

Allergic Contact Dermatitis—Formation, Structural Requirements, and Reactivity of Skin Sensitizers

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Contact allergy is caused by a wide range of chemicals after skin contact. Its clinical manifestation, allergic contact dermatitis (ACD), is developed upon repeated contact with the allergen. This perspective focuses on two areas that have yielded new useful information during the last 20 years: (i) structure–activity relationship (SAR) studies of contact allergy based on the concept of hapten–protein binding and (ii) mechanistic investigations regarding activation of nonsensitizing compounds to contact allergens by air oxidation or skin metabolism. The second area is more thoroughly reviewed since the full picture has previously not been published. Prediction of the sensitizing capacity of a chemical is important to avoid outbreaks of ACD in the population. Much research has been devoted to the development of in vitro and in silico predictive testing methods. Today, no method exists that is sensitive enough to detect weak allergens and that is robust enough to be used for routine screening. To cause sensitization, a chemical must bind to macromolecules (proteins) in the skin. Expert systems containing information about the relationship between the chemical structure and the ability of chemicals to haptenate proteins are available. However, few designed SAR studies based on mechanistic investigations of prohaptens have been published. Many compounds are not allergenic themselves but are activated in the skin (e.g., metabolically) or before skin contact (e.g., via air oxidation) to form skin sensitizers. Thus, more basic research is needed on the chemical reactions involved in the antigen formation and the immunological mechanisms. The clinical importance of air oxidation to activate nonallergenic compounds has been demonstrated. Oxidized fragrance terpenes, in contrast to the pure terpenes, gave positive patch test reactions in consecutive dermatitis patients as frequently as the most common standard allergens. This shows the importance of using compounds to which people are exposed when screening for ACD in dermatology clinics.

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1. Introduction

Contact allergy is caused by a wide range of chemicals after prolonged or repeated contact with the skin. In the western world, 15–20% of the population are allergic to one or more chemicals in their environment (*1*). Allergic contact dermatitis (ACD)¹ is the clinical manifestation of contact allergy and is developed at the site of contact when a sensitized individual is exposed to the allergen in a concentration exceeding the individual threshold. The dermatitis (eczema) can develop anywhere on the body depending on exposure, but the hands

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¹ Abbreviations: ACD, allergic contact dermatitis; ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; DC, dendritic cell; Calc, cinnamic alcohol; EPR, electron paramagnetic resonance; FMO, flavin-containing monooxygenase; FM, fragrance mix; GSH, glutathione; LLNA, local lymph node assay; LC, Langerhans cell; P450, cytochrome P450; PPD, *p*-phenylenediamine; QSAR, quantitative structure–activity relationship; RAI, relative alkylation index; SAR, structure–activity relationship; SI, stimulation index.



Figure 1. "...most people regard other people's skin disease as trivial, but those with skin disease at some time in their life never regard it as trivial" (5).



Figure 2. Patch test technique. A standard series of the most frequent skin sensitizers is applied on the back of a patient with suspected ACD. The sensitizers are mixed in suitable vehicles, most often petrolatum, to nonirritating and nonsensitizing concentrations. The patches remain on the skin for 48 h, whereafter they are removed. Evaluation of possible skin reactions is performed on days 4 and 7 after application of the patch tests. A small eczematous reaction shows that the patient is allergic to the compound applied on that specific spot (4).

are the sites most exposed to chemicals and are thus most affected. Hand eczema causes a significantly reduced health-related quality of life for the affected individual (2) and is also of major economic consequence for society (3) (Figure 1). The diagnosis and therapy of ACD are still about the same as 50 years ago. Contact allergy is diagnosed in dermatitis patients by patch testing using a standard tray of about the 30 most frequent contact allergens, diluted in petrolatum or water (4) (Figure 2). A small eczematous reaction at a test site indicates that the patient is allergic to the compound applied on this site. There is no cure for ACD, and the treatment is still only symptomatic, using anti-inflammatory corticosteroids. As an acquired contact allergy is chronic and only avoidance of the offending agent can prevent one from elicitation of ACD, contact allergy to common allergens (e.g., nickel and fragrances) can cause life-long dermatitis.

For this reason, there is a continuous, ongoing regulatory process to decrease exposure or to forbid the use of potent contact allergens. A recent review with historical examples on actions to control contact allergy epidemics shows that strict regulation of exposure has caused a decrease in new cases of ACD (6). It is important not only to stop epidemics but also to hinder their onset by preventing strong allergens from entering the market. In this protective regulatory work, chemistry-oriented contact allergy research is essential. Especially, two areas have yielded new useful information during the last 20 years: (i) structure–activity relationship (SAR) studies of contact allergy based on the concept of hapten–protein binding and (ii)

mechanistic investigations regarding activation of nonsensitizing compounds to contact allergens by air oxidation or skin metabolism.

Of these areas, SAR/quantitative structure–activity relationship (QSAR) studies and their implication for contact allergy have been frequently reviewed over the years and will therefore not be scrutinized in detail. Instead, references are given to other publications. The second area including activation of nonsensitizing compounds to contact allergens will be more thoroughly reviewed since no review containing a full picture has been published.

2. Mechanisms of Contact Allergy

Contact allergy is regarded as a delayed hypersensitivity reaction (type IV hypersensitivity), which is mediated by antigen-specific T-lymphocytes (7). To develop ACD, an individual must first be sensitized by skin contact with reactive chemicals of low molecular weight (<1000) and appropriate lipophilicity ($\log P \sim 2$) which are able to penetrate into the epidermis. As the chemicals themselves are too small to cause an immunogenic reaction, they must be able to react with macromolecules in the skin (usually considered to be proteins), which thus are haptenated. Reactions may theoretically occur with both soluble proteins and membrane-bound proteins present on the surface of the Langerhans cells (LCs). LCs are the professional antigen-presenting dendritic cells (DCs) present in the epidermis. They become activated by the haptenated protein and migrate via the afferent lymph vessels to the draining lymph nodes where they interact with and present the processed haptenated protein (the antigen) to naive T-cells. These interactions result in the formation of antigen-specific effector and memory T-cell clones, which thereafter circulate in the blood and lymph vessels. Upon renewed contact with the same chemical, memory T-cells are recruited to the site of contact, where interactions between T-cells and antigen-presenting cells can take place directly in the epidermis, thus initializing the inflammatory process (Figure 3).

3. Testing for Allergenic Properties

The prediction of the sensitizing capacity of a chemical is of importance to avoid outbreaks of ACD in the population. The most reliable methods for prediction of sensitizing capacity still involve animal testing. A challenge for scientists in the field is the ban on in vivo testing of cosmetic and toiletry ingredients for skin-sensitizing properties, which will come into force in the European Union in 2013 (8). Hence, much research has been devoted to the development of in vitro and in silico predictive testing methods. Because the mechanism of contact allergy is complex and partly poorly understood, the efforts thus far have shown only limited success (9–11).

3.1. Animal Testing. Currently, the murine local lymph node assay (LLNA) is the recommended and most widely used predictive method for the identification and potency assessment of contact allergens (12). The LLNA has been evaluated and accepted by the U.S. Food and Drug administration (FDA) and is recommended by the Organization for Economic Cooperation and Development (OECD Guideline for the Testing of Chemicals 429. Skin Sensitisation: Local Lymph Node Assay) as a stand-alone test method for the determination of sensitizing capacity. The LLNA offers reliable information for hazard identification and is therefore used in risk assessment in industry (13). In contrast with previously used predictive methods [e.g., the guinea pig maximization test, GPMT, and the Buehler test

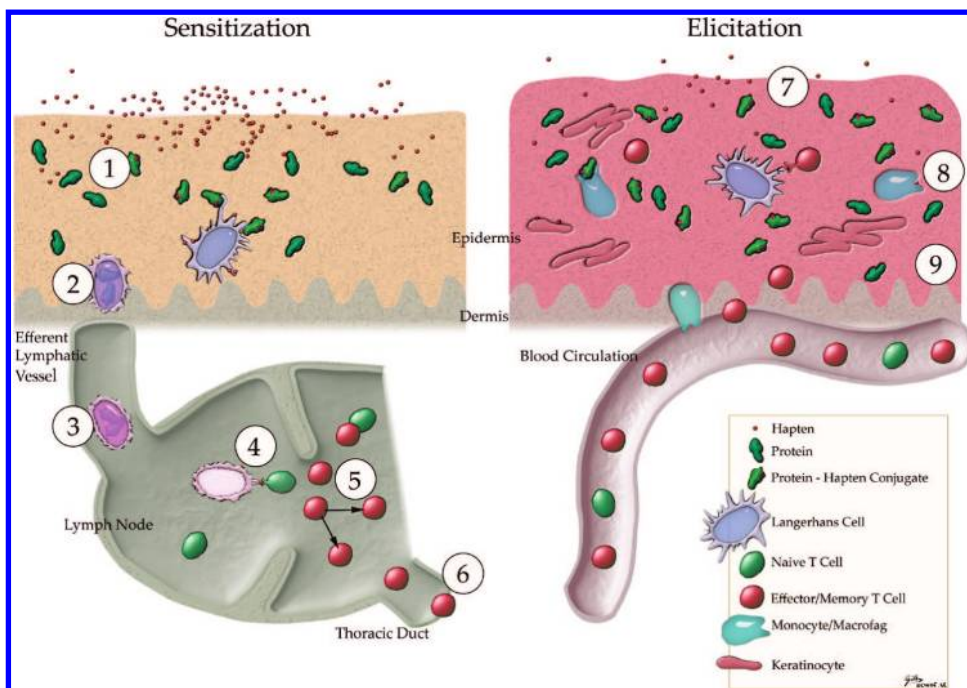


Figure 3. (1) Binding of haptens to proteins and other macromolecules. (2) Internalization of hapten-modified proteins. (3) Hapten-induced activation of LCs that migrate and process hapten–protein complexes. (4) Presentation of antigens by LCs to naive specific T-cells. (5) Proliferation of antigen specific T-cells; memory T-cells are formed. (6) Hapten-specific memory T-cells leave the lymph node and enter the circulation. (7) Re-exposure to the hapten. (8) Release of cytokines and chemokines attracting cells to the skin from the circulation. (9) Inflammatory response within 24–48 h, symptoms of ACD.

in guinea pigs (14)], the LLNA also offers a possibility to determine a quantitative response of the sensitizing effect of a chemical, which makes the results (dose–response relation) from this method useful in QSAR studies. The assay is based on the fact that there is a correlation between the vigor of a proliferative response induced in the local draining lymph nodes by topically applied chemicals and the extent of sensitization developed (15). The results are expressed as a stimulation index (SI), that is, the proliferative response ratio between test and control groups. Test materials that at one or more concentrations cause an SI of 3 or higher are considered to be positive in the LLNA, and calculated EC₃ values (the estimated concentration required to produce an SI of 3) are used to compare the sensitizing capacity of different test materials (16). Thus, in contrast with human patch test methods and the previously used guinea pig tests, the end point in the LLNA is induction and not elicitation. It is important that the LLNA is used with care since it does not clearly discriminate between irritants and allergens, which could result in irritant compounds, typically when tested in high concentrations, being classified as skin sensitizers (17, 18).

3.2. In Vitro Methods. An in vitro approach to predict the skin-sensitizing potential of a chemical should be designed to include its potential to penetrate into viable epidermis, react with macromolecules, and initiate an antigen-specific immune response. Possible metabolic activation of nonreactive compounds must also be considered. A review on in vitro assessments of the sensitizing activity of low molecular weight compounds was recently published (19). The availability of peripheral blood-derived human DCs was a significant improvement in the strive to develop in vitro predictive assays (20–22). Another step forward was the possibility of using the real-time polymerase chain reaction (RT-PCR) instead of direct measurements of proteins to investigate changes in activation markers after induction with a contact allergen (23).

The novel in vitro assays should allow assessments using human cell systems, which would be advantageous, for example,

with regard to the metabolism of prohaptens, which could be species-dependent. Two cell types are predominantly involved in this process, that is, LCs and keratinocytes. Human DCs have been employed to assess the sensitizing potential of various chemical agents in vitro, as exposure to contact allergens is known to alter both cell surface marker profiles and gene expression of immature DCs (19, 24). Recently, Aebly et al. (25) demonstrated that DC exposure to the contact sensitizer 2,4,6-trinitrobenzenesulphonic acid induced a dose-dependent modulation of genes associated with DC maturation (i.e., IL-1 β , AQP3), whereas exposure to the model irritant compound, sodium dodecyl sulphate (SDS), did not induce significant gene regulation. The impact of chemical exposure on DCs and the potential use of such information in the development of cell-based assays for assessing skin sensitization potential of chemicals in vitro have been reviewed by Ryan et al. (10).

Many studies show a clear correlation between the chemical reactivity of a compound (mainly the reactivity towards nucleophiles) and its sensitizing potency (see below). Because binding to the thiol group of cysteine is the one most often associated with sensitizers (26), the ability to quantify conjugation with glutathione (GSH) has been of interest. In a recent study, Aptula et al. (27) investigated the relationship between skin sensitization assessed in the LLNA and a thiol reactivity index based on GSH (pEC₅₀ thiol, EC₅₀ being defined as the concentration of the test substance that gives 50% depletion of the free thiol under standard conditions). As not all sensitizers react with thiols, the authors suggest that this index should be combined with measurements of the compounds' cytotoxicity to the protozoa *T. pyriformis*. When taken together, the assays correctly predict the sensitizing potential of 23 of 24 investigated compounds. However, the number of compounds investigated is small and the experience from the relevance of the protozoa toxicity with regard to skin-sensitizing capacity is limited (27). In addition, the combination of the two methods makes the process rather complicated. A more simplified method has been

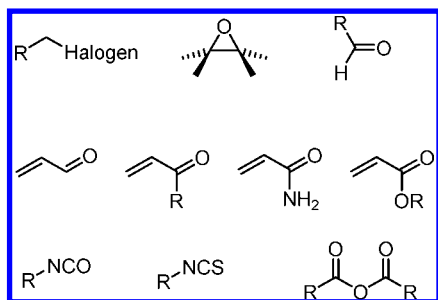


Figure 4. Examples of electrophilic functional groups present in haptens, which can react and bind to nucleophiles present in proteins.

suggested as a first assessment of the skin-sensitizing potency of chemicals (28). In this method, the depletion of unmodified peptide after incubation with a chemical was determined and correlated with the sensitizing potency of the same chemical according to LLNA data. In addition to GSH, peptides containing either cysteine, lysine, or histidine were used. The results obtained showed good correlation for strong and moderate sensitizers and will be further developed (28).

