** |
|---|---|---|---|---|---|---|
| 4-Isopropylaniline (CAS 99-88-7)  | **Sensitizer**<br>Inside AD<br>P=90%<br>Reliability: 2 | **Sensitizer**<br>AD warning<br>P=88%<br>Reliability: 2 | **Sensitizer**<br>Plausible<br>Reliability: 2 | **Sensitizer**<br>outside OPS but inside OPS limits,<br>P=100%<br>Reliability: 2 | **Severe**<br>inside OPS<br>P=100%<br>Reliability: 2 | **Weak Sensitizer**<br>Out of AD<br>Active is: Metabolite<br>Reliability: 2 |
| *p*-Toluidine (CAS 106-49-0)  | Sensitizer<br>Inside AD<br>P=90% | Sensitizer<br>Inside AD<br>88% | Sensitizer<br>Plausible | Sensitizer<br>Inside OPS<br>P=100% | Moderate<br>Inside OPS<br>P=0% | Strong Sensitizer<br>Out of AD<br>Active is: Metabolite |
| 4-Pentylaniline (CAS 33228-44-3)  | Sensitizer<br>Inside AD<br>P= 90% | Sensitizer<br>Inside AD<br>88% | Sensitizer<br>Plausible | Sensitizer<br>Inside OPS<br>P=100% | Moderate<br>Inside OPS<br>P=0% | Strong Sensitizer<br>Out of AD<br>Active is: Metabolite |
| Aniline (CAS 62-53-3)  | Sensitizer<br>Inside AD<br>P = 90% | Sensitizer<br>Inside AD<br>P=88% | Sensitizer<br>Plausible | Sensitizer<br>Inside OPS<br>P=100% | Moderate<br>Inside OPS<br>P=0% | Weak Sensitizer<br>Inside AD<br>Active is: Metabolite |
| *N,N*-Dimethyl-*p*-benzenediamine (CAS 99-98-9)  | Sensitizer<br>Inside AD<br>P=90% | Sensitizer<br>Inside AD<br>P=90% | Sensitizer<br>Plausible | Sensitizer<br>Inside OPS<br>P=97.5% | Severe<br>Inside OPS<br>P=100% | Strong Sensitizer<br>Inside AD<br>Active is: Metabolite |
| *m*-Toluidine (CAS 108-44-1)  | Sensitizer<br>Inside AD<br>P = 90% | Sensitizer<br>Inside AD<br>P=88% | Sensitizer<br>Plausible | Sensitizer<br>Inside OPS<br>P=100% | Moderate<br>Inside OPS<br>P=0% | Weak Sensitizer<br>out of AD<br>Active is: Metabolite |

Note on highlighting: predictions highlighted in green affirm experimental findings (see Table 1a and 1b); predictions highlighted in red contradict experimental findings. Further discussion is included below.

[1] Danish QSAR Database (internal version): MC Score = internal score, the breakpoint between Neg (negative) and Pos (positive) is 25. P = probability that positive prediction is correct. See appendices for detailed prediction and Applicability Domain information

[2] VEGA/CAESAR Skin Sensitisation model v.2.1.5. See appendices for detailed prediction and Applicability Domain information

[3] DEREK Knowledge Base 2012 1.0 (Lhasa Ltd, Leeds, UK). See appendices for detailed prediction information

[4] TOPKAT v6.2, as implemented in the Accelrys Discovery Suite software. This first classifier model distinguishes between Sensitizers and non-sensitizers. The manual states that a score of >70% should be interpreted as a positive prediction of skin sensitisation potential The OPS (Optimal Prediction Space) is a multivariate statistical TOPKAT indication of model applicability domain. See appendices for detailed information.

[5] TOPKAT v6.2 as implemented in the Accelrys Discovery Suite software. This second skin sensitisation classifier model distinguishes between severe and mild/moderate skin sensitizers. It can be used to get an indication of the skin sensitisation potential for those substances which are predicted to be a sensitizer by the previous TOPKAT module. The manual states that a score of P>70% should be interpreted as a prediction of SEVERE skin sensitisation. The OPS (= Optimal Prediction Space) is a multivariate statistical TOPKAT indication of model applicability domain.

