

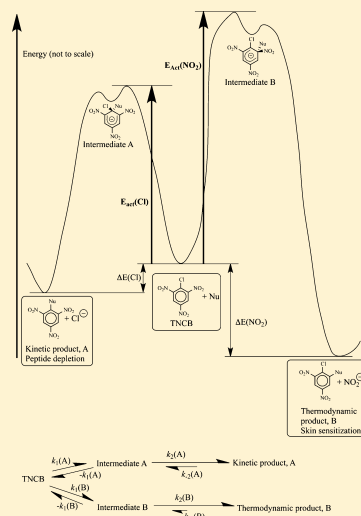
# Electrophilic Reactivity and Skin Sensitization Potency of $S_NAr$ Electrophiles

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**ABSTRACT:** We published in 2011 a quantitative mechanistic model (QMM) for skin sensitization potency of  $S_NAr$  electrophiles in the mouse local lymph node assay (LLNA). In this model, potency was correlated with a combination of  $\sigma^*$  for the leaving group and the total  $\sigma^-$  values of the other substituents in the aromatic ring. Shortly afterward Natsch et al. published a kinetic study in which rate constants were determined for reactions of  $S_NAr$  electrophiles with the cysteine-based peptide Ac-RFAACAA (Cys-peptide) that is used in the direct peptide reactivity assay (DPRA), and correlations were sought between these rate constants and sensitization potency in the LLNA. These two publications together have enabled the present study, aiming to develop a linear free energy relationship (LFER) correlating Cys-peptide reactivity with a reactivity parameter (RP) based on a combination of  $\sigma^*$  and  $\sigma^-$  substituent constants and, by analyzing differences between the QMM based on RP and the QMM based on Cys-peptide rate constants, to gain further insights into the underlying chemistry of skin sensitization. For the 2,4-dinitro-X-substituted benzenes (DNXB), the rate constants of Natsch et al. are well correlated with the reactivity parameter used in our earlier work, with two outliers. These are the compounds with X = F and X = SCN, which are both substantially more reactive toward Cys-peptide than predicted from comparison of their RP values with those of the other DNXB compounds. These two compounds are both negative outliers from a correlation of sensitization potency with experimental rate constants, but fit well to the correlation of sensitization potency with RP values. With these two compounds excluded, sensitization potency is well correlated with the experimental rate constants for the DNXB compounds (X =  $SO_3^-$ , I, Br, Cl) together with 2,4-dichloro-1-nitrobenzene and 1,3,4,5-tetrachloro-2,6-dicyanobenzene. The regression equation is  $pEC_3 = 0.88 \log k + 4.03$ ,  $R^2 = 0.966$ . The implication of DNFB being an outlier is that the model Cys-peptide nucleophile is substantially more sterically hindered than the cutaneous nucleophile(s) involved in the sensitization process. The pattern seen with 2,4-dinitrothiocyanatobenzene suggests that this compound reacts as an  $S_NAr$  electrophile in the sensitization process, but by a different pathway, acting as a CN transfer agent, with the model Cys-peptide. For two further compounds, 2,4,6-trinitrochlorobenzene and 2,4,6-trinitrobenzenesulfonate, the Cys-peptide rate constants are well predicted by the reactivity parameter based on displacement of the Cl or  $SO_3^-$  substituent, but their sensitization potency is underestimated by both the Cys-peptide rate constant and this reactivity parameter. However, potency of these two compounds is well predicted by a reactivity parameter calculated on the basis of displacement of the 2-nitro group. This is interpreted as a case of sensitization being driven by the thermodynamically favored rather than the kinetically favored reaction product.



## INTRODUCTION

It is well established that the ability (or lack thereof) of chemicals to act as skin sensitizers is strongly related to their ability to react covalently with skin proteins.<sup>1,2</sup> Although the nature and location of the target proteins is not clearly established, reactivity parameters based on simple model nucleophiles have often proved adequate to model structure–potency relationships, and it has been demonstrated that within reaction mechanistic domains quantitative mechanistic models (QMMs) can be developed based on such parameters.<sup>1,3–5</sup>

The first reported reactivity–potency relationship for skin sensitization was carried out in the 1930s on  $S_NAr$  electrophiles (although the term  $S_NAr$  had not been coined at that time),<sup>6</sup> the most well-known of these being 2,4-dinitrochlorobenzene

(DNCB). This relationship was qualitative, the ability or inability to sensitize being found to correspond to the ability or inability to react with aniline in methanol at reflux temperature over 2 h. Subsequently this qualitative relationship was modified and extended to further  $S_NAr$  electrophiles, so as to discriminate between sensitizers and nonsensitizers on the basis of substituent constants<sup>7</sup> or QM parameters.<sup>8</sup> Later, with the introduction of the murine local lymph node assay (LLNA), quantitative sensitization potency data became available for several  $S_NAr$  electrophiles, in the form of EC3 values, EC3 being the concentration required, on application to the mouse ear, to produce a 3-fold

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Table 1. Reactivity Parameters and LLNA Potency for  $S_NAr$  Electrophiles<sup>a</sup>