So far, the *in vitro* methods described are in most cases able to identify strong and moderate sensitizers. Because many contact allergens of clinical importance are weak sensitizers, increasing the sensitivity of these methods is an important challenge for future work. A trivial but fundamental problem in all cellular/protein-based assays is the water solubility of the test chemicals, since most skin sensitizers known today are lipophilic organic compounds. Another problem is the stability of the compound during the cellular experiments, which are carried out over several hours or days. Furthermore, no *in vitro* methods developed are able to also account for the activation of a nonsensitizing compound to a hapten. The activation can take place outside the body by air oxidation or photoactivation or in the skin due to metabolism.

3.3. In Silico Methods. Also, *in silico* methods have been extensively used in recent years, and these methods are discussed separately (see below).

4. Hapten-Protein Interaction

Chemically, the skin can best be described as a nucleophilic environment, rich in water and with electron-rich nucleophilic functional groups present in the proteins. The nucleophiles of primary interest are thiols ($-SH$) and primary amines ($-NH_2$) present in the side chains of the amino acids cysteine and lysine, respectively (26). Amino acids such as histidine ($=N-$), methionine ($-S-$), and tyrosine ($-OH$) also have nucleophilic properties.

Most contact allergens are electrophiles, and just like the nucleophiles, they are characterized by their functional groups. Figure 4 shows the most common functional groups of known electrophilic haptens. Lists of potential haptens with these and other related functional groups have recently been published (26, 29–31).

In vitro reactivity studies using model nucleophiles and various electrophiles have shown a certain degree of specificity in the reactions (32–36). We recognize five basic types of reactions: S_N2 reactions, S_NAr reactions, Schiff base formations, Michael type additions, and acylation reactions. Alkyl halides, epoxides, and sulfonic acid esters easily undergo S_N2 reactions. Aliphatic aldehydes and some diketones can form Schiff bases with the primary amino groups of the side chain of lysine. A variety of α,β -unsaturated aldehydes, ketones, amides, and

esters are involved in Michael type additions. Michael type acceptors are the most easily identified haptens leading to skin sensitization. Acylation reactions can occur with anhydrides and also with isocyanates and isothiocyanates.

All of these reactions have been linked to skin sensitization and elicitation, and toxicity predictions of compounds with electrophilic functional groups have been extensively discussed in recent reviews (26, 31, 37). It should be observed that the experimental evidences for these types of reactions to occur are mostly derived from *in vitro* reactions between haptens and model peptides (26).

During the last decade, the possibility of a radical mechanism involved in antigen formation has attracted increased interest (38–41). Hydroperoxides, formed by air oxidation of many commonly used chemicals, have been shown to be strongly sensitizing and clinically important contact allergens. They are known to easily form radicals by cleavage of their labile O–O bond (see below). Theoretically, the hydroperoxides could either be specific haptens or form nonspecific antigens. In the case of a nonspecific action, structurally different hydroperoxides would form the same immunogenic protein by acting as oxidizing agents able to oxidize functional groups in proteins. However, no general cross-reactivity was observed in sensitization studies of different hydroperoxides in guinea pigs, showing that the nonspecific mechanism is unlikely. Instead, a similarity in the overall structure of the antigen is needed for cross-reactivity of structurally dissimilar hydroperoxides (42). Thus, the activity of hydroperoxides as haptens is likely to involve radical reactions either by direct formation of covalent bonds with proteins by radical coupling or, as in the case of allylic hydroperoxides, radical rearrangements to form epoxides acting as electrophilic haptens. Indications on both reaction pathways have been presented (39) and are discussed more in detail below.

A third type of contact allergens is metal ions. The ions of nickel, cobalt, and chromium are common contact allergens due to frequent exposure at work and in daily life. The metal ions of iron, zinc, and silver are rarely implicated, even though many individuals are also exposed to these ions. An explanation for this is that allergenic metal ions form stable coordination complexes with proteins, which are recognized as nonself. There are indications that antigen formation can take place by direct interaction of nickel ions with the natural peptide residing in the major histocompatibility complex (MHC) class II binding region on the surface of the LCs (43–45). The hapten-protein bonds formed in the electrophile-nucleophile reactions and the radical reactions are likely to be strong covalent bonds. As for the allergenic metal ions, the coordination between the electron-rich ligands (O, N, and S) of proteins and the nickel, cobalt, or chromium ions lead to highly stable complexes.

5. SARs and QSARs in Contact Allergy

5.1. SAR Studies. Already in 1869, it was proposed that the biological activity of a compound is a function of its chemical structure (46). Today, we see the biological activity as a function of the structural and physicochemical properties of the compounds. This also applies to skin sensitizers (47). What is probably the earliest SAR study correlating chemical reactivity and skin sensitization was reported by Landsteiner and Jacobs in 1936 (48). They found a clear correlation between chemical reactivity of a series of halogenated nitrobenzene derivatives and their allergic response in guinea pigs. In the same study, it was also proposed that the formation of a hapten-protein complex is a prerequisite for the development of ACD. Up until the middle of the 1980s, reports on contact allergy of various

materials or chemicals were in the form of case reports without much systematic understanding. The modern concept of SAR in contact allergy was pioneered by Dupuis and Benezra (49). They and others have shown that the structural requirements for haptens are highly specific.

Most SAR studies in ACD give detailed structural information but only semiquantitative data on the biological activity. Because QSARs have predictive potential, much research has been performed to develop QSARs for skin sensitization (50–56). The first such QSAR model was published by Roberts and Williams in 1982, where they established correlations between the sensitizing capacity and the physicochemical parameters for a series of alkylating agents (57).

5.2. QSAR Studies. The construction of QSARs for skin sensitization has been facilitated by the development of the LLNA method (12, 15), as it allows the use of quantitative potency data for contact allergens (58). Estimations of sensitizing potency have for many years been obtained from guinea pigs; however, this animal model gives only semiquantitative data. Today, the establishment of QSARs and the development of computer-based expert systems in combination with a collection of databases of skin-sensitizing potencies of known contact allergens are rapidly evolving. Reliable and validated QSAR models are needed to meet the legislative demands to minimize the use of animal testing to assess skin sensitization potencies. To validate QSARs, a series of principles has been defined by the OECD (59).

QSAR models can be divided into mechanism-based (local models) and empirically derived statistical (global models) approaches. Attempts to construct mechanism-based QSARs for skin sensitization have often been based on the relative alkylation index (RAI). The RAI is used to quantify the relative degree of haptenation as a function of the dose given, the chemical reactivity expressed as the relative rate constant for reactions with a model nucleophile (often *n*-butylamine), and the hydrophobicity (expressed as calculated log*P* values). The general form for the RAI equation is

$$\text{RAI} = \log D + a \log k + b \log P$$

where *D* is the dose, *k* is the relative rate constant, and *P* is the octanol/water partition coefficient. The constants *a* and *b* are determined experimentally and are only valid for series of compounds reacting according to the same mechanism. Homologous series of compounds representing different chemical groups, for example, aldehydes and diketones (60–62), sultones (57, 63), alkyl alkansulfonates (64–67), phenyl benzoates (68), halo- and pseudohalobenzenes (69), dimethyl butyrolactones (70), and urushiol analogues (71), have been analyzed using RAI.

The main limitations with the use of RAI to estimate allergenic capacity are that it only gives good results for series of compounds reacting according to the same mechanism. In addition, the RAI does not take the true skin penetration into account, which means that the amount that actually reaches the target for reaction in the skin is not considered.

As mentioned above, mainly homologous series of compounds have been used to construct QSARs. As it is probably not possible to obtain a generally applicable QSAR equation suitable for all types of compounds, structurally diverse sets of chemicals need to be used to improve the predictability of the QSAR approach. However, not only structural diversity should be considered; diverse mechanisms responsible for the skin sensitization might be even more important. Attempts in this direction have been done using the recently published TOPS-

MODE approach (topological substructural molecular descriptors) (72, 73), which involves the development of linear QSARs using the spectral moments of a molecular bond matrix. This matrix is built on bond contributions that can be used to identify potential pharmacophores and/or toxicophores. Bond weights are computed to account for the contributions from different physicochemical properties, for example, hydrophobicity, polar surface area, molar refractivity, molecular polarizability, van der Waals radii, and atomic charges. Using this approach, a model was developed based on 93 compounds, which was validated using external validation sets. It has also been claimed that the TOPS-MODE models can be used to identify prohaptens as potentially sensitizing chemicals.

To allow predictive studies on skin sensitization for structurally heterogeneous series of compounds, Li et al. have developed so called four-dimensional (4D) fingerprint categorical QSAR models for sensitizers and nonsensitizers (30). The models are based on LLNA data in combination with molecular information regarding the composition of atoms, the shape, the size, the bonding topology, and the conformational flexibility of the different compounds in the database. Predictive models based on 4D fingerprints have been established, which can distinguish non/weak sensitizers from strong/extreme sensitizers. To account for the complicated process of skin sensitization descriptors such as hydrophobicity (skin penetration), electrophilicity, and polarity (reactivity towards skin proteins), size, shape, and steric interactions (T-cell receptor recognition) constitute the different fingerprints. The main limitation with this method is that the 4D fingerprints become more abstract and less transparent than other descriptors generally used to construct QSAR models.

5.3. Expert Systems. Today, different expert systems are available for the prediction of the sensitizing capacity of contact allergens (74). They contain information about the relationship between the chemical structure and the ability of chemicals to haptenate proteins. Derek for Windows (Deductive Estimation of Risk from Existing Knowledge) is one such expert system used for the prediction of mutagenicity, carcinogenicity, and skin sensitization (56, 75–78). Derek for Windows is a rule-based system that is composed of a set of molecular substructures correlated to a type of chemical reactivity that has been associated with skin sensitization and used as structural alerts. The set of structural alerts includes potential acylating or alkylating/aryllating agents, “Michael electrophiles” (α,β -unsaturated carbonyl compounds), aldehydes, free radical generators, thiol-exchange agents, or precursors thereof. The list of rules is constantly upgraded, users can add their own rules, and new features to allow a more reliable identification of potential health hazard have been added (79). One such feature is the link to the metabolism prediction program Meteor (75, 80). However, the treatment of metabolic activation of prohaptens needs to be improved, as several identified prohaptens are not recognized by the program (35, 81). Another important feature in Derek for Windows is the consideration of skin permeability, which is estimated based on the molecular weight and the calculated log*P* values (octanol/water) of compounds of interest.

TOPKAT (Toxicity Prediction by Komputer Assisted Technology) is used for the prediction of several acute and chronic toxicity end points, including skin sensitization. The program is built on different modules dependent on toxicity end point; two modules are related to skin sensitization: one for nonsensitizers vs sensitizers and the other for weak/moderate vs strong sensitizers. To ensure reliable predictions, the query structure used in a search is always analyzed to make sure that it is

covered by the optimum prediction space (OPS) (82). TOPKAT predictions are derived by using linear free energy relationships coupled to statistical regression analysis. Instead of using molecular orbital methods as a basis for modeling, TOPKAT uses electrotopological descriptors to combine the electronic character with the topological environment of each atom in a molecule (83). The TOPKAT models for skin sensitization have been developed based only on data obtained in guinea pigs.

Another statistically based expert system is CASE/Multi-CASE (Computer Automated Structure Evaluation) (84–87). The program identifies structural fragments of compounds that are associated with specific biological activities independently of the mechanism of action. It has been used to predict, for example, mutagenesis, carcinogenesis, and dermal and respiratory sensitization. The structural fragments are used to assess the probability of activity of untested compounds as well as to estimate their potency by generating multivariate regression equations based on structural fragments and log*P*. Models of skin sensitization have been constructed based on combined data obtained from animal and human studies (88).

TIMES-SS (TIssue Metabolism Simulator for Skin Sensitization) is an expert system considered to be a hybrid between knowledge-based and statistically based systems (89, 90). The program is used to construct QSAR models to estimate skin sensitization potency by also considering skin metabolism (both phase I and phase II metabolism) and the potential interaction of reactive metabolites (or the parent compound) with skin proteins. Three-dimensional QSAR models are used to evaluate the reactivity for compounds containing different alerting groups. These submodels are based on different physicochemical, steric, and electronic parameters.

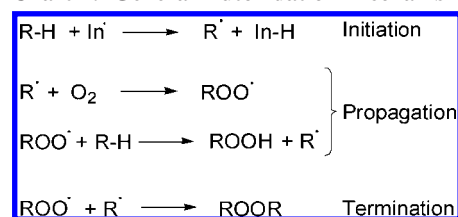
Derek for Windows, TOPKAT, and TOPS-MODE have recently been validated according to the OECD validation principles for QSAR models. The performance of the programs was evaluated against a data set of 210 compounds. It was shown that all three models could predict skin-sensitizing chemicals reasonably well, but none succeeded to give useful results for nonsensitizers (11). Also, TIMES-SS has been evaluated according to the OECD principles. In this evaluation, an external data set containing new LLNA data of 40 compounds was compared with predictions made by the program. The program was found to perform well as predicted values were in concordance with the experimental data. For a detailed discussion, see Patlewicz et al. (91).

Although considerable efforts have been put into the development of reliable predictive QSAR models, further improvements are still necessary. More experimental work to assess important properties will be needed, and more refined models have to be developed, which include skin penetration, chemical reactivity, and metabolism. No truly predictive QSAR models for skin sensitization potency can be established without access to carefully determined experimental data from well-designed structure–activity experiments. It is necessary not only to use commercial compounds but also to be able to design and synthesize adequate series of compounds based on the SAR/QSAR to be investigated.

6. Activation of Nonsensitizing Compounds by Air Oxidation

Some of the more significant accomplishments regarding chemistry and contact allergy during the last 20 years are related to the oxidation of nonallergenic compounds to potent contact allergens. Most organic compounds can undergo autoxidation, a reaction that can be defined as the insertion of oxygen into a

Chart 1. General Autoxidation Mechanism



C–H bond forming a hydroperoxide (ROOH). Autoxidation is a free radical chain reaction (Chart 1).

The relative success of this slow reaction lies in the biradical character of the ground state of oxygen. The oxygen acts as a fast and efficient radical quencher, which results in the formation of a peroxy radical (ROO[•]). The overall rate of the reaction is determined by the ease of hydrogen abstraction in the second step of the propagation reaction, which affords the hydroperoxide (ROOH) and a new radical (R[•]) that feeds the radical chain reaction. This step is generally selective, and hydrogens in, for example, benzylic, allylic, and tertiary positions or next to heteroatoms such as O and N are preferably abstracted, as this yields particularly stable radicals.

6.1. Turpentine. In the middle of the 20th century, extensive investigations were performed on the highly allergenic turpentine, a low-boiling liquid consisting of a mixture of monoterpenes obtained from coniferous trees. Both painters and physicians in Sweden observed that French turpentine had a lower tendency to cause ACD as compared to Scandinavian turpentine, and it was shown that this was related to the higher Δ³-carene content in the Scandinavian products (92). Further investigations concluded that “the eczematogenic factor is probably formed from a monomolecular hydroperoxide of Δ³-carene” (93). However, the authors reported no isolation and no structure elucidation of the active compound (Figure 5) (94).