[6] Predictions from the TIMES-Skin Sensitisation model v16.18 . For the interpretation of the predictions (red/green highlight) the prediction of non- or weak-sensitizer was interpreted (in accordance with the interpretation of LLNA potency classes for classification and labelling purposes) as Non-sensitizer and mild/moderate-, strong- and extreme-sensitizer were considered Sensitizers. If the TIMES prediction of weak sensitizer is interpreted as Sensitizer (for classification and labelling) all predictions, with the exception of *m*-toluidine would be correct.

***MULTICASE MC4PC (version 2.4.1.4)***

The substance is predicted to be very active in the commercial model A33 "Allergic contact dermatitis". The identified alert is the aromatic amine in the *para* position to the substitution. The alert is based on 20 molecules of which 18 are active. The Danish QSAR Model predicts all analogues substituted in the *para* position to be "very active" (internal score 59-67, the breakpoint between positive and negative is 25) whereas those that are not substituted in the *para* position (*m*-toluidine and aniline) are still predicted to be "moderately active" (internal score 39; hence the model is able to discriminate between substitution in the *para* and *meta* positions). The two metabolites proposed by the OECD QSAR Toolbox (see Table 1a) were also predicted to be "very active". The prediction was inside the applicability domain of the model and considering the predictive performance for the structural analogues, is judged to be of good quality (Reliability 2: reliable with restrictions).

***VEGA / CAESAR (version 2.1.5, program downloaded from the Vega site (Vega, 2013))***

The compound is predicted to be a sensitizer (Active: 0.88, Inactive: 0.12), however with an applicability domain warning (some atom-centered fragments of the compound have not been found in the compounds of the training set, or are rare fragments). The applicability domain indicator is borderline (0.79 where 0.80 is considered inside the domain). Nevertheless, the predictions from the VEGA / CAESAR model for the structural analogues are all inside the applicability domain (as defined by the VEGA / CAESAR model), and the predictions for the *para*- and non-substituted anilines are all correct. These results indicate that the QSAR model is capable of predicting this type of substance (*para*-substituted anilines) with high reliability (Reliability 2: reliable with restrictions).

***DEREK for Windows prediction***

4-Isopropylaniline was predicted to be a PLAUSIBLE skin sensitizer based on the structural alert "Aromatic primary or secondary amine" (alert number 427). The DEREKfW prediction "PLAUSIBLE" means that there is a structural alert in the assessed compound for skin sensitisation and all other requirements described in the alert description are fulfilled. Based on this positive DEREKfW prediction and the correct predictions of the structural analogues (except for the *meta*-substituted analogue), 4-isopropylaniline is predicted to be a skin sensitizer (Reliability 2: reliable with restrictions).

***TOPKAT 6.2***

The TopKat model predicts both the substance of interest as well as the aniline analogues to be skin sensitizers. Again, the predictions are correct for the *para*- and un-substituted anilines, whereas the *meta*-substituted aniline is a False Positive. All predictions for the analogues were well within the TopKat defined Optimal Prediction Space (OPS). The prediction from the TOPKAT 6.2 model for 4-isopropylaniline was not within the OPS, but still considered within the OPS limits, i.e. still within the applicability domain. The prediction from the TOPKAT Sensitizer vs. non-sensitizer model is therefore considered reliable (Reliability 2: reliable with restrictions). The TOPKAT Severe vs Mild/moderate sensitizers model prediction that 4-isopropylaniline will be a Severe sensitizer is slightly less reliable, as the prediction for the analogue 4-pentylaniline has to be considered wrong.

***TIMES Skin Sensitisation model (version 16.18 with autoxidation)***

The target substance is predicted to be a weak skin sensitizer, where the activity is due to autooxidation to a hydroperoxide and metabolism to an aromatic nitroso compound (see section "Substance profile"). However, the structure was out of the model structure domain. If a prediction is out of the applicability domain this does not necessarily mean that the prediction is therefore not valid, or is incorrect. It indicates

that the uncertainty about the reliability of the model is increased, as the performance statistics from the training and/or validation datasets might not be applicable to this specific substance. All models set the applicability domain thresholds differently and some are stricter than others. For example, the TIMES-SS requires 100% of the fragments to be covered correctly as well as key physico-chemical parameters, and this check is done for the generated metabolites as well followed by an alert performance (response space) check. Other models set cut-offs for fragments space lower (at ~80%, for example). Therefore variation in the parameters employed in a model's internal determination of whether a substance falls within its applicability domain requires consideration in examining relative reliability across models. Given the conservative applicability domain categorization that is applied in the TIMES-SS model (see also Teubner *et al*, 2013) and the fact that the model correctly predicts the skin sensitizing property of the analogous substances, a reliability score of 2 is assigned (reliable with restrictions).

