COMMITTEE ON THE TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Recommendations of the Bystander Risk Assessment Working Group report concerning skin sensitisation from exposure to pesticides

BACKGROUND

1. A joint working group of the Advisory Committee on Pesticides (ACP) and the Committee on Toxicity (COT) was set up in 2010 to review the methods used in the regulatory assessment of potential health risks to bystanders and residents from the application of pesticides. This group, the Bystander Risk Assessment Working Group (BRAWG), published its report in 2012 (BRAWG Report, Appendix 1). The main aims of the working group were to review the current approach to modelling exposures for bystanders and residents in the light of current scientific knowledge, and also to review the approach used to assess the risks arising from exposures, in the light of current knowledge.

2. The BRAWG report defines bystanders and residents in the following terms:-

Bystanders are persons who are located within or directly adjacent to the area where PPP (plant protection product) application or treatment is in process or has recently been completed; whose presence is quite incidental and unrelated to work involving PPPs, but whose position might lead them to be exposed; and who may not choose or be able to take action to avoid or control exposure.

Residents are persons who live, work or attend a school or other institution adjacent to an area that is or has been treated with a PPP; whose presence is quite incidental and unrelated to work involving PPPs but whose position might lead them to be exposed; who may not choose or be able to take action to avoid or control exposure; and who might be in the location for 24 hours per day.

3. In considering risk assessment, the working group noted a concern that some individuals may become sensitised to pesticides. The report states that risk factors for sensitisation are, in general, not well understood, and that further work is needed to characterise and quantify the potential of pesticide formulations in current use to induce skin sensitisation in humans. BRAWG made three specific recommendations on skin sensitisation. These are expressed in the following paragraphs of the BRAWG report:-

- Paragraph 5.27: The group **recommends** that research be conducted on the extent to which current or new formulations may change the ability of chemicals to act as sensitisers
- Paragraph 5.28: The group recognises the importance of the local lymph node assay (LLNA) in providing more quantitative estimates of sensitising potency, but **recommends** that further work be undertaken to characterise better the LLNA potency of formulations currently used, and the influence of co-formulants on sensitisation. The relationship between such potency estimates and human risk is unclear and the group **recommends** that further work be undertaken to define this relationship.

4. The Government has accepted all the recommendations of the working group (Government Response to BRAWG Report, Appendix 2). It has stated that it will discuss with the COT how the above recommendations could be taken forward.

SKIN SENSITISATION AND PESTICIDES

5. The section on skin sensitisation in the BRAWG Report first discusses possible acute local effects of pesticides, such as irritation of directly exposed tissues. It then discusses allergic sensitisation as a result of exposure and re-exposure to sensitising chemicals. Paragraphs 4.60 to 4.68 (pages 27-28) of the Report discuss issues arising from irritation and skin sensitisation evoked by pesticide formulations.

Acute local effects of pesticides

6. Paragraph 4.61 of the BRAWG Report states that bystander and resident exposure to pesticides is most likely to be to diluted products, and acute effects such as skin or eye irritation, localised to directly exposed tissues, can occur if the exposure is at sufficiently high concentration for a sufficient period of time. The current approach to assessing local effects of chemicals is set out in the Dangerous Preparations Directive (1999/45/EC). The Directive states that, "The systematic assessment of all the dangerous health effects is expressed by means of concentration limits, expressed as a weight/weight percentage, … and in conjunction with the classification of the substance". Thus, if a chemical is classified as "irritant", a concentration limit of 20% weight/weight applies to that chemical when used in a product. If a chemical is classified as "sensitising" (see below), then a concentration limit of 1% weight/weight applies. The derivation of these "trigger" concentrations is based upon expert opinion. If an in-use dilution of a chemical, as specified on the label of a product, is above the trigger value for its classification, then a specific assessment of the dilution has to be performed.