| chemical name                       | CAS       | $\sigma^*$ | $\Sigma\sigma^-$ | RP   | log $k$ | pEC3 <sub>obs</sub> | pEC3 <sub>calc</sub> |
|-------------------------------------|-----------|------------|------------------|------|---------|---------------------|----------------------|
| DNFB                                | 70-34-8   | 3.21       | 2.48             | 3.25 | 0.98    | 3.76                | 3.68                 |
| DNCB                                | 97-00-7   | 2.96       | 2.48             | 3.19 | -0.65   | 3.70                | 3.51                 |
| DNBB                                | 584-48-5  | 2.84       | 2.48             | 3.16 | -0.47   | 3.46                | 3.43                 |
| DNIB                                | 709-49-9  | 2.46       | 2.48             | 3.07 | -0.44   | 3.24                | 3.18                 |
| DNTB                                | 1594-56-5 | 3.43       | 2.48             | 3.30 | 2.20    | 3.68                | 3.82                 |
| DNBS                                | 89-02-1   | 0.81       | 2.48             | 2.67 | -2.33   | 2.09                | 2.06                 |
| TCPN                                | 1897-45-6 | 2.96       | 2.98             | 3.69 | 0.65    | 4.82                | 4.91                 |
| DCNB                                | 611-06-3  | 2.96       | 1.60             | 2.31 | -3.47   | 0.98                | 1.05                 |
| TNCB                                | 88-88-0   | 2.96       | 3.72             | 4.43 | 2.00    | 3.69                | 6.97                 |
| TCNB, NO <sub>2</sub> -displacement |           | 4.25       | 2.16             | 3.18 |         |                     | 3.45                 |
| TNBS                                | 2508-19-2 | 0.81       | 3.72             | 3.91 | 0.53    | 2.99                | 5.52                 |
| TNBS, NO <sub>2</sub> -displacement |           | 4.25       | 2.02             | 3.04 |         |                     | 3.06                 |

<sup>a</sup>EC3 in mol/100 g;  $k$  in  $s^{-1} M^{-1}$ . The pEC3<sub>obs</sub> and log  $k$  values are from Natsch et al.<sup>11</sup> Some of these pEC3 values are slightly different from those given in Roberts et al.,<sup>9</sup> but the differences are marginal. The pEC3<sub>calc</sub> values are calculated from eq 4.

proliferation of lymphocytes in the auricular lymph node. Using these data, a quantitative mechanistic model (QMM) was developed correlating EC3 values with substituent constants:<sup>9</sup>

$$pEC3 = 2.48\Sigma\sigma^- + 0.60\sigma^* - 4.51 \quad (1)$$

This can be written as

$$pEC3 = 2.48(\Sigma\sigma^- + 0.24\sigma^*) - 4.51 \quad (1a)$$

The term  $\Sigma\sigma^- + 0.24\sigma^*$  is a composite reactivity parameter (RP) in which  $\sigma^*$  is the Taft substituent constant for the leaving group and  $\Sigma\sigma^-$  is the sum of the Hammett  $\sigma^-$  values for the other ring substituents.<sup>9</sup> References 7 and 9 give a fairly detailed overview of  $S_NAr$  chemistry and structure-reactivity relationships. A more comprehensive coverage is given by Terrier.<sup>10</sup>

Almost simultaneously with the publication of this QMM,<sup>9</sup> Natsch et al.<sup>11</sup> reported a kinetic study in which rate constants were measured for reactions of the same set of compounds with a cysteine-based peptide (Cys-peptide) and compared against LLNA EC3 values. The two approaches are very similar: in each case, relationships are looked for between reactivity parameters and potency in the LLNA, the difference being in the nature of the reactivity parameters. Here we compare the findings from the two approaches, aiming to develop a linear free energy relationship (LFER) correlating Cys-peptide reactivity with a reactivity parameter (RP) based on a combination of  $\sigma^*$  and  $\sigma^-$  substituent constants and, by analyzing differences between the QMM based on RP and the QMM based on Cys-peptide rate constants, to gain further insights into the underlying chemistry of skin sensitization.

## MATERIALS AND METHODS

Skin sensitization data for the  $S_NAr$  electrophiles and rate constants for their reactions were taken from the publication by Natsch et al.<sup>11</sup> Substituent constants ( $\sigma$  and  $\sigma^*$  values) were taken from the compilation by Perrin et al.<sup>15</sup> or estimated by the methods recommended by them. Regression analyses of the data to derive QMMs were performed using MINITAB 16 statistical software (MINITAB Ltd., Coventry, U.K.).

## RESULTS

**Linear Free Energy Relationship (LFER) for Reactivity of  $S_NAr$  Electrophiles toward Cys-Peptide.** Table 1 shows the  $\sigma^*$  values, the  $\Sigma\sigma^-$  values, the RP values ( $RP = \Sigma\sigma^- + 0.24\sigma^*$ ), and the log  $k$  values ( $k$  = second order rate constant for reaction with Cys-peptide) for all the  $S_NAr$  electrophiles

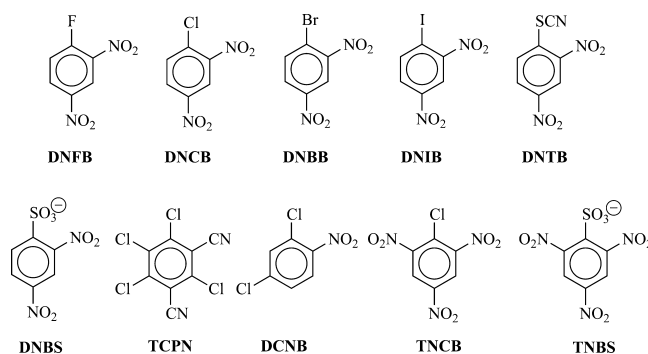


Figure 1.  $S_NAr$  electrophiles.

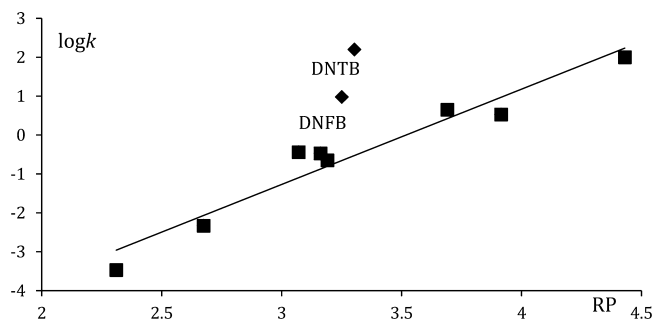


Figure 2. LFER plot of log  $k$  vs RP ( $\Sigma\sigma^- + 0.24\sigma^*$ ). DNFB and DNTB are excluded from the regression.

investigated by Natsch et al.<sup>11</sup> These  $S_NAr$  electrophiles were also investigated in our earlier QMM study<sup>9</sup> and are shown in Figure 1.