As TIMES-SS gives a prediction on a scale of skin sensitisation potency (non- weak-, mild/moderate-, strong- and extreme- sensitizer) an interpretation in binary terms (sensitizer / non-sensitizer) is needed. If the interpretation of LLNA potency for classification and labelling purposes is applied, a TIMES prediction of non- or weak-sensitisation would be interpreted as Non-sensitizer. The prediction for 4-isopropylaniline is then that it is not a skin sensitizer (for classification and labelling purposes). The prediction for the analogue aniline is then consequently incorrect, but the predictions for *N*,*N*-dimethyl-*p*-aniline and *m*-toluidine would be correct. If a TIMES prediction of weak-sensitizer is interpreted meaning that the substance is a sensitizer for classification and labelling purposes, all *para*-substituted aniline analogues are predicted correct, but *m*-toluidine is a false positive prediction, similar to the predictions of (all) the other QSAR models[1].

***Quantitative Predictivity of Combination of Multiple QSAR Models***

As no single QSAR model is considered valid as a stand-alone replacement of an animal test, there is the need to assess the (QSAR) evidence in combination. The question is whether two or more positive predictions from QSAR models actually give a higher probability that the conclusion will be correct. This has been analysed in a recent publication by Rorije et al (2013) using Bayesian statistics. The procedure uses the individual predictive performance (sensitivity, specificity) of models, tests and assays to predict the outcome of an LLNA. Bayesian statistics are applied to calculate the probability that a prediction from a battery of tests and/or models will correctly predict the outcome of an LLNA. This probability is a quantitative measure of the reliability of a prediction when (QSAR) models are combined. What is additionally needed is an indication of what is considered sufficient reliability to allow replacement of the animal test. This can be deduced from the reliability/reproducibility of the *in vivo* GPMT test correctly predicting the outcome of an LLNA (and vice versa). Both *in vivo* test results are accepted as stand-alone results under the EU regulation REACH, and also in the OECD CoCAM process. Therefore the probability with which the GPMT can predict the outcome in the LLNA can be considered sufficient. The probability that a substance is tested positive in the GPMT, and will also be tested positive in an LLNA was calculated in a rigorous official validation study of the LLNA to be **83%** (NICEATM-ICCVAM, 1999). A battery of alternative tests and/or models can therefore be considered sufficiently reliable to replace an *in vivo* test result if it reaches at least this threshold probability of 83%.

In the analysis the individual predictive performance (sensitivity, specificity) of DEREKfW, TIMES-SS and the OECD QSAR Toolbox alerts is evaluated, *without taking into account any applicability domain information*. This means that TIMES-SS predictions which were considered to be out of the applicability domain were *included* in the calculation of the predictive statistics in this analysis. The calculated Bayesian probability that a substance will test positive in the LLNA test, given that these three models agree with each other (table 6 in Rorije et al, 2013), is **85.9%.** This is already above the probability of 83% with

[1] Note that these QSAR predictions are not aligned with GHS criteria, as discussed in detail in Teubner et al. (2013).

which a positive GPMT can predict a positive LLNA outcome. The actual observed percentage of correct predictions of the battery of these three models, without taking into account applicability domain information, when all three models predict positive, was **89.5%,** using a set of 522 substances for which LLNA results are available.
The additional evidence from positive CAESAR, MultiCASE and TOPKAT model predictions are likely to increase this probability even further, however statistics were not calculated for these specific models (Rorije, 2013).

**Conclusion**

A weight of evidence analysis of the experimental data on structural analogues, and QSAR model predictions, gives a strong indication that 4-isopropylaniline (CAS 99-88-7) would very likely be a skin sensitizer.