7. There are potentially concerns about the use of these trigger concentrations and their safety. For sensitisers for instance, it is assumed that, if their concentration is below 1% w/w in a diluted product, there is no further issue regarding their safety or sensitising potency. The use of this1% w/w dilution as a suitable trigger concentration is based upon expert opinion, and on the assumption that dilutions below 1% are not likely to be harmful. However, the alternative would be to perform an assessment of every in-use dilution of a chemical available, which might prove to be too costly and laborious a process.

Allergic sensitisation to pesticides

8. Allergic contact dermatitis (ACD) is a common environmental health problem, and develops in two phases – in the first "induction" phase, when the skin of a susceptible individual is exposed to sufficient amounts of a chemical sensitiser (allergen), a primary cutaneous immune response occurs (Gerberick 2000). There may be no visible changes in the skin at this point, but the individual is now sensitised to the allergen. On subsequent contact with the same allergen, at the same or a different skin site, an accelerated and more aggressive secondary immune response occurs at the site of contact, and this is the second "elicitation"

phase of the allergic response. There are visible changes in the skin as a result of this response.

9. The biological events which lead to sensitisation are now relatively well understood. In the induction phase, a small molecular weight chemical (called a hapten) contacts the skin and penetrates into the viable epidermis, where it reacts either directly or indirectly with proteins to form an antigenic complex (Robinson 2000). Dendritic cells, and Langerhans cells in particular, are then mobilised, take up the antigen and migrate via the lymphatics to draining lymph nodes. There they present the antigen to responsive T-lymphocytes, which divide and differentiate into memory cells. The induction phase of skin sensitisation is associated with the activation of both CD4 (T helper) and CD8 (T cytotoxic) cells (Gerberick 2000). In the second, elicitation, phase, re-exposure to the inducing chemical (or a cross-reacting chemical) re-activates the T-lymphocyte memory cells, which release cytokines and chemokines and initiate the inflammatory response, characterised by the common clinical manifestations, redness and swelling, of allergic contact dermatitis (ACD).

10. All allergenic chemicals show dose-response and threshold characteristics, although these can vary markedly from individual to individual (Robinson 2000). Other factors which can influence these characteristics include inherent potency, the duration and frequency of exposure, and the vehicle in which the chemical is contained. Robinson points out that a chemical which is a contact allergen can be formulated into a consumer product, as long as it is at a level that produces an acceptably low incidence of sensitisation. One other critical factor in the development of sensitisation is dose of chemical per unit area (Kimber 2008); experiment has shown that it is the dose per unit area of skin, rather than the total amount of chemical delivered, that is a key metric in the acquisition of sensitisation. Thus, a mass of substance that can produce sensitisation may not necessarily cause elicitation if it is administered over a larger area of skin.

CHARACTERISATION OF SENSITISING POTENTIAL OF FORMULATIONS

The Local Lymph Node Assay (LLNA)

11. One of the main tests in current use to examine whether a chemical might be a skin sensitiser is the mouse local lymph node assay (LLNA). The guidelines for testing procedures are set out in OECD guideline 429 (OECD 429). The LLNA is now currently used routinely for chemical hazard/risk assessment in the EU, and is the required test for plant protection products (personal communication from HSE Chemicals Regulation Directorate, 23 July 2014).

History of the LLNA

12. The LLNA was developed during the 1990s and, by the early 2000s, papers were being published which described the advantages of the assay, the validation process for it, and why it could be the preferred method for regulators (Kimber 1992; Gerberick 2000; Basketter 2002). The major strength of the assay is that the clonal expansion of T lymphocytes and, in particular, the vigour of the proliferative response in draining lymph nodes, correlates closely with the extent to which skin

sensitisation develops. The LLNA is based on the measurement of the response induced in lymph nodes. It should be noted that it is the induction phase that is assessed in this assay. There are significant advantages of the LLNA assay over other assays for skin sensitisation with respect to animal welfare.