Figure 2 shows a plot of log  $k$  vs RP. The equation of the regression line is the LFER for reaction of  $S_NAr$  electrophiles with Cys-peptide:

$$\log k = 2.45(\pm 0.25)RP - 8.62(\pm 0.85) \quad (2)$$

where  $n = 8$ ,  $R^2 = 0.940$ , adj  $R^2 = 0.930$ ,  $s = 0.46$ , and  $F = 94$ .

The plot shows good linearity over the whole reactivity range, covering 5 orders of magnitude. There are two outliers: 2,4-dinitrofluorobenzene (DNFB) and 2,4-dinitrothiocyanatobenzene (DNTB). These two compounds, omitted from the regression (eq 2), are both more reactive to Cys-peptide than would be predicted from their substituent constants (RP). The atypical behavior of these two compounds are rationalized in the following sections.

**DNFB.** As discussed in detail in our previous paper,<sup>9</sup> the relative reactivity of DNFB, compared to other DNXB compounds, depends on the nature of the attacking nucleophile. If there is significant steric interaction between the attacking nucleophile and the X-group, DNFB has an advantage over the other DNXBs because of the small size of the fluorine atom. This steric advantage is not captured by the normal  $\sigma^*$  value for F ( $\sigma^*$  is a purely electronic parameter), and consequently, DNFB is a positive outlier in LFER plots of reactivity vs  $\sigma^*$ . To allow for this effect, adjusted values  $\sigma_{\text{adjF}}^*$  can be used. For reactions with simple nucleophiles, appropriate  $\sigma_{\text{adjF}}^*$  values are methoxide ion, 4.30; piperidine, 4.70; thiophenoxide ion, 3.55; and normal nonadjusted value, 3.21.<sup>9</sup> The adjustment required for thiophenoxide is much less than for the harder nucleophiles methoxide and piperidine: this is rationalized by the argument that for a soft nucleophile the transition state is formed relatively early, when the nucleophilic reaction center is still too distant for substantial steric interaction with the X-group.<sup>12</sup> However, this clearly does not apply to Cys-peptide. For Cys-peptide, a  $\sigma_{\text{adjF}}^*$  value of 5.99 would be required to bring DNFB into line. This is much larger than the  $\sigma_{\text{adjF}}^*$  values corresponding to the simple nucleophiles listed above. Since the reaction center of Cys-peptide is on a short branch of a long open chain multibranched molecule (Figure 3), steric interactions between the X-group

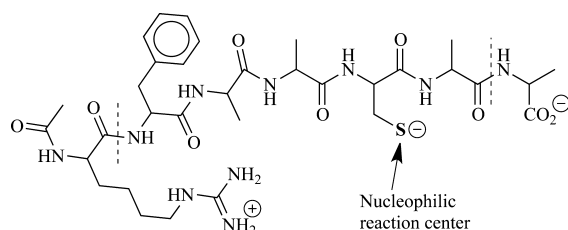
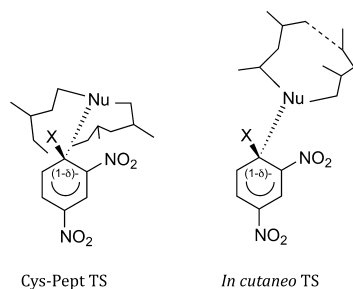


**Figure 3.** Structure of Cys-peptide. Broken vertical lines show amide bonds hydrolyzed in the formation of the  $m/z$  506.3 reaction product with DNTB.

of DNXB and Cys-peptide are likely to be substantial, in this case the interactions being between the X-group and the peptide chains rather than with the nucleophilic reaction center, as shown in Scheme 1. Consequently, for reaction with Cys-peptide, DNFB has a large steric advantage over the other  $S_N\text{Ar}$

#### Scheme 1. Transition States (TS) for $S_N\text{Ar}$ Reactions<sup>a</sup>



<sup>a</sup>In the Cys-peptide transition state, the peptide chains attached to the nucleophilic reaction center are flexible and can interact sterically with the X group. Avoidance of conformations enabling these steric interactions leads to a large negative entropy of activation. For the proposed in cutaneo transition state, the peptide chains attached to the nucleophilic center are constrained by participation in the protein tertiary structure and are unable to adopt conformations giving significant steric interaction with the X group.

electrophiles considered here. All of the other  $S_N\text{Ar}$  electrophiles are substantially less reactive to Cys-peptide than they would be but for the steric interactions with the leaving group.

**DNTB.** This compound is more than 500 times more reactive to Cys-peptide than predicted by eq 2. This suggests to us that DNTB reacts with Cys-peptide predominantly by a different pathway, not  $S_N\text{Ar}$ . This is consistent with the Supporting Information given by Natsch et al.:<sup>11</sup> analysis by LC-MS revealed peaks with  $m/z$  values at “917.4 (minor peak). Adduct with HSCN elimination (i.e.  $S_N\text{Ar}$  reaction product), 506.3 and 776.4. No explanation, peptide cleavage?”

The 776.4 peak is consistent with a reaction product in which the  $-\text{SH}$  group of Cys-peptide has been converted to  $-\text{SCN}$ , corresponding to the ionized SH group of Cys-peptide attacking the central carbon atom of the  $-\text{SCN}$  group of DNTB, with expulsion of 2,4-dinitrothiophenolate ion. The 506.3 peak is consistent with the 776.4 compound having lost an amino acid unit from each end of the chain by hydrolysis, i.e., Ac-RFAAC(CN)AA has been hydrolyzed to FAAC(CN)A. Reaction of DNTB by attack of nucleophiles at the central C atom of the  $-\text{SCN}$  group is well established in the literature.<sup>13,14</sup>

Thus, the experimental LC/MS evidence of Natsch et al.,<sup>11</sup> supported by the literature, indicates that with Cys-peptide DNTB reacts mainly, not as an  $S_N\text{Ar}$  electrophile, but as a  $-\text{CN}$  transfer agent.