4-isopropylaniline is structurally related to *p*-toluidine (CAS 106-49-0), aniline (CAS 62-53-3), and 4-pentylaniline (CAS 33228-44-3), which are known skin sensitisers. All of these substances, except aniline, are *para*-substituted and are predicted to form reactive nitrosamines. The analogous substance *N,N*-dimethyl-*p*-benzenediamine (CAS 99-98-9) also tests positive for skin sensitisation but is judged to be an outlier in the category. The analogous substance *m*-toluidine (CAS 108-44-1), which is substituted at the *meta* position and therefore less suitable for read-across purposes, is not thought to form reactive nitrosamine (nor could it form a reactive quinone methide imine), and tests negative for skin sensitisation. Further, there are positive QSAR predictions from five different models which are deemed reliable (reliability 2: reliable with restrictions). In addition, there are profiler alerts in the OECD QSAR Toolbox consistent with the current knowledge as e.g. presented in the DEREK knowledge database on metabolic activation in the skin for aromatic amines.

Statistically, the probability that a substance for which a battery of QSAR models is in agreement in their positive predictions will be around 90% correct in predicting a positive outcome in the LLNA. This is well above the (statistical) reliability of experimental tests that is implicitly accepted in regulatory frameworks.

**References**

Argese, E., Bettiol, C., Fasolo, M., Zambon, A., Agnoli, F. 2002. *Substituted aniline ineraction with submitochondrial particles and quantitative structure-activity relationships*. Biochimica et Biophysica Acta 1558: 151-160.

Ashby, J., Hilton, J., Dearman, R. J., Callander, R. D., Kimber, I. 1993. *Mechanistic relationship among mutagenicity, skin sensitisation, and skin carcinogenicity*. Environmental Health Perspectives 101 (1): 62

Doerge, D.R., Corbett, M.D. 1991. *Peroxygenation mechanism for chloroperoxidase-catalyzed N-oxidation of arylamines*. Chemical Research in Toxicology 4(5):556-60.

Dossou, K.G., Sicard, C., Kalopissis, G., Reymond, D., Schaefer, H. 1985. *Method for assessment of experimental allergy in guinea pigs adapted to cosmetic ingredients.* Contact Dermatitis 13(4): 226-34.

ECHA (2013). European Chemicals Agency's REACH data Dissemination website. Accessed 2013; http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances

Estrada E., Patlewicz G., Gutierrez Y., From Knowledge Generation to Knowledge Archive. *A General Strategy Using TOPS-MODE with DEREK To Formulate New Alerts for Skin Sensitisation*. J. Chem. Inf. Comput. Sci., 2004, 44, 688-698.

EU 2004. *European Union Risk Assessment Report for Aniline (CAS 62-53-3)*. European Communities, 2004. Available at http://esis.jrc.ec.europa.eu/doc/risk_assessment/REPORT/anilinereport049.pdf

Eyer P., *Reactions of Oxidatively Activated Arylamines with Thiols: Reaction Mechanisms and Biologic Implications: An Overview*. Environmental Health Perspectives, 102(Suppl 6): 123-132 (1994), 126-127.

Greim H (2004). *p*-Toluidin. In: Gesundheitsschädliche Arbeitsstoffe, Toxikologisch-arbeitsmedizinische Begründungen von MAK-Werten, Loseblattsammlung, 39. Lfg. DFG, Deutsche Forschungsgemeinschaft, Wiley-VCH Verlag, Weinheim.

Gross, K,C. & Seybold, P.G. 2000. *Substituent effects on the physical properties and pKa of aniline.* International Journal of Quantum Chemistry 80: 1107-1115.

Hopkins J.E., Naisbitt D.J., Humphreys N., Dearman R.J., Kimber I., Park B.K., *Exposure of mice to the nitroso metabolite of sulfamethoxazole stimulates interleukin 5 production by $CD4^+$ T-cells*, Toxicology, 2005, 206(2), 221-231

IARC 2010. *Some Aromatic Amines, Organic Dyes, and Related Exposures. General discussion of common mechanisms for aromatic amines*, p. 41-54. WHO

Kayser, D., Schlede, E. 2001. *Chemikalien und Kontaktallergie – Eine bewertende zusammenstellung.* Springer Auslieferungs GmbH, Heidelberg, 644 pp.