LLNA method

13. The standard LLNA protocol uses young adult, 6 to 16-week old, female CBA/Ca strain mice. Groups of 4 or 5 mice are treated by application to the back of both ears with several concentrations (which do not result in excessive local irritation or systemic toxicity) of a chemical for three days (Basketter 2002). A control group is treated with vehicle alone. There is a two-day rest period, and on the sixth day after initiation of exposure all the mice receive an intravenous injection into the tail vein of ^{3}H-labelled thymidine. Five hours later the animals are killed, the draining auricular lymph nodes are excised and pooled for each experimental group or for each individual animal, and a cell suspension is prepared. The incorporation of radioactive thymidine into the activated immune cells is measured by β-scintillation counting. For each concentration of the test material, a stimulation index (SI) is derived, relative to the concurrent vehicle control. Chemicals which, at one or more test concentrations, induce an SI of 3 or more are classified as skin sensitisers. The amount of chemical required to induce an SI of 3 is known as the EC3 (Effective Concentration 3) which can be estimated from the LLNA dose-response data.

Key strengths of the LLNA

14. A key advantage of the LLNA EC3 value is that it gives an estimate of the relative potency of a sensitiser. EC3 values can be expressed as the percentage concentration of test chemical required, or as dose required for induction per unit area of skin (described in Loveless 2010). Considering percentages, it follows that a chemical with a low EC3 value, for example, 0.02% or 0.04%, is a strong sensitiser, because a very small amount of substance is required to induce a sensitisation response. Chemicals with high EC3 values, for example, 75%, are considered as weak sensitisers.

15. The LLNA has been shown to be a relevant model for identifying chemicals with the potential to cause skin sensitisation (Basketter 2007). Validation studies have been carried out over a number of years, with the EC3 measurements found to be reproducible in both intra- and inter-laboratory evaluations, and stable over time. Several independent groups of researchers have also shown that EC3 values correlate closely with data on relative human skin sensitisation potency (Schneider 2004; Basketter 2005a).

Databases containing LLNA data

16. Compilations of LLNA data have been made by several research groups (for example, Gerberick 2005 provides a database of 211 individual chemicals comprising LLNA data; a second compilation described in Kern 2010 includes an additional 108 substances with LLNA data). Thus, appreciable databases containing information on LLNA results have been gradually built up over time.

17. Besides information on individual active ingredients, some data are also available on formulations. One large publicly available database of LLNA results for

pesticide formulations was published in 2010 by ICCVAM, the Interagency Coordinating Committee on the Validation of Alternative Methods in the USA (ICCVAM 2010). Paragraph 4.66 of the BRAWG report states that there were details on 104 tested products in this database, of which 54% were classified as sensitisers. However, not all formulations were identified by the name of the active substance, and it was difficult for the working group to ascertain how representative the tested formulations were of those approved in the UK.

18. Currently in Europe, registration data for chemicals is submitted through REACH (a European regulation for the Registration, Evaluation, Authorisation and Restriction of Chemicals) to the ECHA, the European Chemicals Agency, and LLNA results, where available on a substance, are included on the ECHA database. The ECHA database is publicly accessible. The HSE also has a database of studies which have been received by them (personal communication from HSE Chemicals Regulation Directorate, 17 April 2014).

19. Data requirements for individual PPPs are specified in EU regulations. New requirements for active substances are described in regulation EU 283/13, and state that a study must be carried out to provide information on the potential of the active substance to cause sensitisation, unless the active substance is already a known sensitiser, and that the LLNA should be used for this purpose. A separate regulation, EU 284/13, describes the requirements for products, specifically for plant protection products. This also states that the formulation must be tested using the LLNA, unless the active substances or co-formulants in the mixture are already known to have sensitising properties.

CHARACTERISATION OF RELATIVE SENSITISING POTENCY OF CHEMICALS

Current classification

20. The current classification of substances for sensitisation potential is still, at the moment, binary – a substance either is, or is not, classified as a skin sensitiser. Use of the LLNA has demonstrated the fact that contact allergens can vary by up to four or five orders of magnitude in terms of their relative potency to cause sensitisation (Loveless 2010), yet the qualitative classification scheme remains. Thus, a substance that induces a 3-fold or greater increase in lymph node proliferation at a test concentration of 0.5% is classified as a sensitiser in the same way as another chemical that may require a 50% concentration to bring about an SI of 3.