#### QMMs for Sensitization Potency of $S_N\text{Ar}$ Electrophiles.

Table 1 shows the pEC3 values of the  $S_N\text{Ar}$  electrophiles discussed here. Figure 4 shows a plot of pEC3 vs  $\log k$  (pEC3

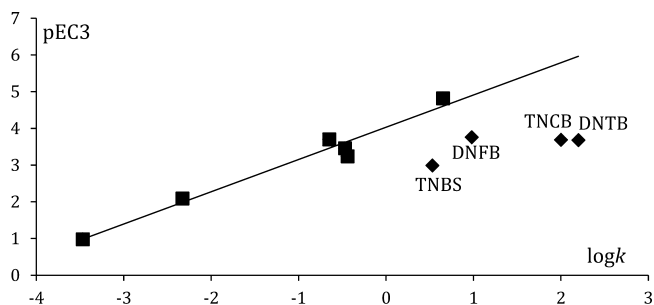


**Figure 4.** QMM plot based on measured rate constants: pEC3 vs  $\log k$ .

values and  $\log k$  values as reported by Natsch et al.<sup>11</sup>). Linear regression analysis gives the QMM:

$$\text{pEC3} = 0.88(\pm 0.08) \log k_{(\text{Cys-peptide})} + 4.03(\pm 0.15) \quad (3)$$

where  $n = 6$ ,  $R^2 = 0.966$ ,  $\text{adj } R^2 = 0.957$ ,  $s = 0.28$ , and  $F = 114$ .

The two tri-nitro compounds TNCB and TNBS are negative outliers, as previously found by Natsch et al.<sup>11</sup> and rationalized by the suggestion that they may largely get quenched in the surface layers of the skin. DNFB and DNTB are also negative outliers. As regards DNFB, this suggests that DNFB has less of a steric advantage in its reaction in cutaneo than it does in its reaction with Cys-peptide. As regards DNTB, since it reacts with Cys-peptide mainly by a non- $S_N\text{Ar}$  mechanism, there is no reason to expect it to fit the Cys-peptide reactivity QMM for  $S_N\text{Ar}$  electrophiles.

Figure 5 shows a plot of pEC3 vs the reactivity parameter RP ( $= \Sigma \sigma^- + 0.24 \sigma^*$ ) with the normal  $\sigma^*$  value of 3.21 used for F. Linear regression analysis gives the QMM:

$$\text{pEC3} = 2.79(\pm 0.11) \text{RP} - 5.39(\pm 0.33) \quad (4)$$

where  $n = 8$ ,  $R^2 = 0.992$ ,  $\text{adj } R^2 = 0.990$ ,  $s = 0.12$ , and  $F = 706$ .

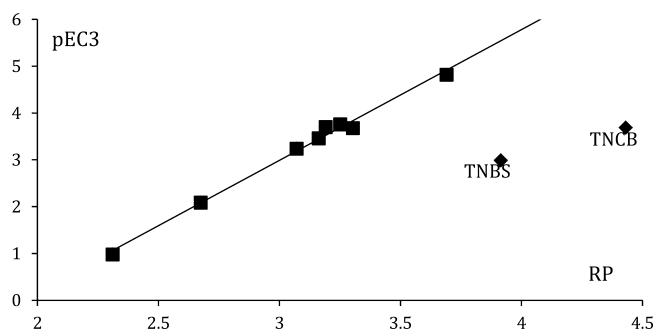


Figure 5. QMM plot based on calculated reactivity parameters: pEC3 vs RP.

Again the two trinitro compounds are negative outliers, as we found in our previous analysis based on substituent constants.<sup>9</sup> At the time (2011) we rationalized this by the argument that for these compounds with three strong activating groups, the intermediate is stabilized to such an extent that the departure of the leaving group from the intermediate becomes the rate determining step, i.e., this step occurs more slowly than the reversible formation of the intermediate. Since the reactivity parameter RP models the rate of formation of the intermediate, it overpredicts the overall rate if the departure of the leaving group is rate determining, and consequently, in this situation eq 4 overpredicts the potency in the LLNA.

From the findings of Natsch et al.<sup>11</sup> we can now infer a simpler and, to us, more convincing explanation for why TNBC and TNBS are negative outliers to eq 4. For both compounds, Natsch et al.<sup>11</sup> observed not only displacement of chloride ion or sulphite ion, but also displacement of a nitro group as nitrite ion. We therefore calculated RP values for TNBC and TNBS based on the assumption that the leaving group is one of the nitro groups ortho to the chloro or sulpho substituent.

For TNBC this calculation is straightforward:

$$\begin{aligned}\Sigma\sigma^- &= 2\sigma^-(m\text{-NO}_2) + \sigma^-(o\text{-Cl}) \\ &= 2 \times 0.74 + 0.68 \\ &= 2.16\end{aligned}$$

$$0.24\sigma^* = 0.24 \times 4.25 = 1.02$$

$$\text{RP} = \Sigma\sigma^- + 0.24\sigma^* = 3.18$$

For TNBS we need to apply a similar calculation, using  $\sigma^-(o\text{-SO}_3^-)$  in place of  $\sigma^-(o\text{-Cl})$ . However, Perrin et al.<sup>15</sup> do not give a value for  $o\text{-SO}_3^-$ , so we have to estimate it by analogy with the  $\text{SO}_2^-$  substituent, for which Perrin et al.<sup>15</sup> give the values  $\sigma_m = -0.02$  and  $\sigma_o^- = 0.21$ , i.e., a difference of 0.23. Assuming this difference applies to the  $\text{SO}_3^-$  substituent, we estimate  $\sigma^-(o\text{-SO}_3^-)$  as

$$\sigma(m\text{-SO}_3^-) + 0.23 = 0.31 + 0.23 = 0.54$$

This gives  $\text{RP} = 3.04$ .