Kleniewska D (1975). *Studies on hypersensitivity to "para group"*. Berufsdermatosen 23:31-36, cited in Greim 2004.

Kleniewska D and Maibach H (1980). *Allergenicity of Aminobenzene compounds: structure function relationships*. Dermat. Beruf Umwelt **28**, 11-13

Mekenyan, O., Patlewicz, G., Dimitrova, G., Kuseva, C., Todorov, M., Stoeva, S., Kotov, S., Donner, E.M. 2010. *Use of genotoxicity information in the development of integrated testing strategies (ITS) for skin sensitisation*. Chemical Research in Toxicology 23(10):1519-40.

NICEATM-ICCVAM, 1999. *The Murine Local Lymph Node Assay: A Test Method for Assessing the Allergic Contact Dermatitis Potential of Chemicals/Compounds.* The Results of an Independent Peer Review Evaluation Coordinated by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and the National Toxicology Program Center for the Evaluation of Alternative Toxicological Methods (NICEATM), NIH Publication No. 99-4494. Available from http://iccvam.niehs.nih.gov/docs/immunotox_docs/llna/llnarep.pdf

OECD 2005. *SIDS Initial Assessment of p-Toluidine (CAS 106-49-0)*, OECD, Paris. Available at http://webnet.oecd.org/HPV/UI/handler.axd?id=c00130f6-16e4-46de-8e0f-c9d6f525c38a

OECD 2003. *SIDS Initial Assessment Report for m-Toluidine (CAS 108-44-1)*. http://www.inchem.org/documents/sids/sids/108441.pdf

OECD 2013. *SIDS Initial Assessment Report for 4-Isopropylaniline (CAS 99-88-7)*. http://webnet.oecd.org/HPV/UI/SIDS_Details.aspx?key=3bf77dbe-2bf3-4c59-bb3d-36f9c5ffe43f&idx=0

Price, S.M., & Shupack, J.L. 1978. *Allergic contact dermatitis due to N,N-dimethyl-para-phenylenediamine in bacteriology technicians.* Cutaneous Medicine for the Practitioner 21(3):330-2.

Patlewicz *et al*. 2010. *Can mutagenicity information be useful in an Integrated Testing Strategy (ITS) for skin sensitisation?* SAR and QSAR in Environmental Research, 21: 7, 619 – 656.

Payne, M.P. & Walsh, P.T. 1994. *Structure-activity relationships for skin sensitisation potential: development of structural alerts for use in knowledge-based toxicity prediction systems.* Journal of Chemical Information and Computer Sciences 34: 154-161.

Roberts, D.W., Patlewicz, G., Dimitrov, S.D., Low, L.K., Aptula, A.O., Kern, P.S., Dimitrova, G.D., Comber, M.I., Phillips, R.D., Niemelä, J., Madsen, C., Wedebye, E.B., Bailey, P.T., Mekenyan, O.G. 2007. *TIMES-SS--a mechanistic evaluation of an external validation study using reaction chemistry principles*. Chemical Research in Toxicology 20(9):1321-30.

Rorije E., Aldenberg T., Buist H., Kroese D., Schüürmann G. (2013) *The OSIRIS Weight of Evidence approach: ITS for Skin Sensitisation.* Regulatory Pharmacology and Toxicology, accepted for publication. Available online: http://www.sciencedirect.com/science/article/pii/S0273230013000895

Rosenkranz, H.S., Klopman, G., Zhang, Y.P., Graham, C., Karol, M.H. 1999. *Relationship between allergic contact dermatitis and electrophilicity.* Environmental Health Perspectives 107(2): 129–132.

Teubner, W., Mehling, A., Schuster, A.M., Guth, K., Worth, A., Burton, J., van Ravenzwaay, B., Landsiedel, R. 2013. *Computer models versus reality: how well do in silico models currently predict the skin sensitisation potential of a substance.* Regulatory Toxicology and Pharmacology 67(3): 468-485.