6.2. Fragrance Terpenes. During the last 20 years, fragrance contact allergy has become a major problem due to an increased use of fragrancized products (95). Furthermore, it has been revealed that oxidation products from oxidative degradation of commonly used fragrance terpenes are frequent causes of contact allergy (96). Oxidative degradation of fragrance terpenes, usually referred to as maturation, is a process in the manufacture of fine fragrances that has been known and used since ancient times.

Contact allergy to naturally occurring monoterpenes gained renewed interest at the end of the 1980s when citrus oil, consisting of 98% limonene (1), was advertised as an environmentally friendly alternative to ordinary industrial solvents. At the time, little was known about its toxicological effects (97). However, new investigations showed that limonene autoxidizes on storage and handling to form a contact allergenic mixture (98). The major components in the oxidation mixture were identified (compounds 2–6), and especially, the hydroperoxides 2 and 3 were shown to be potent sensitizers (Figure 6) (99–102). Extensive patch test studies in more than 8000 patients in various dermatology clinics in Europe revealed that autoxidized limonene is a frequent cause of contact allergy (96, 103, 104).

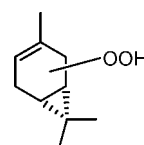


Figure 5. Δ³-Carene hydroperoxide proposed as the major sensitizer in Scandinavian turpentine.

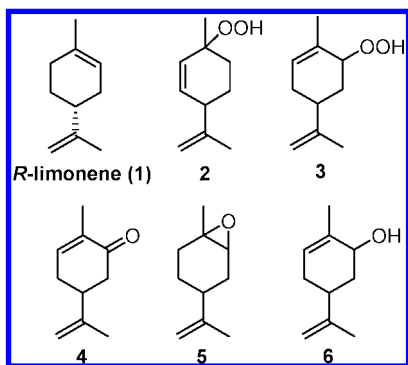


Figure 6. Limonene and identified oxidation products after autoxidation.

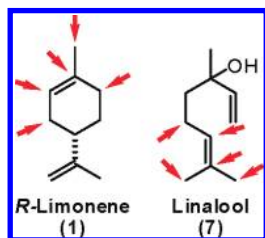


Figure 7. Possible positions for hydroperoxide formation in *R*-limonene (1) and linalool (7).

The new findings prompted the start of a directed research regarding the effects of autoxidation of different fragrance terpenes, that is, linalool (7), geraniol (17), and β -caryophyllene (24), and the impact on the sensitizing capacity of these compounds. Many terpenes are excellent targets for autoxidation, as they contain numerous easily abstractable allylic protons, a prerequisite for the autoxidation chain reaction (Figure 7). Thus, they are able to form hydroperoxides upon air exposure. However, in some cases, the hydroperoxides formed are not sufficiently stable and are immediately degraded to less sensitizing secondary oxidation products (e.g., alcohols and α,β -unsaturated carbonyl compounds). In addition, it was found that the ability of the studied terpenes to form stable hydroperoxides can be correlated with the sensitizing capacity of the corresponding autoxidized mixtures.

6.3. Limonene (1). Limonene (1) can form a number of allylic hydroperoxides considering the most substituted double bond (Figure 7). However, in the oxidation mixture, only two hydroperoxides (2 and 3, Figure 6) were detected (100, 102). Both animal and human data indicate that 2 and 3 are the main offending agents responsible for the increased sensitizing potential of autoxidized limonene, despite the fact that the investigated autoxidation mixtures (10 weeks of air exposure) only contain in total 5–6% of 2 and 3 (101, 102).

6.4. Linalool (7). Linalool (7) from lavender oil is the most frequently used fragrance terpene. The autoxidative degradation of linalool has been thoroughly investigated with regard to chemical mechanisms. It was found that various allergenic oxidation products (8–16), with emphasis on the hydroperoxides 8–10, are formed upon air exposure of linalool (105, 106). Clinical studies revealed that autoxidized linalool is also a frequent sensitizer in dermatitis patients (107). When linalool autoxidizes, the formation of a number of hydroperoxides is theoretically possible (Figure 7) (106). In air-exposed linalool, two stable hydroperoxides (8 and 9, Figure 8) were detected in concentrations of 15 and 4%, respectively, in a mixture exposed to air for 45 weeks. A third hydroperoxide (10) is probably also formed in small amounts, as its degradation product (13) was detected in the oxidation mixture. Hydroperoxide 10 could not

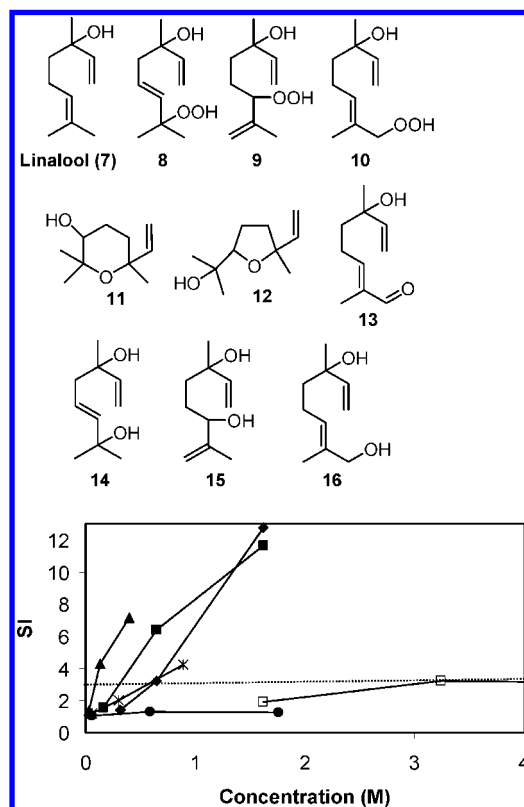


Figure 8. Linalool and identified oxidation products after autoxidation. Dose response curves for linalool hydroperoxides 8 and 9 (\blacktriangle), linalool air-exposed for 45 weeks (\blacksquare), linalool air-exposed for 10 weeks (\blacklozenge), 13 (*), 14 (\bullet), and linalool 7 (\square) tested in the LLNA. Concentrations are given in molar. The horizontal, dotted line marks an SI of 3, the cut-off limit for a compound to be considered a sensitizer.

be directly detected, which likely is due to its poor stability. The stability of 8, the major hydroperoxide, has been confirmed by theoretical calculations (108).

No epoxides were detected in the linalool oxidation mixture; instead, nonallergenic pyran (11) and furan derivatives (12) were identified, which are believed to originate from an epoxide formed from 8 (Figure 8) (106). On the basis of LLNA experiments and clinical studies, the hydroperoxides were shown to be the main contributors to the allergenic effect of autoxidized linalool. The concentration of the hydroperoxides in the oxidation mixture increased from 4% in the sample air exposed for 10 weeks to 15% in that air exposed for 45 weeks, and the sensitizing capacity of the oxidation mixture increased accordingly (Figure 8).

6.5. Geraniol (17). Geraniol (17) is one of the main constituents of rose oil. It is also present in the fragrance mix I (FM), used for screening of contact allergy in dermatitis patients. However, in comparison with other fragrance chemicals in the FM I, it rarely reveals any cases of ACD (109). Investigations of the autoxidative degradation of geraniol showed only small amounts formed of hydroperoxide 18, whereas secondary oxidation products [i.e., geranial (19), neral (20), 21, 22, 23, and hydrogen peroxide and alcohols, Figure 9] were found in larger amounts (110). As was found for limonene (1) and linalool (7), a higher sensitizing capacity was observed for oxidized geraniol as compared to the pure substance (110).

The autoxidation pattern of geraniol differs from that of limonene and linalool. All carbon atoms in the chemical structure of geraniol have the possibility to host a hydroperoxide. Of specific interest is the allylic position at C1 (Figure 9), which

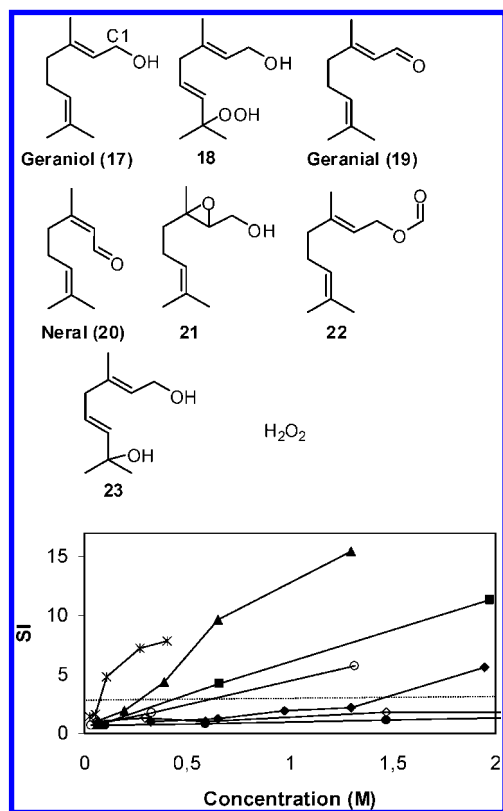


Figure 9. Geraniol and identified oxidation products after autoxidation. Dose response curves for geraniol hydroperoxide **18** (*), geraniol air-exposed for 10 weeks (**▲**), geranial **19** (**■**), neral **20** (**○**), geraniol **17** (**◆**), geraniol alcohol **23** (**◇**), and hydrogen peroxide **24** (**●**) tested in the LLNA. Concentrations are given in molar. The horizontal, dotted line marks an SI of 3, the cut-off limit for a compound to be considered a sensitizer.

is able to form a radical stabilized by both a double bond and a hydroxyl group. A hydroperoxide formed in this position was not isolated; however, it is believed to be the precursor of the secondary oxidation products **19**, **20**, and hydrogen peroxide found in the oxidation mixture (Figure 9) (110). Even though only approximately 1% of **18** (Figure 9) was detected in the mixture, its impact on the sensitizing capacity of autoxidized geraniol seems to be important. Other contributors to the increase of sensitizing potential in oxidized geraniol are geranial (**19**) and neral (**20**), compounds of moderate sensitizing capacity in the LLNA (Figure 9).

6.6. β -Caryophyllene (24). β -Caryophyllene (**24**) is a sesquiterpene from clove oil and is commonly used as a fragrance chemical. In studies of its susceptibility toward autoxidation, it was found to decompose with the same rate as found for limonene (**1**); however, no hydroperoxides could be identified in the oxidation mixture (111). Instead, β -caryophyllene oxide (**25**, Figure 10) was detected in a concentration of 10% after 1 week of oxidation. This indicates that also for β -caryophyllene, the oxidation reaction is directed toward the most substituted double bond. Furthermore, it shows that the hydroperoxides formed are unstable and rapidly rearrange to epoxides (111). Two hydroperoxides of β -caryophyllene (**26** and **27**, Figure 10), synthetically prepared and tested in the LLNA, were found to be strong sensitizers analogous to previously tested hydroperoxides. When testing air-exposed β -caryophyllene (10 weeks), it was found to be weakly sensitizing, which correlates with the absence of hydroperoxides in the oxidation mixture. Epoxide **25** was shown to be a moderate sensitizer in guinea pig studies

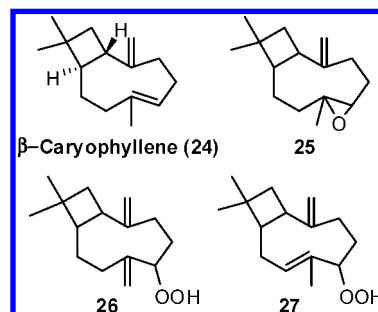


Figure 10. β -Caryophyllene, its oxidation product β -caryophyllene oxide (**25**), and synthesized β -caryophyllene hydroperoxides (**26** and **27**).

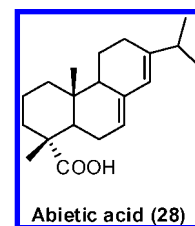


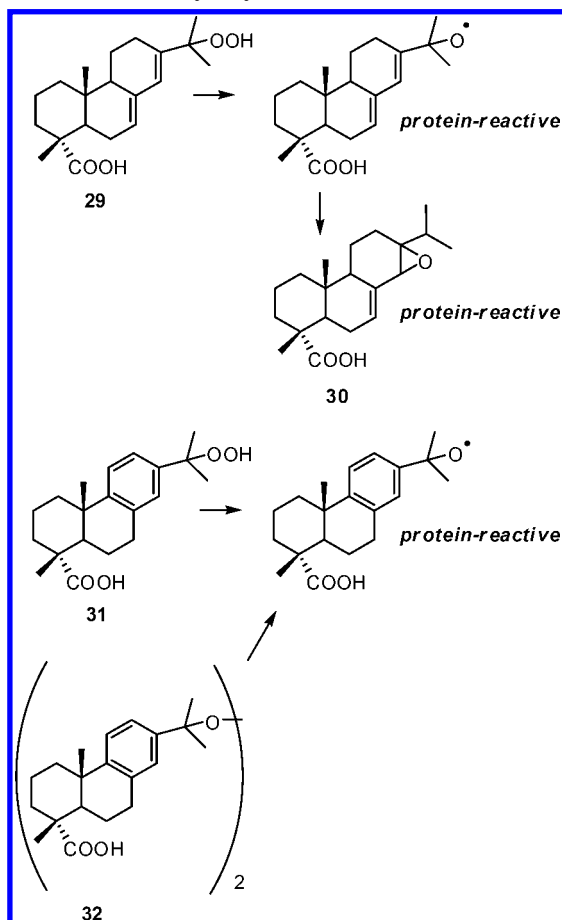
Figure 11. Chemical structure of abietic acid (**28**).

(111). In addition, only few reactions to autoxidized β -caryophyllene were observed in clinical studies (107).

6.7. Colophony. Colophony (rosin) is the nonvolatile fraction of the exudates from coniferous trees, and its main constituent is abietic acid (**28**, Figure 11). Because of its low costs and good technical properties, rosin is widely used in industrial and daily life products. However, it is also one of the most common causes of contact allergy, and many cases of ACD from colophony are reported in the literature (112). Different rosin types are used interchangeably and are often chemically modified. Unmodified colophony is a complex mixture of diterpenoid acids (i.e., resin acids, ca. 90%), diterpene alcohols, aldehydes, and hydrocarbons (112). Abietic acid (**28**) has been described as the allergenic constituent (113). Because **28** is not an electrophile, its sensitizing capacity was questioned when investigations regarding the allergenic properties of colophony started 20 years ago. It was found that highly purified **28** is nonallergenic (114) but rapidly autoxidizes forming a hydroperoxide (**29**, Scheme 1), which subsequently was identified as a major allergen of colophony (115). A variety of other oxidation products from abietic acid and dehydroabietic acid (the other major resin acid in colophony) were isolated and identified, some of which were shown to be sensitizers in guinea pig studies (112). Clinical investigations have shown that patch testing with **29** detects about 50% of the patients with contact allergy to colophony (116, 117).