Using these RP values based on displacement of the nitro group, we can calculate pEC3 values from eq 4. These are shown, and compared with the experimental values, in Table 1. The experimental pEC3 values agree well with the values calculated on the basis of  $-\text{NO}_2$  being the leaving group in the reaction leading to sensitization.

Including TNBC and TNBS in the regression, with their RP values calculated on the basis of  $-\text{NO}_2$  being the leaving group, gives a QMM, not significantly different from eq 4, but now covering all 10  $\text{S}_\text{N}\text{Ar}$  electrophiles studied:

$$\text{pEC3} = 2.81(\pm 0.12)\text{RP} - 5.44(\pm 0.36) \quad (5)$$

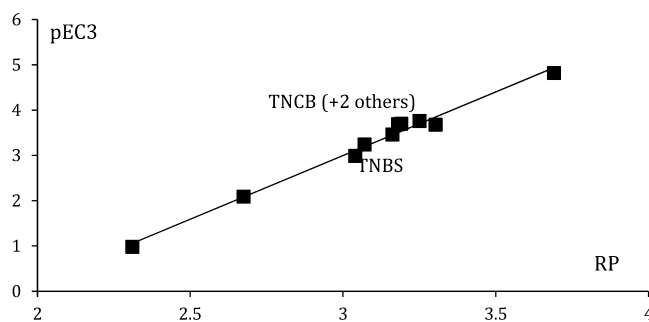


Figure 6. QMM plot based on calculated reactivity parameters: pEC3 vs RP, with RP based on  $\text{NO}_2$  as the leaving group for TNBC and TNBS.

where  $n = 10$ ,  $R^2 = 0.987$ ,  $\text{adj } R^2 = 0.985$ ,  $s = 0.13$ , and  $F = 594$ . This is shown in Figure 6.

In contrast to the situation with the log  $k$ -based QMM (Figure 4), for the RP-based QMMs both DNFB and DNTB are well predicted. Since the RP is based on the assumption that the  $\text{S}_\text{N}\text{Ar}$  mechanism applies and does not take account of steric effects, the fact that DNFB and DNTB fit this QMM indicates the following:

- The in cutaneo nucleophile(s) involved in sensitization by  $\text{S}_\text{N}\text{Ar}$  electrophiles is/are not sterically hindered.
- The in cutaneo nucleophile(s) involved in sensitization by  $\text{S}_\text{N}\text{Ar}$  electrophiles react(s) with DNTB by the  $\text{S}_\text{N}\text{Ar}$  pathway, rather than by nucleophilic attack at the carbon atom of the SCN group.

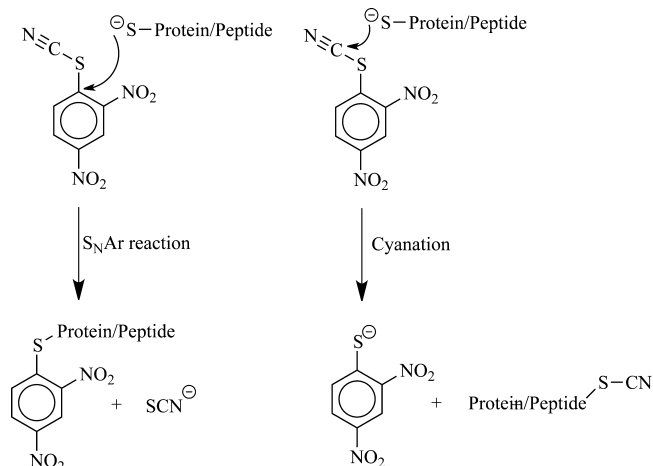
## DISCUSSION

Overall the high statistical quality of the RP-log  $k$  LFER (eq 2) supports the reliability of the Cys-peptide kinetic method used by Natsch et al.<sup>11</sup> and the validity of RP, a combination of Hammett and Taft substituent constants, as a reactivity parameter for  $\text{S}_\text{N}\text{Ar}$  electrophiles. The high statistical quality of both the kinetics-based QMM (eq 3) and the RP-based QMM (eq 4) demonstrates a strong quantitative correlation between in chemico reactivity to Cys-peptide and in cutaneo reactivity to the protein nucleophile(s) involved in skin sensitization by  $\text{S}_\text{N}\text{Ar}$  electrophiles. However, there are some outliers to these models, and these provide insights into the differences in chemical properties between Cys-peptide and the in cutaneo nucleophiles. Below we discuss each case in turn.

**DNFB.** The fact that DNFB potency is well predicted by its RP but not by its log  $k$  (Cys-peptide) value indicates a difference between the in cutaneo nucleophile(s) and Cys-peptide: Cys-peptide is substantially sterically hindered, whereas the in cutaneo nucleophile is not hindered. This can be explained by the open chain nature of Cys-peptide (Figure 3), whereas the in vivo nucleophile is presumably part of a protein tertiary structure with the  $\text{CH}_2\text{SH}$  group (if that is the nucleophilic center) exposed. DNFB is 43 times as reactive to Cys-peptide as predicted by the LFER (eq 2) based on the other leaving groups. This factor of 43 gives a minimum value for the degree to which the other  $\text{S}_\text{N}\text{Ar}$  electrophiles are less reactive toward Cys-peptide than they would be in the absence of any steric effect greater than that experienced by DNFB. Consequently, the in cutaneo nucleophile(s) involved in sensitization by  $\text{S}_\text{N}\text{Ar}$  electrophiles must be at least 43 times as reactive as Cys-peptide.