US National Library of Medicine, Haz-Map, updated December 2012. Available at http://hazmap.nlm.nih.gov/category-details?table=copytblagents&id=6377

**Appendices (SEPARATE DOCUMENTS)**

***I. QSAR predictions from the Danish EPA QSAR database (MultiCASE MC4PC)***

***II. QSAR predictions from the DEREK nexus v.1.5***

***III. QSAR predictions from VEGA/CAESAR v2.1.5***

***IV. QSAR predictions from TOPKAT v6.2 Skin Sensitisation models***

***V. QSAR predictions from TIMES Skin Sensitisation (OASIS) v.16.18***

***VI. QMRF for the Multicase skin sensitisation model in the Danish QSAR database***

***VII. QMRF and QPRF for the DEREK skin sensitisation model***

**Annex 1: Physico-chemical Properties of 4-Isopropylaniline[1]**

| Property | Value | Reliability |
|---|---|---|
| Physical state/appearance | Pale yellow clear liquid | 2 |
| Melting point | <-100 °C | 2 |
| Boiling point | 226-227 °C at 745 mmHg | 2 |
| Density | 0.953 g/cm$^3$ at 20 °C | 2 |
| Vapour pressure | 5.62 Pa at 25 °C[1)] | 1 |
| Water solubility | 2390 mg/L at 20 °C[2)] | 1 |
| Partition coefficient between octanol and water | log $K_{ow}$ = 2.3 at 25 °C[3)] | 1 |
| Dissociation constant | pKa=5.00 at 25 °C | 1 |
| Soil adsorption coefficient | log $K_{oc}$ =2.53 [4)]<br>KOCWIN | 2 |
| Henry's Law constant | 0.318 Pa.m$^3$/mol at 20 – 25 °C [5)]<br>0.375 Pa.m$^3$/mole at 25 °C [6)]<br>HENRYWIN | 2<br><br>2 |

Table Notes:
Vapour pressure at 25 °C was extrapolated by the following regression expression, which was obtained from the results of a test according to OECD test-guieline104: "Vapour pressure: Gas saturation method" in compliance with GLP.
log P (Pa) = -3062.69/T + 11.0224
Test was conducted according to OECD test-guideline 105: "Water solubility: flask method" in compliance with GLP.
Test was conducted according to OECD test-guideline 107: "Partition coefficient (n-octanol /water): Shake flask method" in compliance with GLP.
The value is estimated by MCI method.
Henry's law constant is calculated by vapour pressure of 5.62 Pa at 25 °C divided by water solubility of 2390 mg/L at 20 °C.
The value is estimated by bond method.

[1] Taken from OECD, 2013; for references, refer to OECD, 2013.

**Annex 2: Analogue Results**

The five selected analogues are presented individually below with information on skin metabolism, skin sensitisation, mutagenicity, mechanistic considerations and a qualitative judgment on their suitability for read-across to 4-isopropylaniline for skin sensitisation.

***p*-Toluidine (CAS 106-49-0)**

**Skin metabolism:** No information on skin metabolism is available in the OECD SIDS (OECD 2005). The REACH registration on the ECHA dissemination website contains references to *in vitro* assays which confirm that *N*-oxidation is a possible metabolic pathway for *p*-toluidine (e.g. Doerge & Corbett, 1991). The skin metabolism simulator in the OECD QSAR Toolbox (v3.0) predicts four potential skin metabolites with the active *C*-nitroso metabolite as one of them. The positive result in the sensitisation test and some mutagenic response in the presence of S9 also indicates *N*-oxidation. In conclusion, there are strong indications that *N*-oxidation is a relevant metabolic pathway in the skin.

**Skin sensitisation:** *p*-Toluidine was concluded to be a skin sensitizer at OECD SIAM 21 (OECD 2005). Patch test was performed with 10 guinea pigs using a 2 % *p*-toluidine petrolatum solution and occlusive dressing for induction. 14 days later, 4 concentrations for the challenge procedure were used: 2 %, 1 %, 0.5 %, 0.25 %. *p*-Toluidine was evaluated as sensitizing because 8/10 guinea pigs showed a positive reaction in the highest concentration (2 %). 6/10, 4/10 and 0/10 animals showed a positive reaction after challenge with 1, 0.5 or 0.25% *p*-toluidine. The positive control was served by *p*-phenylene diamine (Kleniewska and Maibach, 1980). This study was rated a Klimisch score of 2.