6.8. Alcohol Ethoxylates. Ethoxylated nonionic surfactants are widely used in household and industrial cleaners, topical pharmaceuticals, cosmetics, and laundry products. Polyethers, for example, ethoxylated surfactants and polyethylene glycols, are highly susceptible towards air oxidation as the ether oxygens will stabilize intermediary radicals involved (118, 119). Investigations of a chemically well-defined alcohol ethoxylate, C₁₂E₅ (**33**, Figure 12), showed that polyethers form complex mixtures of oxidation products when exposed to air (compounds **34–38**, Figure 12). Sensitization studies in guinea pigs revealed that the pure nonoxidized surfactant itself is nonsensitizing but that many of the investigated oxidation products are sensitizers. Two hydroperoxides (**37** and **38**) were identified in the oxidation mixture, but only **38** was stable enough to be isolated. It was found to be a strong sensitizer in LLNA. The formation of other

Scheme 1. Possible Reaction Pathways for Formation of Protein–Hapten Complexes for Identified Colophony Allergens: 15-Hydroperoxyabiatic Acid (29), 13,14(α)-Epoxyabiatic Acid (30), 15-Hydroperoxydehydroabiatic Acid (31), and 15-Peroxydehydroabiatic Acid (32)^a



^a The proposed reactivity is based on cross-reactivity studies in guinea pigs.

hydroperoxides was indicated by the detection of their corresponding aldehydes in the oxidation mixture (120–124).

On the basis of the lower irritancy, nonionic surfactants are often preferred to ionic surfactants in topical products. However, their susceptibility towards autoxidation also increases the irritation (125). Because of their irritating effect, it is difficult to diagnose ACD to these compounds by patch testing (126).

6.9. Hydroperoxide Antigens and Radical Formation. Hydroperoxides are believed to form antigens via a radical mechanism, starting with the homolytic cleavage of the O–O bond. The resulting alkoxy radical may either bind directly with a protein or rearrange to an epoxide to form a hapten–carrier complex (Scheme 1).

It has been argued that the hydroxy radical (HO[•]) might act as a hapten, thus affording an unspecific allergic response. However, no such unspecific responses were observed when performing cross-reactivity studies with hydroperoxides in guinea pigs (42). For example, no cross-reactivity was seen when animals sensitized to cumene hydroperoxide (39) were challenged with limonene hydroperoxide (2) and vice versa. Interestingly, animals sensitized to 39 also reacted to the cyclohexene hydroperoxide 40 (Table 1). As the rearrangement of the cumene alkoxy radical to its corresponding epoxide is energetically unfavorable, these data indicate that the major

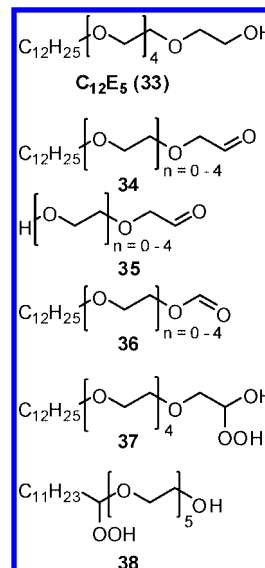


Figure 12. Primary and secondary oxidation products identified after autoxidation of pentaerythritol glycol mono-*n*-dodecyl ether, C₁₂E₅ (33): alkyl polyethyleneglycol aldehydes (34), hydroxyaldehydes (35), alkyl polyethyleneglycol formates (36), 1-hydroperoxy-3,6,9,12,15-pentaoxaheptacosan-1-ol (37), and 16-hydroperoxy-3,6,9,12,15-pentaoxaheptacosan-1-ol (38). According to guinea pig studies, the formates (36) were nonsensitizers, while the other investigated oxidation compounds were allergenic.

Table 1. Cross-Reactivity Pattern of Structurally Diverse Hydroperoxides in Sensitization Tests in Guinea Pigs^a

Induction ¹	Challenge ¹	39	2
39		+2	-3
2		-	+
40		+	-

^a (1) Sensitization studies performed in guinea pigs according to Freund's complete adjuvant test (129). (2) Significant positive reactions in animals induced and challenge tested with cumene hydroperoxide (39). (3) No significant reactions in animals induced with limonene hydroperoxide (2) when challenge tested with cumene hydroperoxide (39).

pathway for the formation of the hapten–protein complex goes via a direct addition of the alkoxy radicals to the protein (42). This is in agreement with the cross-reactivity pattern observed in guinea pig studies of hydroperoxides 29 and 31 and peroxide 32. These compounds can all form antigens directly via alkoxy radicals (Scheme 1). The radicals from 29, but not from 31 and 32, can also rearrange to epoxides, which could function as

Table 2. Classes of Prohaptens and Proposed Reactive Intermediates and Bioactivation Pathways

prohaptent	reactive intermediate(s)	biotransformation(s)	example(s)
aliphatic amines	aldehydes, imines, iminium ions	hydroxylation of α -carbon followed by elimination of water or amine	3-dimethylaminopropylamine (138) ethylenediamine (139)
alkenyl halides	epoxides, acyl chlorides, aldehydes, GSH conjugates	epoxidation, oxidation followed by halogen migration, GSH conjugation	trichloroethylene (140, 141)
<i>p</i> -alkylated phenols	<i>p</i> -quinone methides, semiquinone radicals	one- and two- electron oxidations	isoeugenol (142)
aromatic amines	nitrenium ions, nitroso compounds, quinonediimines, quinones, semiquinoneimine radicals	one- and two-electron oxidations, <i>N</i> -hydroxylation followed by oxidation or phase II conjugations	2-aminophenol (143) PPD (144–146) sulfamethoxazole (147)
azo dyes	probably the same as for aromatic amines	reduction of azo bonds, oxidations	disperse blue 124, disperse orange 3, disperse red 1 and disperse yellow 3 (148, 149)
catechols	<i>o</i> -quinones, semiquinone radicals	one- and two-electron oxidations	eugenol (142) isopropenyl caffeate (150) lauryl gallate (151) urushiols (152)
conjugated dienes	allylic epoxides	epoxidation	α -terpinene (133) α - and β -phellandrene (133)
hydroquinones primary alcohols	<i>p</i> -quinones, semiquinone radicals aldehydes	one- and two-electron oxidations oxidation	hydroquinone (38, 144) CALc (153) hydrocortisone (154)
polyaromatic hydrocarbons (PAHs)	arene epoxides	epoxidation	benzo[<i>a</i>]pyrene (143, 155) 7,12-dimethylbenz[<i>a</i>]anthracene (143, 155)
α,β -unsaturated oximes	allylic nitrosoalkenes	epoxidation followed by rearrangement	carvoxime (81)
vinylbenzenes	epoxides	epoxidation	styrene (156) vinyl pyridine (143)

electrophilic haptens (39). Epoxide **30** was found to be allergenic and cross-reacted with **29** but not with **31** in guinea pig tests (39). However, animals sensitized to **31** cross-reacted with **32**. Because **31** cannot rearrange to form epoxides and therefore might bind to the protein as an alkoxy radical, the assembled results indicate that **29** as well as other allylic hydroperoxides can react with proteins either as a radical or after rearrangement to epoxides. Another possible route for protein modification is the trapping of the rearranged carbon centered epoxy radical by a protein. Evidence for the importance of this pathway has been presented in studies using chemical trapping experiments (40, 127) and electron paramagnetic resonance (EPR) experiments (41, 128).

7. Bioactivation of Prohaptens

The skin was, for a long time, considered to be an inert barrier that protects the body from the environment. However, since the 1980s, the biotransformation of inherently nonprotein-reactive chemicals to highly reactive intermediates (usually referred to as bioactivation or metabolic activation) in the skin has gained increased interest. Today, numerous studies show that the human skin is able to metabolize endogenous and exogenous compounds and that both phase I and phase II enzymes are present in the skin (130). Cutaneous enzymes that catalyze phase I transformations include the cytochrome P450 (P450) mixed-function oxidase system, alcohol and aldehyde dehydrogenases (ADHs and ALDHs), monoamine oxidases (MAOs), flavin-containing monooxygenases (FMOs), and hydrolytic enzymes. Acyltransferases, GSH S-transferases, UDP-glucuronosyltransferases, and sulfotransferases are examples of phase II enzymes that have been shown to also be present in human skin (130). These enzymes are known to catalyze both activating and deactivating biotransformations (131, 132), but the influence of the reactions on the allergenic activity of skin

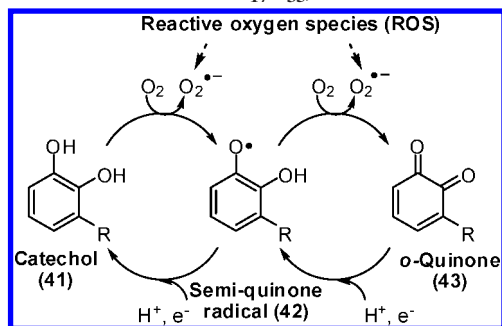
sensitizers has only been briefly studied. Compounds that are bioactivated to haptens are in the following referred to as prohaptens.

Few mechanistic investigations of prohaptens have so far been published, for example, the studies on alkenes (35, 133, 134), oximes (36), and the allylic primary alcohol cinnamic alcohol (CALc) (54) (135–137). However, on the basis of knowledge of xenobiotic bioactivation reactions, clinical observations, and/or in vivo and in vitro studies of sensitizing capacity and chemical reactivity, numerous skin-sensitizing prohaptens can be recognized and grouped into chemical classes (Table 2). Identified prohaptens include natural products (e.g., urushiols, **41**, and α -terpinene, **66**), dyes [e.g., *p*-phenylenediamine (PPD), **48**, and disperse blue 124], flavor and fragrances (e.g., CALc, **54**, and eugenol), drugs (e.g., sulfamethoxazole and hydrocortisone), and industrially used chemicals (e.g., styrene and ethylenediamine).

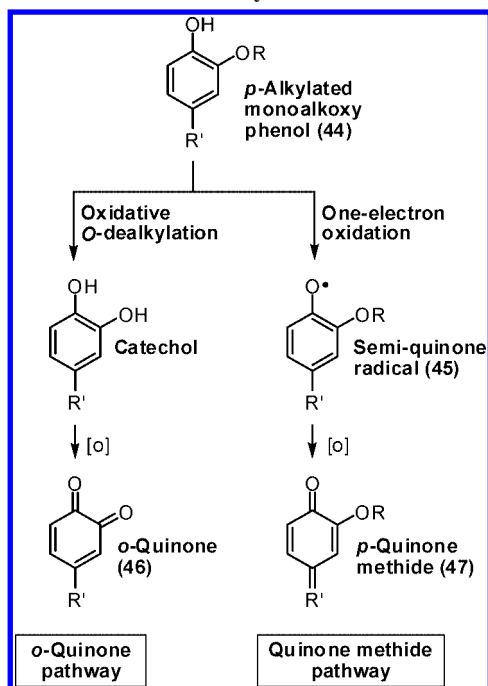
7.1. Catechols and Hydroquinones. Urushiols found in plants of the *Toxicodendron* genus (e.g., poison oak, ivy, and sumac) growing mainly in North America were among the first suspected prohaptens. In the United States, 50% or more of the population may be sensitized to urushiols (157). Urushiols are catechols (**41**) substituted with a C15 or C17 linear alkyl chain. They are generally considered to form strongly sensitizing and highly protein-reactive *o*-quinones (**43**) by oxidation in the skin (Scheme 2) (49).

It has also been suggested that the semiquinone radical (**42**) may act as a hapten and that the reactive oxygen species (ROS) formed in the redox process generates oxidative stress leading to tissue damage and an increased inflammation reaction (38, 132). The oxidation of catechols and hydroquinones to *o*- and *p*-quinones may be enzymatic or the result of nonenzymatic processes in the skin. Monoalkoxy phenols form reactive intermediates by one-electron oxidation to give a semiquinone

Scheme 2. Proposed Mechanism for Bioactivation of Catechols (R = H) and Urushiols (R = Linear Alkyl Chain of C₁₅H₂₅, C₁₅H₂₇, C₁₅H₂₉, C₁₅H₃₁, C₁₇H₂₉, C₁₇H₃₁, C₁₇H₃₃, or C₁₇H₃₅)



Scheme 3. Proposed Bioactivation for *p*-Alkylsubstituted Monoalkoxy Phenols^a

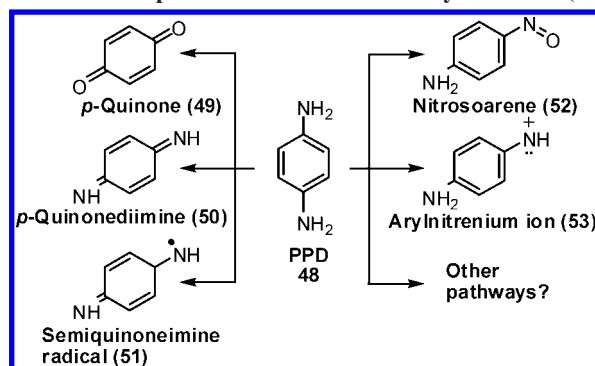


^a R = alkyl and R' = alkyl or alkenyl.

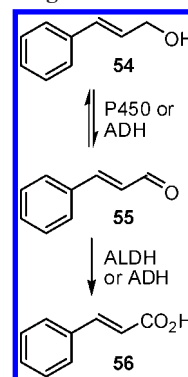
radical (**45**, Scheme 3) or by P450-mediated oxidative dealkylation followed by oxidation to the corresponding quinone (**46**, *o*-quinone pathway, Scheme 3) (132, 142). Moreover, the dialkoxy urushiol analogue pentadecylveratrole was found to be a nonsensitizer (158), showing that an increase in the number of metabolic transformations leading to a reactive intermediate leads to a drop in the allergenic activity of a compound. Furthermore, monoalkoxy phenols having a *p*-alkyl substituent (**44**) may be oxidized to form reactive quinone methides (**47**, quinone methide pathway, Scheme 3). This has been proposed for isoeugenol, a commonly used fragrance compound (142). Quinone methides may be formed from alkylbenzenes after phase I *p*-hydroxylation followed by two-electron oxidation (159).