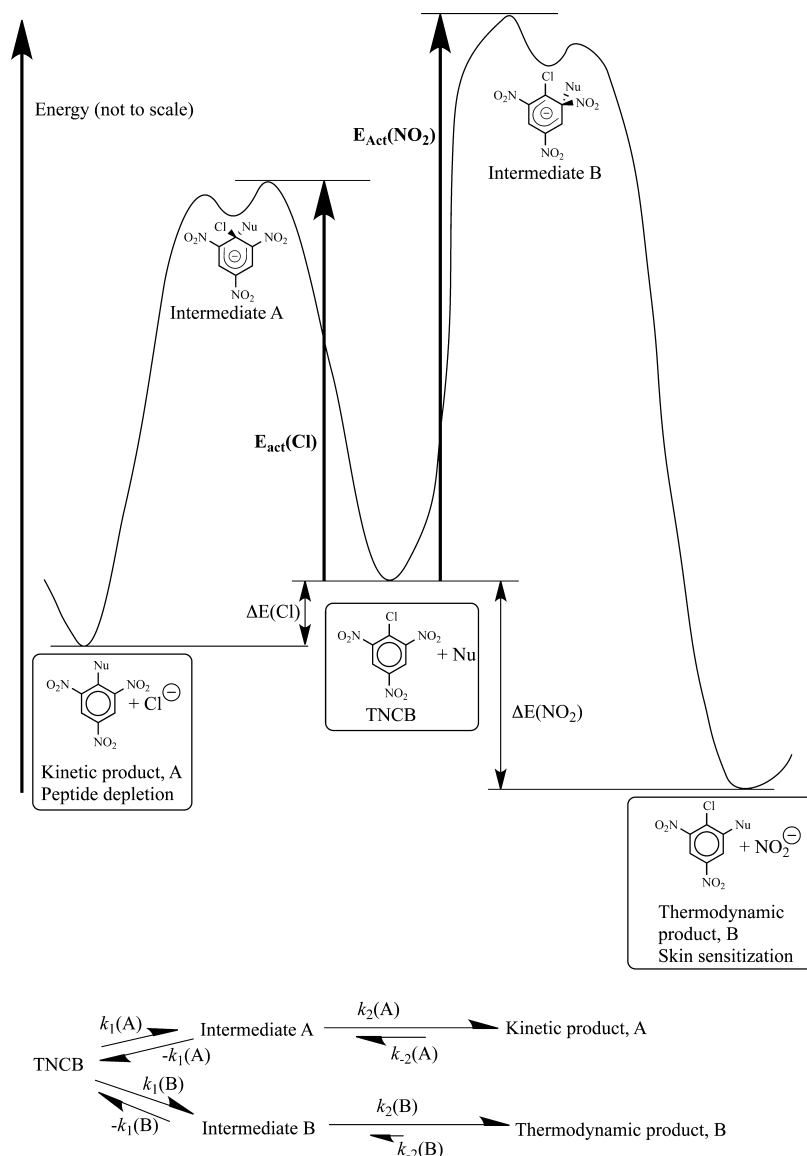
**DNTB.** DNTB reacts with Cys-peptide predominantly by a non- $\text{S}_\text{N}\text{Ar}$  mechanism, by attack at the C of the SCN group, and this explains why its reactivity is underpredicted by the LFER.

Scheme 2. Reactions of DNTB with Thiolate Nucleophiles



It is reasonable to presume that this electrophilic reaction site is less sterically hindered than the aromatic carbon that is attacked in the S<sub>N</sub>Ar reaction. However, the LLNA potency of DNTB is well predicted from its RP by the S<sub>N</sub>Ar QMM. This implies that in the reaction leading to sensitization, DNTB reacts as an S<sub>N</sub>Ar electrophile. The fact that Cys-peptide and the in cutaneo nucleophile(s) have different reaction pathways with DNTB (Scheme 2) may again reflect the much greater steric effects applying to Cys-peptide.

**TCNB and TNBS.** For these two compounds, the most reactive electrophilic center is the carbon atom bearing the Cl or SO<sub>3</sub><sup>−</sup> group, and consistent with this, with the RP calculated on this basis, TNCB and TNBS fit the LFER for Cys-peptide reactivity (eq 2). However, the Cys-peptide reaction product mixtures contain not only the Cl or SO<sub>3</sub><sup>−</sup> displacement product but also the NO<sub>2</sub> displacement product,<sup>11</sup> and we find that TNCB and TNBS fit the sensitization QMM when we base RP

Scheme 3. Reaction Pathways for TNCB<sup>a</sup>

<sup>a</sup>This scheme also applies to TNBS, with SO<sub>3</sub><sup>−</sup> in place of Cl. One of the *o*-NO<sub>2</sub> groups is shown as being displaced in the thermodynamic pathway; displacement of the *p*-NO<sub>2</sub> group is an alternative possibility. On the basis of RP values, referring to the lower part of the scheme,  $k_1(A) > k_1(B)$ , so A is the earlier dominant product. However, if  $k_{-2}(A)$  is significant (consistent with a high RP value for A) and  $k_{-2}(B)$  is insignificant (consistent with a low RP value for B), the final dominant product is the more thermodynamically stable B.