In addition the following study with humans is reported in the SIAR (OECD 2005): 58 dermatitis patients, known to be hypersensitive to *p*-phenylene diamine, were patch tested with 2 % *p*-toluidine in yellow paraffin. 63.8 % (37) of the patients showed positive reactions (Kleniewska, 1975). The study is not assignable because only patients with dermatitis and already sensitized to *p*-phenylene diamine were included in the test.

**Mutagenicity:** *p*-Toluidine does not induce point mutations in the vast majority of *in vitro* Ames tests (a positive result is reported for the strain TA100 with hamster S9). In Chinese hamster lung cells *p*-toluidine is clastogenic in the presence but not in the absence of S9-mix (OECD 2005).

**Structural and mechanistic considerations:** *p*-Toluidine is substituted in the *para* position and the methyl substituent has a weak electron donating property which is comparable to 4-isopropylaniline. $E_{LUMO,}$ which is used as a descriptor for hydrogen bonding capacity, is very similar to that of 4-isopropylaniline.

**Conclusion:** *p*-Toluidine is judged to be a very close analogue to 4-isopropylaniline and suitable to use for read-across for skin sensitisation.

**4-Pentylaniline (CAS 33228-44-3)**

**Skin metabolism:** No test data for skin metabolism (or any other type of metabolism) have been identified. The skin metabolism simulator in the OECD QSAR Toolbox (v3.0) predicts seven potential skin metabolites with the active *C*-nitroso metabolite as one of them. The positive result in the LLNA test also indicates a potential for *N*-oxidation in the skin. In conclusion, there are indications that *N*-oxidation is a relevant metabolic pathway in the skin.

**Skin sensitisation:** 4-pentylaniline was found to be a strong sensitizer in the LLNA test (Roberts *et al*, 2007).

**Mutagenicity:** No information is available.

**Structural and mechanistic considerations:** 4-Pentylaniline is substituted in the *para* position and the pentyl substituent has a weak electron donating property which is comparable to 4-isopropylaniline. $E_{LUMO,}$ which is used as a descriptor for hydrogen bonding capacity, is very similar to that of 4-isopropylaniline.

**Conclusion:** 4-Pentylaniline is judged to be a close analogue to 4-isopropylaniline and suitable to use for read-across for skin sensitisation.

***m*-Toluidine (CAS 108-44-1)**

**Skin metabolism:** According to a BUA report cited in OECD (2005), *m*-toluidine is rapidly absorbed via the gastrointestinal tract, via skin and is metabolized by ring hydroxylation. However, other evidence suggests that *N*-oxidation is also a relevant metabolic pathway. The OECD SIDS for *m*-toluidine (OECD 2003) cites an older study in which the protein reactive metabolite, *m*-nitrosotoluene is measured in blood after a single injection of 111.1 mg *m*-toluidine-HCl/kg b.w. to dogs. The skin metabolism simulator in the OECD QSAR Toolbox (v3.0) predicts four potential skin metabolites with the active *C*-nitroso metabolite as one of them. However, negative results in the LLNA test and in 14 genotoxicity *in vitro* assays suggest that *N*-oxidation may play a smaller role compared to ring hydroxylation metabolism for *m*-toluidine.

**Skin sensitisation:** A GLP compliant LLNA test (OECD 429) is reported in the REACH registration dossier on the ECHA dissemination website for REACH registered substances. Dermal application of 2, 10 and 50% of *m*-toluidine on both ears of female NMR mice for three consecutive days did not show an increase in the stimulation indices for cell counts or for weights of the draining lymph nodes. Hence, according to this assay the substance has no sensitisation potential.

**Mutagenicity:** 14 *in vitro* studies are presented in the REACH registration dossier on the ECHA disseminated website for REACH registered substances. No mutagenic potential has been identified in these studies. The conclusion in the OECD SIDS is that *m*-toluidine is considered not to be genotoxic (OECD 2003).

**Structural and mechanistic considerations:** *m*-Toluidine is substituted in the *meta* position and thereby differs from 4-isopropylaniline, which is substituted in *para* position. However, the methyl substituent has a weak electron donating property which is comparable to 4-isopropylaniline. $E_{LUMO,}$ which is used as a descriptor for hydrogen bonding capacity, is also very similar to that of 4-isopropylaniline.