7.2. Aromatic Amines. Aromatic amines are closely related to catechols and hydroquinones with PPD (**48**, Scheme 4) as a prominent example. PPD is a frequently occurring and potent skin sensitizer commonly used in hair dyes (160). It was initially thought to be activated in the skin to 1,4-benzoquinone (**49**) and its trimer, Bandrowski's base (49, 161). However, later studies showed only a limited degree of cross-reactivity for **49** and Bandrowski's base in PPD-sensitized individuals, suggesting

Scheme 4. Proposed Bioactivation Pathways for PPD (48**)**



Scheme 5. Proposed Mechanism of Bioactivation of Calc (54**) to Cinnamic Aldehyde (**55**) by P450 or ADH Followed by Detoxification to Cinnamic Acid (**56**) by ALDH or ADH Acting as a Dismutase**

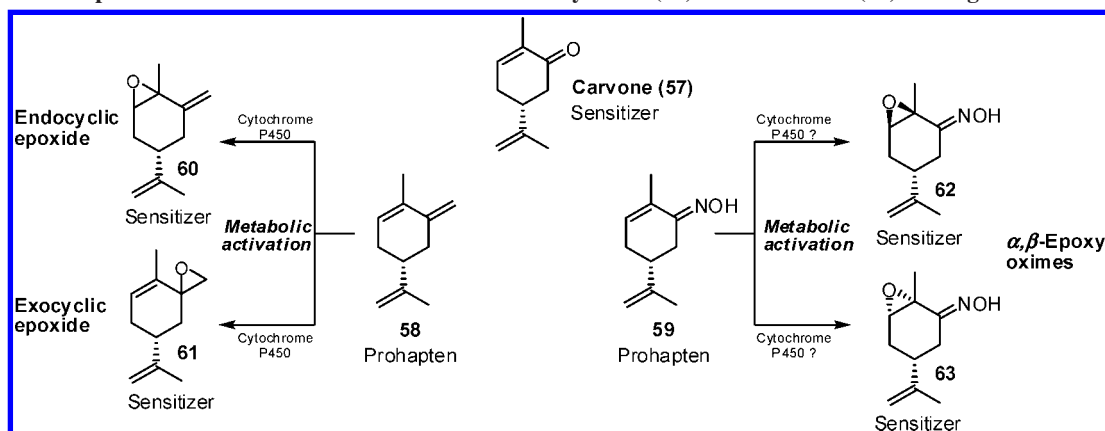


that these compounds are not the only reactive intermediate formed from PPD (146, 162, 163). PPD is known to readily autoxidize to a variety of degradation products (164, 165), and *p*-quinonediimines (**50**) (166) and semiquinoneimine radicals (**51**) (known as Würster radicals) (167) are examples of intermediates of potential importance in contact allergy to PPD and related compounds (Scheme 4).

Aromatic amines are also known to be metabolically activated via *N*-hydroxylation, leading to the formation of *N*-hydroxylamines (132). These intermediates can be converted to either reactive nitrosoarenes (**52**) after oxidation by P450 or FMOs or undergo phase II conjugations (acetylation and sulfation), ultimately leading to the formation of reactive aryl nitrenium ions (**53**, Scheme 4). It has been suggested that PPD could undergo similar activation reactions in the skin (131). However, metabolism studies using human liver microsomes showed no evidence for the formation of mono-oxygenated metabolites or for enzyme-mediated covalent binding of ¹⁴C-PPD to microsomal protein (168).

7.3. Activated Primary Alcohols. The flavor and fragrance chemical Calc (**54**) has been the subject of several studies of cutaneous metabolic activation (135–137, 169). It is an allylic primary alcohol, which has been suggested to be metabolically activated to the corresponding aldehyde, the known skin sensitizer cinnamic aldehyde (**55**, Scheme 5) (170). In addition, metabolism studies using human skin confirmed that Calc is converted to **55** and subsequently oxidized to cinnamic acid (**56**) (135). On the basis of enzyme inhibition studies using pyrazoles, it was hypothesized that **54** is mainly activated by cutaneous ADHs. Later studies using an enriched cocktail containing the major P450 enzymes found in human skin revealed that P450 is also involved in the bioactivation of **54** (134). Hydrocortisone is a β -keto primary alcohol that has been

Scheme 6. Proposed Mechanisms of Bioactivation of a Methylidene (58) and an Oxime (59) Analogue of Carvone (57)



shown to be bioactivated to its corresponding allergenic aldehyde (154). This anti-inflammatory corticosteroid is used for treatment of ACD.

7.4. Alkenes and Oximes. Conjugated dienes and α,β -unsaturated oximes represent two previously unknown classes of prohaptens. The synthetic methylidene and oxime (58 and 59, Scheme 6) analogues of the known skin sensitizer carvone (57), an α,β -unsaturated ketone terpenoid (Scheme 6), were initially thought to be weak or nonsensitizers as they are poor electrophiles (35, 81). From a chemical reactivity point of view, it was proposed that their sensitizing capacity would be in the order $57 > 59 > 58$. Unexpectedly, the result was $59 > 58 > 57$ (35, 81). It was likely considered that 58 and 59 were activated by skin metabolism.

As epoxidation of double bonds is a common bioactivation pathway for alkenes, two allylic epoxides (60 and 61) were proposed as the reactive intermediates formed from the 58. These epoxides were found to possess both sensitizing capacity in vivo and in vitro and chemical reactivity toward Pro-His-Cys-Lys-Arg-Met, a hexapeptide containing the most common nucleophilic amino acids (35, 134). Epoxides 60 and 61 were also found to elicit a contact allergic response in guinea pigs sensitized to 58 (35). Incubation of 58 with liver microsomes as well as with an enriched skinlike P450 enzyme cocktail confirmed that 58 is metabolically activated to 60 and 61 and revealed that this activation involves P450 (35, 134). Furthermore, a SAR study of potentially prohaptenic alkenes (64–70) demonstrated that conjugated dienes in or in conjunction with a six-membered ring are prohaptens, whereas related alkenes containing isolated double bonds or an acyclic conjugated diene were weak or nonsensitizing compounds (Figure 13) (133). This difference in sensitizing capacity of conjugated dienes as compared to alkenes with isolated double bonds was found to be due to the high reactivity and sensitizing capacity of the allylic epoxides metabolically formed from conjugated dienes. It was also demonstrated that the naturally occurring monoterpenes β -phellandrene (64), α -phellandrene (65), and α -terpinene (66), present in, for example, tea tree oil, are prohaptens (Figure 13) (133).

Few metabolism studies on alkyl oximes are found in the literature. Studied alkyl oximes include acetoxime and methyl ethyl ketoxime, which are metabolically converted to nitronates (171, 172), and cyclohexanone oxime, which is subsequently hydrolyzed to cyclohexanone (173). The importance of the in vivo hydrolysis of 59 to 57 was also proposed in elicitation studies in guinea pigs, observed by a partial cross-reactivity between the oxime and the carvone (81). However, as 57 is a considerably weaker sensitizer than its oxime counterpart, an

alternative bioactivation pathway of 59 was proposed (81). Liver microsomal incubations in the presence of GSH revealed the formation of electrophilic mono-oxygenated metabolites. Hence, three putative metabolites were proposed as follows: two diastereomeric α,β -epoxy oximes (62 and 63) and a nitro analogue (36). When tested in the LLNA, α,β -epoxy oximes 62 and 63 were found to be considerably more potent sensitizers as compared to 59, whereas the nitro compound was void of activity at the concentrations tested. Elicitation experiments also demonstrated a cross-reactivity between the less sterically hindered α,β -epoxy oxime diastereomer (62) in mice sensitized to 59. Furthermore, compounds 62 and 63 were found to be

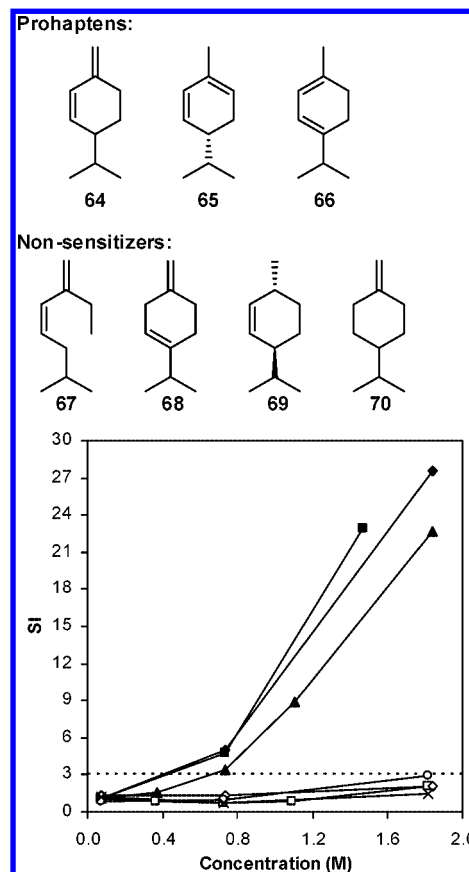
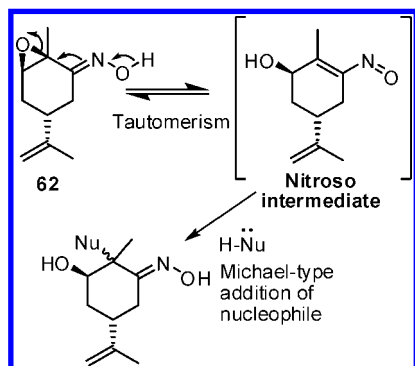


Figure 13. Structure-allergenic activity study of potentially prohaptenic alkenes (64–70). Dose-response curves for alkenes 64 (■), 65 (◆), 66 (▲), 67 (□), 68 (◇), 69 (○), and 70 (×) tested in the LLNA. Concentrations are given in molar. The horizontal, dotted line marks an SI of 3, the cut-off limit for a compound to be considered a sensitizer. The conjugated dienes 64–66 are sensitizers, and alkenes 67–70 are non-sensitizing compounds.

Scheme 7. Proposed Mechanism for the Formation and Reactivity of Nitroso Intermediates Formed from α,β -Epoxy Oximes



highly reactive toward GSH and Pro-His-Cys-Lys-Arg-Met. The strong sensitizing potency of **62** and **63** as well as their high chemical reactivity were unexpected and led to the speculation that the mechanism of nucleophilic addition to α,β -epoxy oximes was more complex than direct nucleophilic attack on the epoxide. Reactivity experiments with *N*-acetylcysteine methyl ester confirmed this hypothesis and presented strong indications that α,β -epoxy oximes likely exist in a rapid equilibrium with their corresponding nitroso tautomers (Scheme 7) (36).

8. Outlook

Contact allergy is the most frequent manifestation of adverse health effects caused by the interaction of chemicals in our environment with the immune system. It is an important occupational and environmental health problem, and more than 3000 chemicals have been shown to cause skin sensitization (174). Prevention from exposure of skin-sensitizing chemicals is of utmost importance, as an acquired contact allergy is life long and only symptomatic treatment of ACD is available. A review with historical examples on actions to control contact allergy epidemics was recently published (6). Regulatory work and legislation to decrease or eliminate exposure of potent contact allergens are important, since environmental exposure is of major importance for the development of sensitization. For nickel and chromium (as chromate), strict regulation of exposure has decreased the number of new cases of ACD (6 and refs therein).

To stop the introduction of new sensitizers on the market, the ultimate challenge is to design predictive nonanimal test methods. Today, no such method exists that is sensitive enough to detect weak allergens and robust enough to be used for routine screening in industry. In the development of predictive models or systems, it is important to use appropriate animal models, chemicals of known purity, and carefully designed SAR/QSAR studies. The compounds have to be chosen in such a way that they can give the possibility of answering the questions posed. To that end, it is necessary to use not only commercial compounds but also to be able to design and synthesize adequate series of compounds based on the SAR/QSAR to be investigated. Furthermore, the fact that many compounds are not allergenic themselves but rather are activated via various processes to form sensitizers must be considered. With regard to this, more basic research is needed on the chemical reactions involved in the antigen formation and the immunological mechanisms in the skin. Currently, the only alternative predictive methods for practical use in the identification of prohaptens are knowledge-based expert systems. These systems can give

structural alerts for potentially prohaptenic structures, but as they are based on current knowledge, they will only be able to give a warning signal for known classes of prohaptens. Hence, continuing efforts towards the study of xenobiotic bioactivation in the skin are important for the development of successful predictive assays. This includes identification and quantification of cutaneous xenobiotic-metabolizing enzymes, identification and mechanistic investigations of novel classes of prohaptens, and exploration of SARs for known prohaptens. Unfortunately, ACD is regarded as a trivial condition by those not affected. Therefore, the number of researchers engaged in fundamental experimental mechanistic research of contact allergy is limited as compared to those in other areas of toxicology and allergology.

For screening of contact allergy and diagnosis of ACD, the currently used patch test technique detects contact allergy only to the tested chemicals. However, to be able to reveal causes of ACD, it is important to test with the compounds in which people really come in contact. This is clearly shown in the case of fragrance terpenes, where the formation of allergenic compounds due to autooxidation on handling and storage was not previously considered. These fragrance materials had in most cases been patch tested in their nonoxidized form; consequently, very few reactions were observed (175–177). After the new understanding of the importance of autooxidation, screening performed in thousands of patients at dermatology clinics in Europe revealed that 2–3% of the tested patients reacted to the oxidized materials (96, 103, 104, 107). These figures are of the same magnitude as those of the most common allergens in the standard tray of contact allergens.

Regulation of the peroxide content of terpenes and terpenoid materials is considered within the EU. At present, the fragrance industry is working to prevent autooxidation from occurring at production. Also, the handling and storage of products containing compounds prone to autooxidation should be improved to diminish the exposure to the allergenic oxidation products. This illustrates the importance of basic research to increase knowledge of the mechanisms of contact allergy and thereby influence society to decrease the exposure of sensitizing chemicals.

References

- (1) Nielsen, N. H., Linneberg, A., Menne, T., Madsen, F., Frolund, L., Dirksen, A., and Jorgensen, T. (2001) Allergic contact sensitization in an adult Danish population: Two cross-sectional surveys eight years apart (the Copenhagen Allergy Study). *Acta Dermato-Venereol.* 81, 31–34.
- (2) Wallenhammar, L. M., Nyfjall, M., Lindberg, M., and Meding, B. (2004) Health-related quality of life and hand eczema—A comparison of two instruments, including factor analysis. *J. Invest. Dermatol.* 122, 1381–1389.
- (3) Serup-Hansen, N., Gudum, A., and Munk-Sørensen, M. (2004) Valuation of chemical related health impacts. Environmental Project No. 929, Ministry of the Environment, Environmental Protection Agency.
- (4) Wahlberg, J. E., and Lindberg, M. (2006) Patch testing. In *Contact Dermatitis*, 4th ed. (Frosch, P. J., Menné, T., and Lepoittevin, J.-P., Eds.) pp 366–390, Springer, Berlin.
- (5) Ryan, T. J. (1987) The confident nude or whither dermatology. *Dermatol. Pract.* 5, 8–18.
- (6) Pontoppidan, J., Johansen, J. D., and Menne, T. (2007) Contact allergy epidemics and their controls*. *Contact Dermatitis*, 185–195.
- (7) Rustemeyer, T., van Hoogstraten, I. M. W., von Blomberg, B. M. E., and Scheper, R. J. (2006) Mechanisms in allergic contact dermatitis. In *Contact Dermatitis* (Frosch, P. J., Menné, T., and Lepoittevin, J. P., Eds.) pp 11–43, Springer, Berlin, Heidelberg.
- (8) (2003) EU: Directive 2003/15/EC of the European Parliament and of the Council of 27 February 2003 amending Council Directive 76/768/EEC on the approximation of the laws of the Member States relating to cosmetic products ("7th Amendment to the European Cosmetics Directive"). *Off. J. Eur. Union L.* 66, 26–35.