on NO<sub>2</sub> displacement (Table 1, eq 5). This seems to be a straightforward case (although not confidently predictable in advance without the experimental data on the reaction product compositions) of kinetic control vs thermodynamic control, as illustrated in Scheme 3 for TNCB. The most reactive site in TNCB is at the Cl position, and the Cys-peptide is initially depleted predominantly by reaction at this position via reactions  $k_1(A)$  and  $k_2(A)$  rapidly reaching an equilibrium position. However, the initial Cl-displacement product A is gradually transformed, via reactions  $k_{-2}(A)$ ,  $k_{-1}(A)$ ,  $k_1(B)$ , and  $k_2(B)$  to the thermodynamically more stable NO<sub>2</sub> displacement product. In the Cys-peptide depletion experiments, the reaction sequence  $k_{-2}(A)$ ,  $k_{-1}(A)$ ,  $k_1(B)$ , and  $k_2(B)$  does not have time to reach completion, so both adducts are observed. The in cutaneo nucleophile(s), as argued above, is/are more reactive, and the formation of the stable NO<sub>2</sub>-displacement product is what leads to sensitization. This interpretation is based on formation of the Cl or SO<sub>3</sub><sup>-</sup> displacement product A being reversible and formation of the NO<sub>2</sub> displacement product B being essentially irreversible. For present purposes, we can define “essentially irreversible” as “the reverse reaction is too slow to occur to a significant extent in the time scale of the process under consideration.” The reaction product A has three *o*-/*p*-NO<sub>2</sub> activating groups, and the ipso group is -SR ( $s^*$  for -SMe is 1.56, larger than  $s^*$  for the SO<sub>3</sub><sup>-</sup> group in TNBS), so the product A should have high reactivity (higher than that of TNBS) as an S<sub>N</sub>Ar electrophile; therefore, displacement of the SR group by attack of chloride ion or sulphite ion is not unreasonable. There is literature evidence that chloride ion and sulfite ion can act as nucleophiles in S<sub>N</sub>Ar reactions,<sup>10,16–18</sup> so it is reasonable to consider the Cl and SO<sub>3</sub><sup>-</sup> displacement reactions of TNCB and TNBS with thiols RS<sup>-</sup> as being significantly reversible. The equilibrium will lie heavily on the TN(SR)B side (RS<sup>-</sup> being a much more reactive nucleophile than Cl<sup>-</sup>); so in terms of cysteine peptide depletion the reverse reaction will not be significant, but in terms of the product distribution (in the cys peptide reaction) and protein haptentation (in the skin sensitization process), the reverse reaction together with an alternative, essentially irreversible, pathway has a significant impact. In contrast, the nitro-group displacement reaction leading to B can be considered as essentially irreversible: the reaction product B has low reactivity (RP values 2.53 for B(Cl) and 2.39 for B(SO<sub>3</sub><sup>-</sup>), similar to DCNB, 2.31), and nitrite ion is a relatively poor nucleophile compared to thiolate.<sup>16</sup>

## CONCLUSIONS

The analysis presented here provides further demonstration of the strong dependence of sensitization potency on reaction chemistry.

From comparison of the behavior of DNFB in the QMMs based on Cys-peptide kinetics (positive outlier) and on the reactivity parameter RP (well fitted), we have been able to infer a significant difference between the model Cys-peptide and the in cutaneo nucleophile(s) involved in skin sensitization: the nucleophilic reaction center in the latter is much less sterically hindered and in consequence is estimated to be at least 43 times as reactive as the model Cys-peptide. This interpretation rationalizes the differing behavior of DNTB in the model Cys-peptide system (reaction by a non-S<sub>N</sub>Ar pathway and positive outlier in the kinetics-based QMM) as compared to in cutaneo (potency well fitted by the S<sub>N</sub>Ar QMM based on RP). This finding is also consistent with the report by Elbayed et al.<sup>19</sup> that reactions of allergens with protein nucleophiles in reconstructed

human epidermis are much faster than reactions in solution with model peptides or proteins.

The findings with TCNB and TNBS are interpreted as a case of sensitization being driven by the thermodynamically favored rather than the kinetically favored reaction product. This is to our knowledge the first such case to be identified. As a general principle, we suggest that such cases may be, if not confidently predicted, at least noted as possible, where (a) the rate constant for the reverse reaction of the kinetic product can be predicted, or shown experimentally, to be similar in magnitude to those for known sensitizers in the same reaction mechanistic domain; (b) the rate constant for the formation of the thermodynamic product can be predicted, or shown experimentally, to be similar in magnitude to those for known sensitizers in the same reaction mechanistic domain. It is worth noting, however, that failure to predict a case of thermodynamically driven sensitization will lead to overestimation of potency, not underestimation.

OECD has published a series of reports on “The Adverse Outcome Pathway for Skin Sensitization Initiated by Covalent Binding to Proteins,” which presents the available mechanistic knowledge of the sensitization response within an adverse outcome pathway (AOP).<sup>20,21</sup> There are AOP-based risk assessment approaches under development,<sup>22</sup> where AOP is used as the mechanistic basis for physiologically- and mechanistically-based toxicokinetic–toxicodynamic models of the sensitization response. The importance of reactivity as a determinant of sensitization potency and the value of quantitative reactivity data as an input for predicting potency is well recognized.<sup>2,4,11,23,24</sup> In this article, we have demonstrated how quantitative reactivity data can be used to model sensitization potency in the LLNA. LLNA EC3 values for already tested chemicals can be useful in risk assessment for human exposure. In the same way, the predicted EC3s from QMMs like the one here have a useful role to play in risk assessment. Although our model has been developed using LLNA potency data, the same principles apply for sensitization potency in general, and the model should predict relative potencies of S<sub>N</sub>Ar chemicals for other skin sensitization end-points including human sensitization.

Finally, we note that, although the focus of this article is on mechanism-based modeling of skin sensitization potency, S<sub>N</sub>Ar chemistry is currently an area of interest in the field of protein immobilization and biocatalytic enzyme chip technology,<sup>25</sup> and some of our findings may also be of relevance in this field.

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### Notes

The authors declare no competing financial interest.

## ABBREVIATIONS

QMM, quantitative mechanistic model; LLNA, local lymph node assay; DPRA, direct peptide reactivity assay; LFER, linear free energy relationship; RP, reactivity parameter; DNXB, 2,4-dinitro-X-substituted benzenes; DNCB, 2,4-dinitrochlorobenzene; DNFB, 2,4-dinitrofluorobenzene; DNBB, 2,4-dinitrobenzobenzene; DNIB, 2,4-dinitroiodobenzene; DNTB, 2,4-dinitrothiocyanatobenzene; DNBS, 2,4-dinitrobenzenesulfonic acid; TCPN, tetrachloroisophthalonitrile; DCNB, 2,4-dichloronitrobenzene; TNCB, 2,4,6-trinitrochlorobenzene; TNBS, 2,4,6-trinitrobenzenesulfonic acid; OECD, organization for economic cooperation and development; AOP, adverse outcome pathway