**Conclusion:** There are some indications that (at least quantitatively) differences exist in metabolism between *m*-toluidine and 4-isopropylaniline. Hence, read-across from *m*-toluidine to 4-isopropylaniline should be performed with caution.

**Aniline (CAS 62-53-3)**

**Skin metabolism:** According to the EU Risk Assessment Report for aniline (EU 2004), no information is available on skin metabolism from animal studies. However, non-dermal toxicokinetic studies demonstrate that the protein reactive metabolite nitrozobenzene is generated, although the quantity seems to be route and species specific (EU 2004). The skin metabolism simulator in the OECD QSAR Toolbox (v3.0) predicts two potential skin metabolites with the active *C*-nitroso metabolite as one of them. In addition, positive findings in two of three sensitisation studies, and the induced potential for *in vitro* mutagenicity by addition of S9, support the generation of a protein reactive metabolite.

**Skin sensitisation:** According to the EU Risk Assessment Report (EU 2004), animal data revealed a mild to moderate sensitisation rate. In 2/3 guinea pig tests a positive rate of 10% and 50% are documented. In

the test revealing a 50% positive result 20% aniline was used for challenge, while the tests demonstrating weak or negative results used very low challenge concentrations (challenge with 10% aniline resulted in 1/10 sensitised animals, challenge with 1% aniline in no sensitisation at all). In humans positive reactions have also been reported, mainly in patients suffering from eczematous dermatitis. The positive reactions are often associated with *para*-group compound cross reactivity. In addition, in humans aniline shows cross-reactivity to substances of the *para*-substituted compound group, which has to be considered as a hazard by itself. Based on animal and human data, aniline has a harmonised classification for skin sensitisation in the EU and as such is labelled with the R-phrase R 43 "May cause sensitisation by skin contact".

**Mutagenicity:** According to EU (2004) aniline is negative in routine bacterial mutation tests. In mammalian cell cultures positive effects were obtained with respect to chromosomal effects, SCE and possibly for gene mutations. In general, stronger effects are induced in the presence of an exogenous metabolic activation system than in its absence.

**Structural and mechanistic considerations:** Aniline is unsubstituted and thereby differs from 4-isopropylaniline, which is substituted in the *para* position. $E_{LUMO,}$ which is used as a descriptor for hydrogen bonding capacity, is also very similar to that of 4-isopropylaniline.

**Conclusion:** Aniline is judged to be an acceptable analogue to 4-isopropylaniline.

***N,N*-Dimethyl-*p*-benzenediamine (CAS 99-98-9)**

**Skin metabolism:** No relevant test data have been identified. The skin metabolism simulator in the OECD QSAR Toolbox (v3.0) predicts two potential skin metabolites with the active *C*-nitroso metabolite as one of them. However, the other potential skin metabolite has a different alert for protein binding.

**Skin sensitisation:** The substance was found to be a strong sensitizer in a GPMT (Dossou *et al*. 1985). In addition three cases of allergic contact dermatitis due to *N,N*-dimethyl-*p*-benzenediamine exposure to humans are reported in the literature (Price & Shupack 1978).

**Mutagenicity:** A positive response is recorded in some bacterial strains in AMES but only with metabolic activation (OECD QSAR Toolbox – the original reference could not be located).

**Structural and mechanistic considerations:** *N,N*-Dimethyl-*p*-benzenediamine is substituted in the *para* position. The substituent contains a tertiary amine which has electron withdrawing properties. However, the electron donating property of the two attached methyl groups may counteract this to some degree. $E_{LUMO,}$ which is used as a descriptor for hydrogen bonding capacity, is very similar to that of 4-isopropylaniline.

**Conclusion:** There are potentially differences in the mesomeric interaction from the substituent compared to 4-isopropylaniline. In addition, the metabolites for this chemical have different alerts for protein binding according to TIMES-SS and the OECD QSAR Toolbox (v3.0) (OASIS protein binding). Hence, read-across from *N,N*-dimethyl-*p*-benzenediamine to 4-isopropylaniline should be performed with caution.

**REFERENCES**: see main report reference list