- (9) Ryan, C. A., Hulette, B. C., and Gerberick, G. F. (2001) Approaches for the development of cell-based in vitro methods for contact sensitization. *Toxicol. in Vitro* 15, 43–55.
- (10) Ryan, C. A., Gerberick, G. F., Gildea, L. A., Hulette, B. C., Betts, C. J., Cumberbatch, M., Dearman, R. J., and Kimber, I. (2005) Interactions of contact allergens with dendritic cells: Opportunities and challenges for the development of novel approaches to hazard assessment. *Toxicol. Sci.* 88, 4–11.
- (11) Patlewicz, G., Aptula, A. O., Uriarte, E., Roberts, D. W., Kern, P. S., Gerberick, G. F., Kimber, I., Dearman, R. J., Ryan, C. A., and Basketter, D. A. (2007) An evaluation of selected global (Q)SARs/expert systems for the prediction of skin sensitization potential. *SAR QSAR Environ. Res.* 18, 515–541.
- (12) Kimber, I., Hilton, J., Dearman, R. J., Gerberick, G. F., Ryan, C. A., Basketter, D. A., Scholes, E. W., Ladics, G. S., Loveless, S. E., and House, R. V., et al. (1995) An international evaluation of the murine local lymph node assay and comparison of modified procedures. *Toxicology* 103, 6373.
- (13) Gerberick, G. F., Ryan, C. A., Dearman, R. J., and Kimber, I. (2007) Local lymph node assay (LLNA) for detection of sensitization capacity of chemicals. *Methods* 41, 54–60.
- (14) Andersen, K. E., and Maibach, H. I. (1985) *Contact Allergy Predictive Test in Guinea Pigs*, Vol. 14, Karger, Basel.
- (15) Kimber, I., and Dearman, R. J. (1991) Investigation of lymph node cell proliferation as a possible immunological correlate of contact sensitizing potential. *Food Chem. Toxicol.* 29, 125–129.
- (16) Basketter, D. A., Lea, L. J., Dickens, A., Briggs, D., Pate, I., Dearman, R. J., and Kimber, I. (1999) A comparison of statistical approaches to the derivation of EC₃ values from local lymph node assay dose responses. *J. Appl. Toxicol.* 19, 261–266.
- (17) Montelius, J., Wahlkvist, H., Boman, A., Fernstrom, P., Grabergs, L., and Wahlberg, J. E. (1994) Experience with the murine local lymph node assay: Inability to discriminate between allergens and irritants. *Acta Dermato-Venereol.* 74, 22–27.
- (18) Vohr, H.-W., and Ahr, H. J. (2005) The local lymph node assay being too sensitive? *Arch. Toxicol.* 79, 721–728.
- (19) Vandebriel, R. J., Van Och, F. M., and van Loveren, H. (2005) In vitro assessment of sensitizing activity of low molecular weight compounds. *Toxicol. Appl. Pharmacol.* 207, 142–148.
- (20) Romani, N., Gruner, S., Brang, D., Kampgen, E., Lenz, A., Trockenbacher, B., Konwalinka, G., Fritsch, P. O., Steinman, R. M., and Schuler, G. (1994) Proliferating dendritic cell progenitors in human blood. *J. Exp. Med.* 180, 83–93.
- (21) Romani, N., Reider, D., Heuer, M., Ebner, S., Kampgen, E., Eibl, B., Niederwieser, D., and Schuler, G. (1996) Generation of mature dendritic cells from human blood. An improved method with special regard to clinical applicability. *Journal of immunological methods* 196, 137–151.
- (22) Bender, A., Sapp, M., Schuler, G., Steinman, R. M., and Bhardwaj, N. (1996) Improved methods for the generation of dendritic cells from nonproliferating progenitors in human blood. *J. Immunol. Methods* 196, 121–135.
- (23) Gildea, L. A., Ryan, C. A., Foertsch, L. M., Kennedy, J. M., Dearman, R. J., Kimber, I., and Gerberick, G. F. (2006) Identification of gene expression changes induced by chemical allergens in dendritic cells: opportunities for skin sensitization testing. *J. Invest. Dermatol.* 126, 1813–1822.
- (24) Cruz, M. T., Goncalo, M., Paiva, A., Morgado, J. M., Figueiredo, A., Duarte, C. B., and Lopes, M. C. (2005) Contact sensitizers downregulate the expression of the chemokine receptors CCR6 and CXCR4 in a skin dendritic cell line. *Arch. Dermatol. Res.* 297, 43–47.
- (25) Aebly, P., Wyss, C., Beck, H., Griem, P., Scheffler, H., and Goebel, C. (2004) Characterization of the sensitizing potential of chemicals by in vitro analysis of dendritic cell activation and skin penetration. *J. Invest. Dermatol.* 122, 1154–1164.
- (26) Divkovic, M., Pease, C. K., Gerberick, G. F., and Basketter, D. A. (2005) Hapten-protein binding: From theory to practical application in the in vitro prediction of skin sensitization. *Contact Dermatitis* 53, 189–200.
- (27) Aptula, A. O., Patlewicz, G., Roberts, D. W., and Schultz, T. W. (2006) Non-enzymatic glutathione reactivity and in vitro toxicity: A non-animal approach to skin sensitization. *Toxicol. in Vitro* 20, 239–247.
- (28) Gerberick, G. F., Vassallo, J. D., Bailey, R. E., Chaney, J. G., Morrall, S. W., and Lepoittevin, J. P. (2004) Development of a peptide reactivity assay for screening contact allergens. *Toxicol. Sci.* 81, 332–343.
- (29) Gerberick, G. F., Ryan, C. A., Kern, P. S., Dearman, R. J., Kimber, I., Patlewicz, G. Y., and Basketter, D. A. (2004) A chemical dataset for evaluation of alternative approaches to skin-sensitization testing. *Contact Dermatitis* 50, 274–288.
- (30) Li, Y., Tseng, Y. J., Pan, D., Liu, J., Kern, P. S., Gerberick, G. F., and Hopfinger, A. J. (2007) 4D-fingerprint categorical QSAR models for skin sensitization based on the classification of local lymph node assay measures. *Chem. Res. Toxicol.* 20, 114–128.
- (31) 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.
- (32) Meschkat, E., Barratt, M. D., and Lepoittevin, J. (2001) Studies of the chemical selectivity of hapten, reactivity, and skin sensitization potency. 1. Synthesis and studies on the reactivity toward model nucleophiles of the (13)C-labeled skin sensitizers hex-1-ene- and hexane-1,3-sultones. *Chem. Res. Toxicol.* 14, 110–117.
- (33) Ahlfors, S. R., Sterner, O., and Hansson, C. (2003) Reactivity of contact allergenic haptens to amino acid residues in a model carrier peptide, and characterization of formed peptide-hapten adducts. *Skin Pharmacol. Appl. Skin Physiol.* 16, 59–68.
- (34) Alvarez-Sanchez, R., Basketter, D., Pease, C., and Lepoittevin, J. P. (2003) Studies of chemical selectivity of hapten, reactivity, and skin sensitization potency. 3. Synthesis and studies on the reactivity toward model nucleophiles of the 13C-labeled skin sensitizers, 5-chloro-2-methylisothiazol-3-one (MCI) and 2-methylisothiazol-3-one (MI). *Chem. Res. Toxicol.* 16, 627–636.
- (35) Nilsson, A. M., Bergstrom, M. A., Luthman, K., Nilsson, J. L., and Karlberg, A. T. (2005) A conjugated diene identified as a prohaptens: Contact allergenic activity and chemical reactivity of proposed epoxide metabolites. *Chem. Res. Toxicol.* 18, 308–316.
- (36) Bergstrom, M. A., Luthman, K., and Karlberg, A.-T. (2007) Metabolic epoxidation of an α,β -unsaturated oxime generates sensitizers of extreme potency. Are nitroso intermediates responsible? *Chem. Res. Toxicol.* 20, 927–936.
- (37) Kato, H., Okamoto, M., Yamashita, K., Nakamura, Y., Fukumori, Y., Nakai, K., and Kaneko, H. (2003) Peptide-binding assessment using mass spectrometry as a new screening method for skin sensitization. *J. Toxicol. Sci.* 28, 19–24.
- (38) Schmidt, R. J., Khan, L., and Chung, L. Y. (1990) Are free radicals and not quinones the haptenic species derived from urushiols and other contact allergenic mono- and dihydric alkylbenzenes? The significance of NADH, glutathione, and redox cycling in the skin. *Arch. Dermatol. Res.* 282, 56–64.
- (39) Gafvert, E., Shao, L. P., Karlberg, A. T., Nilsson, U., and Nilsson, J. L. (1994) Contact allergy to resin acid hydroperoxides. Hapten binding via free radicals and epoxides. *Chem. Res. Toxicol.* 7, 260–266.
- (40) Mutterer, V., Arnau, E. G., Karlberg, A. T., and Lepoittevin, J. P. (2000) Synthesis and allergenic potential of a 15-hydroperoxyabiatic acid-like model: Trapping of radical intermediates. *Chem. Res. Toxicol.* 13, 1028–1036.
- (41) Arnau, E. G., Haberkorn, L., Grossi, L., and Lepoittevin, J. P. (2002) Identification of alkyl radicals derived from an allergenic cyclic tertiary allylic hydroperoxide by combined use of radical trapping and ESR studies. *Tetrahedron* 58, 5535–5545.
- (42) Brared-Christensson, J., Matura, M., Backtorp, C., Borje, A., Nilsson, J. L. G., and Karlberg, A. T. (2006) Hydroperoxides form specific antigens in contact allergy. *Contact Dermatitis* 55, 230–237.
- (43) Romagnoli, P., Labhardt, A. M., and Sinigaglia, F. (1991) Selective interaction of Ni with an MHC-bound peptide. *EMBO J.* 10, 1303–1306.
- (44) Moulon, C., Vollmer, J., and Weltzien, H. U. (1995) Characterization of processing requirements and metal cross-reactivities in T cell clones from patients with allergic contact dermatitis to nickel. *Eur. J. Immunol.* 25, 3308–3315.
- (45) Van den Broeke, L. T., Heffler, L. C., Linder, M. T., Nilsson, J. L. G., Karlberg, A. T., and Scheynius, A. (1999) Direct Ni²⁺ antigen formation on cultured human dendritic cells. *Immunology* 96, 578–585.
- (46) Crum-Brown, A., and Fraser, T. R. 1868–9 On the connection between chemical constitution and physiological action. Part 1. On the physical action of the ammonium bases, derived from Strychia, Brucia, Thebaia, Codeia, Morphia and Nicotia. *Trans. R. Soc. Edinburgh* 25, 151–203.
- (47) Lepoittevin, J. P. (1999) Development of structure-activity relationships (SARs) in allergic contact dermatitis. *Cell. Biol. Toxicol.* 15, 47–55.
- (48) Landsteiner, K., and Jacobs, J. (1936) Studies on the sensitization of animals with simple chemical compounds. II. From the Laboratories of The Rockefeller Institute for Medical Research, 625–639.
- (49) Dupuis, G., and Benezra, C. (1982) *Allergic Contact Dermatitis To Simple Chemicals. A Molecular Approach*, Marcel Dekker, New York.
- (50) 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.
- (51) Patlewicz, G. Y., Basketter, D. A., Smith Pease, C. K., Wilson, K., Wright, Z. M., Roberts, D. W., Bernard, G., Arnau, E. G., and Lepoittevin, J.-P. (2004) Further evaluation of quantitative structure-activity relationship models for the prediction of the skin sensitization potency of selected fragrance allergens. *Contact Dermatitis* 50, 91–97.
- (52) Miller, M. D., Yourtee, D. M., Glaros, A. G., Chappelow, C. C., Eick, J. D., and Holder, A. J. (2005) Quantum mechanical structure-activity relationship analyses for skin sensitization. *J. Chem. Inf. Model.* 45, 924–929.
- (53) Sosted, H., Basketter, D. A., Estrada, E., Johansen, J. D., and Patlewicz, G. Y. (2004) Ranking of hair dye substances according to predicted sensitization potency: Quantitative structure-activity relationships. *Contact Dermatitis* 51, 241–254.
- (54) Fedorowicz, A., Zheng, L., Singh, H., and Demchuk, E. (2004) QSAR study of skin sensitization using local lymph node assay data. *Int. J. Mol. Sci.* 5, 56–66.
- (55) Fedorowicz, A., Singh, H., Soderholm, S., and Demchuk, E. (2005) Structure-activity models for contact sensitization. *Chem. Res. Toxicol.* 18, 954–969.
- (56) Barratt, M. D., Basketter, D. A., and Roberts, D. W. (1998) Structure-activity relationships for contact hypersensitivity. In *Allergic Contact Dermatitis—The Molecular Basis* (Lepoittevin, J. P., Basketter, D. A., Goossens, A., and Karlberg, A. T., Eds.) pp 129–154, Springer-Verlag, Berlin.
- (57) 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.
- (58) Basketter, D. A., Roberts, D. W., Cronin, M., and Scholes, E. W. (1992) The value of the local lymph node assay in quantitative structure-activity investigations. *Contact Dermatitis* 27, 137–142.
- (59) Roberts, D. W., Aptula, A. O., Cronin, M. T., Hulzebos, E., and Patlewicz, G. (2007) Global (Q)SARs for skin sensitisation: Assessment against OECD principles. *SAR QSAR Environ. Res.* 18, 343–365.
- (60) Roberts, D. W., and Patlewicz, G. (2002) Mechanism based structure-activity relationships for skin sensitisation—the carbonyl group domain. *SAR QSAR Environ. Res.* 13, 145–152.
- (61) Roberts, D. W., York, M., and Basketter, D. A. (1999) Structure-activity relationships in the murine local lymph node assay for skin sensitization: a,b-Diketones. *Contact Dermatitis* 41, 14–17.
- (62) Patlewicz, G., Roberts, D. W., and Walker, J. D. (2003) QSARs for the skin sensitization potential of aldehydes and related compounds. *QSAR Comb. Sci.* 22, 196–203.
- (63) Roberts, D. W., Williams, D. L., and Bethell, D. (2007) Electrophilic reactions of skin-sensitizing sultones. *Chem. Res. Toxicol.* 20, 61–71.
- (64) Roberts, D. W., and Basketter, D. A. (1990) A quantitative structure activity/dose response relationship for contact allergic potential of alkyl group transfer agents. *Contact Dermatitis* 23, 331–335.
- (65) Roberts, D. W., Fragnals, R., Lepoittevin, J. P., and Benezra, C. (1991) Refinement of the relative alkylation index (RAI) model for skin sensitization and application to mouse and guinea-pig test data for alkyl alkanesulphonates. *Arch. Dermatol. Res.* 283, 387–394.
- (66) Roberts, D. W., and Basketter, D. A. (1997) Further evaluation of the quantitative structure-activity relationship for skin-sensitizing alkyl transfer agents. *Contact Dermatitis* 37, 107–112.
- (67) Roberts, D. W., and Basketter, D. A. (2000) Quantitative structure-activity relationships: Sulfonate esters in the local lymph node assay. *Contact Dermatitis* 42, 154–161.
- (68) Barratt, M. D., Basketter, D. A., and Roberts, D. W. (1994) Skin sensitization structure-activity relationships for phenyl benzoates. *Toxicol. in Vitro* 8, 823–826.
- (69) 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 SNAr electrophiles. *Chem. Res. Toxicol.* 8, 545–551.
- (70) Franot, C., Roberts, D. W., Basketter, D. A., Benezra, C., and Lepoittevin, J. P. (1994) Structure-activity relationships for contact allergenic potential of γ,γ -dimethyl- γ -butyrolactone derivatives. 2. Quantitative structure-skin sensitization relationships for α -substituted- α -methyl- γ,γ -dimethyl- γ -butyrolactones. *Chem. Res. Toxicol.* 7, 307–312.
- (71) Roberts, D. W., and Benezra, C. (1993) Quantitative structure-activity relationships for skin sensitization potential of urushiol analogues. *Contact Dermatitis* 29, 78–83.
- (72) Estrada, E., Patlewicz, G., Chamberlain, M., Basketter, D., and Larbey, S. (2003) Computer-aided knowledge generation for understanding skin sensitization mechanisms: The TOPS-mode approach. *Chem. Res. Toxicol.* 16, 1226–1235.
- (73) Estrada, E., Patlewicz, G., and Gutierrez, Y. (2004) From knowledge generation to knowledge archive. A general strategy using TOPS-MODE with DEREK to formulate new alerts for skin sensitization. *J. Chem. Inf. Comput. Sci.* 44, 688–698.
- (74) Greene, N. (2002) Computer systems for the prediction of toxicity: An update. *Adv. Drug Delivery Rev.* 54, 417–431.
- (75) Greene, N., Judson, P. N., Langowski, J. J., and Marchant, C. A. (1999) Knowledge-based expert systems for toxicity and metabolism prediction: DEREK, StAR and METEOR. *SAR QSAR Environ. Res.* 10, 299–314, 292 plates.
- (76) Ridings, J. E., Barratt, M. D., Cary, R., Earnshaw, C. G., Eggington, C. E., Ellis, M. K., Judson, P. N., Langowski, J. J., and Marchant, C. A. (1996) Computer prediction of possible toxic action from chemical structure: An update on the DEREK system. *Toxicology* 106, 267–279.
- (77) Barratt, M. D., Basketter, D. A., Chamberlain, M., Admans, G. D., and Langowski, J. J. (1994) An expert system rulebase for identifying contact allergens. *Toxicol. in Vitro* 8, 1053–1060.
- (78) Payne, M. P., and Walsh, P. T. (1994) Structure-activity relationships for skin sensitization potential: Development of structural alerts for use in knowledge-based toxicity prediction systems. *J. Chem. Inf. Comput. Sci.* 34, 154–161.
- (79) Langton, K., Patlewicz, G. Y., Long, A., Marchant, C. A., and Basketter, D. A. (2006) Structure-activity relationships for skin sensitization: Recent improvements to Derek for Windows. *Contact Dermatitis* 55, 342–347.
- (80) Testa, B., Balmat, A.-L., Long, A., and Judson, P. N. (2005) Predicting drug metabolism—An evaluation of the expert system METEOR. *Chem. Biodiversity* 2, 872–885.
- (81) Nilsson, A. M., Bergstrom, M. A., Luthman, K., Nilsson, J. L., and Karlberg, A. T. (2005) An α,β -unsaturated oxime identified as a strong contact allergen. Indications of antigen formation via several pathways. *Food Chem. Toxicol.* 43, 1627–1636.
- (82) Enslein, K., Gombar, V. K., Blake, B. W., Maibach, H. I., Hostynek, J. J., Sigman, C. C., and Bagheri, D. (1997) A quantitative structure-toxicity relationships model for the dermal sensitization guinea pig maximization assay. *Food Chem. Toxicol.* 35, 1091–1098.
- (83) Hall, L. H., Mohny, B., and Kier, L. B. (1991) The electrotopological state: structure information at the atomic level for molecular graphs. *J. Chem. Inf. Comput. Sci.* 31, 76–82.
- (84) Rosenkranz, H. S., Cunningham, A. R., Zhang, Y. P., Claycamp, H. G., Macina, O. T., Sussman, N. B., Grant, S. G., and Klopman, G. (1999) Development, characterization and application of predictive-toxicology models. *SAR QSAR Environ. Res.* 10, 277–298.
- (85) Rosenkranz, H. S., Cunningham, A. R., Zhang, Y. P., and Klopman, G. (1999) Applications of the case/multicase SAR method to environmental and public health situations. *SAR QSAR Environ. Res.* 10, 263–276.
- (86) Klopman, G. (1992) MULTICASE. 1. A Hierarchical computer automated structure evaluation program. *Quant. Struct.-Act. Relat.* 11, 176–184.
- (87) Karol, M. H., Graham, C., and Barratt, M. D. (1999) Structure-activity relationship studies of hypersensitivity. *Comments Toxicol.* 7, 59–77.
- (88) Graham, C., Gealy, R., Macina, O. T., Karol, M. H., and Rosenkranz, H. S. (1996) QSAR for allergic contact dermatitis. *Quant. Struct.-Act. Relat.* 15, 224–229.
- (89) Dimitrov, S., Dimitrova, G., Pavlov, T., Dimitrova, N., Patlewicz, G., Niemela, J., and Mekenyan, O. A. (2005) A stepwise approach for defining the applicability domain of SAR and QSAR models. *J. Chem. Inf. Model.* 45, 839–849.
- (90) Dimitrov, S. D., Low, L. K., Patlewicz, G. Y., Kern, P. S., Dimitrova, G. D., Comber, M. H. I., Phillips, R. D., Niemela, J., Bailey, P. T., and Mekenyan, O. G. (2005) Skin sensitization: Modeling based on skin metabolism simulation and formation of protein conjugates. *Int. J. Toxicol.* 24, 189–204.
- (91) Patlewicz, G., Dimitrov, S. D., Low, L. K., Kern, P. S., Dimitrova, G. D., Comber, M. I., Aptula, A. O., Phillips, R. D., Niemela, J., Madsen, C., Wedebye, E. B., Roberts, D. W., Bailey, P. T., and Mekenyan, O. G. (2007) TIMES-SS-A promising tool for the assessment of skin sensitization hazard. A characterization with respect to the OECD validation principles for (Q)SARs and an external evaluation for predictivity. *Regul. Toxicol. Pharmacol.* 48, 225–239.
- (92) Hellerström, S. (1939) Hypersensitivity test in professional eczema. Their applicability and sources of error. *Acta Dermato-Venereol.* 20, 657.
- (93) Hellerstrom, S., Thyresson, N., Blohm, S. G., and Widmark, G. (1955) On the nature of the eczematogenic component of oxidized delta 3-carene. *J. Invest. Dermatol.* 24, 217–224.
- (94) Widmark, G. (1975) Investigations in the field of terpenes. *Svensk Kem. Tidskr.* 69, 175–184.