## ■ REFERENCES

- (1) Roberts, D. W., and Aptula, A. O. (2008) Determinants of skin sensitisation potential. *J. Appl. Toxicol.* 28, 377–387.
- (2) Roberts, D. W., and Williams, D. L. (1982) The derivation of quantitative correlations between skin sensitisation and physicochemical parameters for alkylating agents and their application to experimental data for sultones. *J. Theor. Biol.* 99, 807–825.
- (3) Roberts, D. W., Aptula, A. O., and Patlewicz, G. (2006) Mechanistic applicability domains for non-animal based prediction of toxicological endpoints. QSAR analysis of the Schiff base applicability domain for skin sensitization. *Chem. Res. Toxicol.* 19, 1228–1233.
- (4) Roberts, D. W., Aptula, A. O., and Patlewicz, G. (2007) Electrophilic chemistry related to skin sensitization. Reaction mechanistic applicability domain classification for a published data set of 106 chemicals tested in the mouse local lymph node Assay. *Chem. Res. Toxicol.* 20, 44–60.
- (5) Roberts, D. W., and Natsch, A. (2009) High throughput kinetic profiling approach for covalent binding to peptides: Application to skin sensitization potency of Michael acceptor electrophiles. *Chem. Res. Toxicol.* 22, 592–603.
- (6) Landsteiner, K., and Jacobs, J. (1936) Studies on the sensitization of animals with simple chemical compounds. *J. Exp. Med.* 64, 643–655.
- (7) Roberts, D. W. (1995) Linear free energy relationships for reactions of electrophilic halo- and pseudohalobenzenes, and their application in prediction of skin sensitization potential for  $S_NAr$  electrophiles. *Chem. Res. Toxicol.* 8, 545–551.
- (8) Mekenyan, O., Roberts, D. W., and Karcher, W. (1997) Molecular orbital parameters as predictors of skin sensitization potential of halo- and pseudohalobenzenes acting as  $S_NAr$  electrophiles. *Chem. Res. Toxicol.* 10, 994–1000.
- (9) Roberts, D., Aptula, A. O., and Patlewicz, G. (2011) Chemistry-based risk assessment for skin sensitization: Quantitative mechanistic modeling for the  $S_NAr$  domain. *Chem. Res. Toxicol.* 24, 1003–1011.
- (10) Terrier, F. (2013) *Modern Nucleophilic Aromatic Substitution*, Wiley, Weinheim, Germany.
- (11) Natsch, A., Haupt, T., and Laue, H. (2011) Relating skin sensitizing potency to chemical reactivity: Reactive Michael acceptors inhibit NF- $\kappa$ B signaling and are less sensitizing than  $S_NAr$ - and  $S_N2$ -reactive chemicals. *Chem. Res. Toxicol.* 24, 2018–2027.
- (12) Bartoli, G., and Todesco, P. E. (1977) Nucleophilic substitution. Linear free energy relationships between reactivity and physical properties of leaving groups and substrates. *Acc. Chem. Res.* 10, 125–132.
- (13) Giles, D. E., and Parker, A. J. (1973) Mechanisms of aromatic nucleophilic substitution by the ambident thiocyanate ion. *Aust. J. Chem.* 26, 273–299.
- (14) Reddy, V. A. (1983) Atypical reaction of ‘essential’ sulfhydryl groups of malic enzyme with 2-nitro-5-thiocyanobenzoate and 2,4-dinitrophenylthiocyanate. *Biochim. Biophys. Acta* 743, 268–280.
- (15) Perrin, D. D., Dempsey, B., and Serjeant, E. P. (1981) *pKa Prediction for Organic Acids and Bases*, Chapman and Hall, London.
- (16) Broxton, T. J., Muir, D. M., and Parker, A. J. (1975) Aromatic nucleophilic substitution reactions of ambident nucleophiles. 111. Reactivity of the nitrite ion. *J. Org. Chem.* 40, 3230–3233.
- (17) Lamm, B. (1959) A halogen exchange reaction in a diazonium compound. *Acta Chem. Scand.* 13, 1254–1255.
- (18) Suschitzky, H., and Wakefield, B. J. (2000) Aromatic Fluorine Chemistry at Salford, in *Fluorine Chemistry at the Millennium; Fascinated by Fluorine* (Banks, R. E., Ed.), Elsevier Science, Oxford, U.K.
- (19) Elbayed, K., Berl, V., Debeuckelaere, C., Moussallieh, F.-M., Piotto, M., Namer, I.-J., and Lepoittevin, J.-P. (2013) HR-MAS NMR spectroscopy of reconstructed human epidermis: Potential for the in situ investigation of the chemical interactions between skin allergens and nucleophilic amino acids. *Chem. Res. Toxicol.* 26, 136–145.
- (20) OECD. 2012a. The Adverse Outcome Pathway for skin sensitisation initiated by covalent binding to proteins. Part 1. *Series on Testing and Assessment*. No. 168. ENV/JM/MONO(2012)10/PART1.
- (21) OECD. 2012b. The Adverse Outcome Pathway for skin sensitisation initiated by covalent binding to proteins. Part 2: Use of the AOP to develop chemical categories and integrated assessment and testing approaches. *Series on Testing and Assessment*. No. 168. ENV/JM/MONO(2012)/PART2.
- (22) MacKay, C., Davies, M., Summerfield, V., and Maxwell, G. (2013) From pathways to people: applying the adverse outcome pathway (AOP) for skin sensitisation to risk assessment. *ALTEX* 30, 473–486.
- (23) Aleksic, M., Thain, E., Roger, D., Saib, O., Davies, M., Li, J., Aptula, A., and Zazzaroni, R. (2009) Reactivity profiling: Covalent modification of single nucleophile peptides for skin sensitization risk assessment. *Toxicol. Sci.* 108, 401–411.
- (24) Gutsell, S., and Russell, P. (2013) The role of chemistry in developing understanding of adverse outcome pathways and their application in risk assessment. *Toxicol. Res.* 2, 299–307.
- (25) Voelker, A. E., and Viswanathan, R. (2013) Synthesis of a suite of bioorthogonal glutathione S-transferase substrates and their enzymatic incorporation for protein immobilization. *J. Org. Chem.* 78, 9647–9658.