- (95) Johansen, J., Menne, T., Christophersen, J., Kaaber, K., and Veien, N. (2000) Changes in the pattern of sensitization to common contact allergens in Denmark between 1985–86 and 1997–98, with a special view to the effect of preventive strategies. *Br. J. Dermatol.* 142, 490–495.
- (96) Karlberg, A. T., and Doms-Gossens, A. (1997) Contact allergy to oxidized d-limonene among dermatitis patients. *Contact Dermatitis* 36, 201–206.
- (97) Karlberg, A.-T., and Lindell, B. (1993) Limonene. In *Criteria Documents from the Nordic Expert Group 1993. Nordic Councils of Ministers* (Beije, B., and Lundberg, P., Eds.) pp 207–246. Arbete & Hälsa.
- (98) Karlberg, A. T., Boman, A., and Melin, B. (1991) Animal experiments on the allergenicity of d-limonene-the citrus solvent. *Ann Occup Hyg* 35, 419–426.
- (99) Karlberg, A. T., Magnusson, K., and Nilsson, U. (1992) Air oxidation of d-limonene (the citrus solvent) creates potent allergens. *Contact Dermatitis* 26, 332–340.
- (100) Karlberg, A. T., Shao, L. P., Nilsson, U., Gafvert, E., and Nilsson, J. L. (1994) Hydroperoxides in oxidized d-limonene identified as potent contact allergens. *Arch. Dermatol. Res.* 286, 97–103.
- (101) Karlberg, A. T., Magnusson, K., and Nilsson, U. (1994) Influence of an anti-oxidant on the formation of allergenic compounds during auto-oxidation of d-limonene. *Ann. Occup. Hyg.* 38, 199–207.
- (102) Nilsson, U., Bergh, M., Shao, L. P., and Karlberg, A. T. (1996) Analysis of contact allergenic compounds in oxidized d-limonene. *Chromatographia* 42, 199–205.
- (103) Matura, M., Goossens, A., Bordalo, O., Garcia-Bravo, B., Magnusson, K., Wrangsjö, K., and Karlberg, A. T. (2002) Oxidized citrus oil (R-limonene): A frequent skin sensitizer in Europe. *J. Am. Acad. Dermatol.* 47, 709–714.
- (104) Matura, M., Skold, M., Borje, A., Andersen, K. E., Bruze, M., Frosch, P., Goossens, A., Johansen, J. D., Svedman, C., White, I. R., and Karlberg, A. T. (2006) Not only oxidized R-(+)- but also S-(–)-limonene is a common cause of contact allergy in dermatitis patients in Europe. *Contact Dermatitis* 55, 274–279.
- (105) Skold, M., Borje, A., Matura, M., and Karlberg, A. T. (2002) Studies on the autooxidation and sensitizing capacity of the fragrance chemical linalool, identifying a linalool hydroperoxide. *Contact Dermatitis* 46, 267–272.
- (106) Skold, M., Borje, A., Harambasic, E., and Karlberg, A. T. (2004) Contact allergens formed on air exposure of linalool. Identification and quantification of primary and secondary oxidation products and the effect on skin sensitization. *Chem. Res. Toxicol.* 17, 1697–1705.
- (107) Matura, M., Skold, M., Borje, A., Andersen, K. E., Bruze, M., Frosch, P., Goossens, A., Johansen, J. D., Svedman, C., White, I. R., and Karlberg, A. T. (2005) Selected oxidized fragrance terpenes are common contact allergens. *Contact Dermatitis* 52, 320–328.
- (108) Backtorp, C., Wass, J. R., Panas, I., Skold, M., Borje, A., and Nyman, G. (2006) Theoretical investigation of linalool oxidation. *J. Phys. Chem. A* 110, 12204–12212.
- (109) Schnuch, A., Lessmann, H., Geier, J., Frosch, P. J., and Uter, W. (2004) Contact allergy to fragrances: Frequencies of sensitization from 1996 to 2002. Results of the IVDK*. *Contact Dermatitis* 50, 65–76.
- (110) Hagvall, L., Backtorp, C., Svensson, S., Nyman, G., Borje, A., and Karlberg, A. T. (2007) Fragrance compound geraniol forms contact allergens on air exposure. Identification and quantification of oxidation products and effect on skin sensitization. *Chem. Res. Toxicol.* 20, 807–814.
- (111) Skold, M., Karlberg, A. T., Matura, M., and Borje, A. (2006) The fragrance chemical beta-caryophyllene-air oxidation and skin sensitization. *Food Chem. Toxicol.* 44, 538–545.
- (112) Karlberg, A.-T. (2000) Colophony. In *Handbook of Occupational Dermatology* (Kanerva, L., Elsnér, P., Wahlberg, J. E., and Maibach, H. I., Eds.) pp 509–516. Springer-Verlag, Heidelberg.
- (113) Fossereau, J., Benezra, C., and Maibach, H. (1982) *Occupational Contact Dermatitis. Clinic and Chemical Aspects*, Munksgaard, Copenhagen.
- (114) Karlberg, A. T., Bergstedt, E., Boman, A., Bohlander, K., Liden, C., Lars, J., Nilsson, G., and Wahlberg, J. E. (1985) Is abietic acid the allergenic component of colophony? *Contact Dermatitis* 13, 209–215.
- (115) Karlberg, A. T., Bohlander, K., Boman, A., Hacksell, U., Hermansson, J., Jacobsson, S., and Nilsson, J. L. (1988) Identification of 15-hydroperoxyabietic acid as a contact allergen in Portuguese colophony. *J. Pharm. Pharmacol.* 40, 42–47.
- (116) Karlberg, A. T., and Gafvert, E. (1996) Isolated colophony allergens as screening substances for contact allergy. *Contact Dermatitis* 35, 201–207.
- (117) Karlberg, A.-T., Bråred Christensson, J., Borje, A., Harambasic, E., and Matura, M. (2007) Methyl esterification of 15-hydroperoxyabietic acid does not affect the patch test result in colophonium allergic patients. *Contact Dermatitis* 56, 355–356.
- (118) Donbrow, M. (1987) Stability of polyoxyethylene chain. In *Nonionic Surfactants: Physical Chemistry* (Schick, M. J., Ed.) pp 1011–1067, New York.
- (119) Bergh, M., Magnusson, K., Nilsson, J. L., and Karlberg, A. T. (1997) Contact allergenic activity of Tween 80 before and after air exposure. *Contact Dermatitis* 37, 9–18.
- (120) Bergh, M., Magnusson, K., Nilsson, J. L., and Karlberg, A. T. (1998) Formation of formaldehyde and peroxides by air oxidation of high purity polyoxyethylene surfactants. *Contact Dermatitis* 39, 14–20.
- (121) Bergh, M., Shao, L. P., Hagelthorn, G., Gafvert, E., Nilsson, J. L., and Karlberg, A. T. (1998) Contact allergens from surfactants. Atmospheric oxidation of polyoxyethylene alcohols, formation of ethoxylated aldehydes, and their allergenic activity. *J. Pharm. Sci.* 87, 276–282.
- (122) Bergh, M., Shao, L. P., Magnusson, K., Gafvert, E., Nilsson, J. L., and Karlberg, A. T. (1999) Atmospheric oxidation of poly(oxyethylene) alcohols. Identification of ethoxylated formates as oxidation products and study of their contact allergenic activity. *J. Pharm. Sci.* 88, 483–488.
- (123) Bodin, A., Shao, L. P., Nilsson, J. L., and Karlberg, A. T. (2001) Identification and allergenic activity of hydroxylaldehydes - a new type of oxidation product from an ethoxylated non-ionic surfactant. *Contact Dermatitis* 44, 207–212.
- (124) Bodin, A., Linnerborg, M., Nilsson, J. L., and Karlberg, A. T. (2003) Structure elucidation, synthesis, and contact allergenic activity of a major hydroperoxide formed at autooxidation of the ethoxylated surfactant C12E5. *Chem. Res. Toxicol.* 16, 575–582.
- (125) Bodin, A., Fischer, T., Bergh, M., Nilsson, J. L., and Karlberg, A. T. (2000) Skin irritation from air-oxidized ethoxylated surfactants. *Contact Dermatitis* 43, 82–89.
- (126) Matura, M., Bodin, A., Skare, L., Nyren, M., Hovmark, A., Lindberg, M., Lundeberg, L., Wrangsjö, K., and Karlberg, A. T. (2004) Multicentre patch test study of air-oxidized ethoxylated surfactants. *Contact Dermatitis* 51, 180–188.
- (127) Bezdard, M., Karlberg, A. T., Montelius, J., and Lepoittevin, J. P. (1997) Skin sensitization to linalyl hydroperoxide: support for radical intermediates. *Chem. Res. Toxicol.* 10, 987–993.
- (128) Bezdard, M., Gimenez-Arnau, E., Meurer, B., Grossi, L., and Lepoittevin, J. P. (2005) Identification of carbon-centred radicals derived from linalyl hydroperoxide, a strong skin sensitizer: A possible route for protein modifications. *Bioorg. Med. Chem.* 13, 3977–3986.
- (129) Klecak, G. (1985) The Freund's complete adjuvant test and the open epicutaneous test. In *Contact Allergy Predictive Tests in Guinea Pigs* (Andersen, K. E., and Maibach, H. I., Eds.) pp 152–171, Karger, Basel.
- (130) Smith, C. K., and Hotchkiss, S. A. M. (2001) Enzymes and pathways of xenobiotic metabolism in skin. *Allergic Contact Dermatitis: Chemical and Metabolic Mechanisms*, pp 89–117, Taylor and Francis, London.
- (131) Smith, C. K., and Hotchkiss, S. A. M. (2001) Xenobiotics as skin sensitizers: Metabolic activation and detoxification, and protein-binding mechanisms. In *Allergic Contact Dermatitis: Chemical and Metabolic Mechanisms*, pp 119–205, Taylor and Francis, London.
- (132) Kalgutkar, A. S., Gardner, I., Obach, R. S., Shaffer, C. L., Callegari, E., Henne, K. R., Mutlib, A. E., Dalvie, D. K., Lee, J. S., Nakai, Y., O'Donnell, J. P., Boer, J., and Harriman, S. P. (2005) A comprehensive listing of bioactivation pathways of organic functional groups. *Curr. Drug Metab.* 6, 161–225.
- (133) Bergstrom, M. A., Luthman, K., Nilsson, J. L. G., and Karlberg, A.-T. (2006) Conjugated dienes as prohaptens in contact allergy: In vivo and in vitro studies of structure-activity relationships, sensitizing capacity, and metabolic activation. *Chem. Res. Toxicol.* 19, 760–769.
- (134) Bergstrom, M. A., Ott, H., Carlsson, A., Neis, M., Zwadlo-Klarwasser, G., Jonsson, C. A., Merk, H. F., Karlberg, A. T., and Baron, J. M. (2007) A skin-like cytochrome P450 cocktail activates prohaptens to contact allergenic metabolites. *J. Invest. Dermatol.* 127, 1145–1153.
- (135) Smith, C. K., Moore, C. A., Elahi, E. N., Smart, A. T. S., and Hotchkiss, S. A. M. (2000) Human skin absorption and metabolism of the contact allergens, cinnamic aldehyde, and cinnamic alcohol. *Toxicol. Appl. Pharmacol.* 168, 189–199.
- (136) Cheung, C., Hotchkiss, S. A. M., and Pease, C. K. S. (2003) Cinnamic compound metabolism in human skin and the role metabolism may play in determining relative sensitization potency. *J. Dermatol. Sci.* 31, 9–19.
- (137) Elahi, E. N., Wright, Z., Hinselwood, D., Hotchkiss, S. A. M., Basketter, D. A., and Pease, C. K. S. (2004) Protein binding and metabolism influence the relative skin sensitization potential of cinnamic compounds. *Chem. Res. Toxicol.* 17, 301–310.

- (138) Wright, Z. M., Basketter, P. A., Blaikie, L., Cooper, K. J., Warbrick, E. V., Dearman, R. J., and Kimber, I. (2001) Vehicle effects on skin sensitizing potency of four chemicals: Assessment using the local lymph node assay. *Int. J. Cosmet. Sci.* 23, 75–83.
- (139) Kimber, I., Hilton, J., Dearman, R. J., Gerberick, G. F., Ryan, C. A., Basketter, D. A., Lea, L., House, R. V., Ladics, G. S., Loveless, S. E., and Hastings, K. L. (1998) Assessment of the skin sensitization potential of topical medicaments using the local lymph node assay: An interlaboratory evaluation. *J. Toxicol. Environ. Health A* 53, 563–579.
- (140) Tang, X. J., Li, L. Y., Huang, J. X., and Deng, Y. Y. (2002) Guinea pig maximization test for trichloroethylene and its metabolites. *Biomed. Environ. Sci.* 15, 113–118.
- (141) Lash, L. H., Fisher, J. W., Lipscomb, J. C., and Parker, J. C. (2000) Metabolism of trichloroethylene. *Environ. Health Perspect.* 108, 177–200.
- (142) Bertrand, F., Basketter, D. A., Roberts, D. W., and Lepoittevin, J. P. (1997) Skin sensitization to eugenol and isoeugenol in mice: Possible metabolic pathways involving ortho-quinone and quinone methide intermediates. *Chem. Res. Toxicol.* 10, 335–343.
- (143) Ashby, J., Basketter, D. A., Paton, D., and Kimber, I. (1995) Structure activity relationships in skin sensitization using the murine local lymph node assay. *Toxicology* 103, 177–194.
- (144) Basketter, D. A., and Goodwin, B. F. (1988) Investigation of the prohaptent concept. Cross-reactions between 1,4-substituted benzene derivatives in the guinea pig. *Contact Dermatitis* 19, 248–253.
- (145) Coulter, E. M., Farrell, J., Mathews, K. L., Maggs, J. L., Pease, C. K., Lockley, D. J., Basketter, D. A., Park, B. K., and Naisbitt, D. J. (2007) Activation of human dendritic cells by p-phenylenediamine. *J. Pharmacol. Exp. Ther.* 320, 885–892.
- (146) White, J. M., Kullavanijaya, P., Duangdeeden, I., Zazzeroni, R., Gilmour, N. J., Basketter, D. A., and McFadden, J. P. (2006) p-Phenylenediamine allergy: The role of Bandrowski's base. *Clin. Exp. Allergy* 36, 1289–1293.
- (147) Naisbitt, D. J. (2004) Drug hypersensitivity reactions in skin: understanding mechanisms and the development of diagnostic and predictive tests. *Toxicology* 194, 179–196.
- (148) Seidenari, S., Mantovani, L., Manzini, B. M., and Pignatti, M. (1997) Cross-sensitizations between azo dyes and para-amino compounds. A study of 236 azo-dye-sensitive subjects. *Contact Dermatitis* 36, 91–96.
- (149) Levine, W. G. (1991) Metabolism of azo dyes: implication for detoxication and activation. *Drug Metab. Rev.* 23, 253–309.
- (150) Hansson, C., Ezzelarab, M., and Sterner, O. (1995) Oxidative activation of the propolis hapten isoprenyl caffeate. *Acta Dermato-Venereol.* 75, 34–36.
- (151) Aptula, A. O., Patlewicz, G., and Roberts, D. W. (2005) Skin sensitization: Reaction mechanistic applicability domains for structure-activity relationships. *Chem. Res. Toxicol.* 18, 1420–1426.
- (152) Goetz, G., Meschkat, E., and Lepoittevin, J. P. (1999) Synthesis of a water-soluble analog of 6-methyl-3-N-alkyl catechol labeled with carbon 13: NMR approach to the reactivity of poison ivy/oak sensitizers toward proteins. *Bioorg. Med. Chem. Lett.* 9, 1141–1146.
- (153) Smith Pease, C. K. (2003) From xenobiotic chemistry and metabolism to better prediction and risk assessment of skin allergy. *Toxicology* 192, 1–22.
- (154) Isaksson, M., Bruze, M., Goossens, A., and Lepoittevin, J. P. (2000) Patch-testing with serial dilutions of tixocortol pivalate and potential cross-reactive substances. *Acta Dermato-Venereol.* 80, 33–38.
- (155) Anderson, C., Hehr, A., Robbins, R., Hasan, R., Athar, M., Mukhtar, H., and Elmet, C. A. (1995) Metabolic requirements for induction of contact hypersensitivity to immunotoxic polyaromatic hydrocarbons. *J. Immunol.* 155, 3530–3537.
- (156) Sjöborg, S., Fregert, S., and Trulsson, L. (1984) Contact allergy to styrene and related chemicals. *Contact Dermatitis* 10, 94–96.
- (157) Lovell, C. R. (1993) *Plants and the Skin*, Blackwell Scientific Publications, Oxford.
- (158) Baer, H., Watkins, R. C., and Bowser, T. (1966) Delayed contact sensitivity to catechols and resorcinols. Relationship of structure and immunization procedure to sensitizing capacity. *Immunochemistry* 3, 479–486.
- (159) Marques, M. M., and Beland, F. A. (1997) Identification of tamoxifen-DNA adducts formed by 4-hydroxytamoxifen quinone methide. *Carcinogenesis* 18, 1949–1954.
- (160) White, I. R. (2006) Second cite. Contact allergy to hair dyes is a well-recognized problem. *Clin. Exp. Dermatol.* 31, 724–726.
- (161) Mayer, R. L. (1954) Group sensitization to compounds of quinone structure and its biochemical basis. *Prog. Allergy* 4, 79–172.
- (162) Basketter, D. A., and Liden, C. (1992) Further investigation of the prohaptent concept—Reactions to benzene derivatives in man. *Contact Dermatitis* 27, 90–97.
- (163) Lisi, P., and Hansel, K. (1998) Is benzoquinone the prohaptent in cross-sensitivity among aminobenzene compounds? *Contact Dermatitis* 39, 304–306.
- (164) Picardo, M., Cannistraci, C., Cristaudo, A., De Luca, C., and Santucci, B. (1990) Study on cross-reactivity to the para group. *Dermatologica* 181, 104–108.
- (165) Coulter, E. M., Farrell, J., Mathews, K. L., Maggs, J. L., Pease, C. K., Lockley, D. J., Basketter, D. A., Park, B. K., and Naisbitt, D. J. (2007) Activation of human dendritic cells by p-phenylenediamine. *J. Pharmacol. Exp. Ther.* 320, 885–892.
- (166) Eilstein, J., Gimenez-Arnau, E., Duche, D., Rousset, F., and Lepoittevin, J. P. (2006) Synthesis and reactivity toward nucleophilic amino acids of 2,5-[¹³C]-dimethyl-p-benzoquinonediimine. *Chem. Res. Toxicol.* 19, 1248–1256.
- (167) Eilstein, J., Gimenez-Arnau, E., Rousset, F., and Lepoittevin, J.-P. (2006) Species involved in the reactivity of PPD derivatives with amino acids. *Contact Dermatitis* 55, 9.
- (168) Stanley, L. A., Skare, J. A., Doyle, E., Powrie, R., D'Angelo, D., and Elcombe, C. R. (2005) Lack of evidence for metabolism of p-phenylenediamine by human hepatic cytochrome P450 enzymes. *Toxicology* 210, 147–157.
- (169) Smith, C. K., Cheung, C., Elahi, E. N., and Hotchkiss, S. A. M. (2001) High-performance liquid chromatography method for the quantification of non-radiolabelled cinnamic compounds in analytes derived from human skin absorption and metabolism experiments. *J. Chromatogr. B* 758, 249–264.
- (170) Basketter, D. A. (1992) Skin sensitization to cinnamic alcohol: The role of skin metabolism. *Acta Dermato-Venereol.* 72, 264–265.
- (171) Kohl, C., Schiller, C. D., Gescher, A., Farmer, P. B., and Bailey, E. (1992) Acetoxime is metabolized by human and rodent hepatic cytochrome-p450 enzymes to the genotoxicant and carcinogen propane 2-nitronate. *Carcinogenesis* 13, 1091–1094.
- (172) Volkel, W., Wolf, N., Derelanko, M., and Dekant, W. (1999) Slow oxidation of acetoxime and methylethyl ketoxime to the corresponding nitronates and hydroxy nitronates by liver microsomes from rats, mice, and humans. *Toxicol. Sci.* 47, 144–150.
- (173) Parmar, D., and Burka, L. T. (1991) Metabolism and disposition of cyclohexanone oxime in male F344 rats. *Drug Metab. Dispos.* 19, 1101–1107.
- (174) de Groot, A. C. (1994) *Patch Testing. Test Concentrations and Vehicles for 3700 Chemicals*, Elsevier Science B.V., Amsterdam.
- (175) Fregert, S., and Hjorth, N. (1969) Results of standard patch tests with substances abandoned. *Contact Dermatitis Newsl.* 5, 85–86.
- (176) Santucci, B., Cristaudo, A., Cannistraci, C., and Picardo, M. (1987) Contact dermatitis to fragrances. *Contact Dermatitis* 16, 93–95.
- (177) Frosch, P. J., Pilz, B., Andersen, K. E., Burrows, D., Camarasa, J. G., Dooms-Goossens, A., Ducombs, G., Fuchs, T., Hannuksela, M., and Lachapelle, J. M. (1995) Patch testing with fragrances: Results of a multicenter study of the European Environmental and Contact Dermatitis Research Group with 48 frequently used constituents of perfumes. *Contact Dermatitis* 33, 333–342.

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