21. Attempts have been made to sub-categorise and to rate chemicals according to their relative potency to induce sensitisation. Table 1 below shows the definition of allergenic potency categories based on tenfold EC3 cut-off values, as suggested by the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) in 2003:-

Category	EC3 values* (%)
Extreme	<0.1
Strong	≥ 0.1 to <1
Moderate	≥ 1 to <10
Weak	≥ 10 to ≤100

Table 1
Sub-categorisation of contact allergens on the basis of relative skin sensitisation (adapted from ECETOC 2003 and reproduced from Loveless 2010)

***** EC3 values are defined as the amount of chemical required to induce a threefold increase in lymph node cell proliferation compared to vehicle control values.

22. More recently, the European Chemicals Agency has produced a document, "Guidance on information requirements and chemical safety assessment" (accessed through http://echa.europa.eu), which includes a chapter R.8 on REACH information requirements, published in 2012. The EU expert group advising on REACH proposes a different potency categorisation based on the LLNA to the one above, and this is shown in Table 2 below.

Category	EC3 (%)
Extreme	≤0.2
Strong	>0.2 - ≤2
Moderate	>2

Table 2
Potency categorisation proposed by the EU expert group on skin sensitisation, based on the LLNA (taken from Appendix R.8-10 of the ECHA Guidance on information requirements and chemical safety assessment, Chapter R.8: Characterisation of dose-response for human health)

23. Nevertheless, the authors in Loveless 2010 noted that, "it is not possible currently to define an EC3 value below 100% that would serve as an appropriate threshold for classification and labelling". The possibility remains that even chemicals with comparatively weak sensitisation potential could be able to cause an allergic response in some individuals who are particularly susceptible, and in conditions where there is sufficient and sustained exposure. Thus the difficulties of characterising relative sensitising potency remain.

Classification of skin sensitising potency of preparations

24. A preparation is defined as "a mixture composed of two or more substances which do not react" (Loveless 2010). Many plant protection products (pesticides) are such mixtures, often comprising a single active ingredient and a number of co-formulants. In cases where the preparation itself has not been tested, the classification of the product is based on the individual toxicological profiles of the component ingredients (active substance/s and co-formulants). Proposals have been made to set specific concentration limits for sensitising substances in

preparations; the European Chemicals Agency has made the following recommendations shown in Table 3 below:-

Potency	Concentration limit of sensitising ingredient present (% w/v)
Extreme	0.001
Strong	0.1
Moderate	1.0

Table 3
Recommendations for concentration limits of sensitising ingredients present in preparations (adapted from echa.europa.eu/documents/10162/13562, Table 3.4.2-i, "Skin sensitising potency for substances and recommendations on concentration limits", page 365)

However, sensitisers of this strength may not be approved for use in plant protection products.

25. If a preparation has not been specifically tested, its classification is based on the percentage content and sensitisation potency of its individual ingredients. It is also accepted practice to estimate skin sensitisation potential of a preparation based on data obtained on a preparation of similar composition; the chemical structure and concentration of the reference preparation need to be known. In Loveless 2010, however,the authors suggest that there is a need to develop formulae for classifying preparations based on the concentration of a given ingredient in a preparation, and the sensitising potency of that ingredient.

CHARACTERISATION OF RELATIONSHIP BETWEEN POTENCY ESTIMATES AND HUMAN RISK

REACH guidance on risk assessment

26. The European Chemicals Agency, in its document "Guidance on information requirements and chemical safety assessment" (accessed through http://echa.europa.eu), includes a chapter R.8 on REACH information requirements. The BRAWG report suggests in paragraph 4.68 that an approach such as that set out in this guidance could be considered as a method of defining risk of sensitisation more clearly.

The characterisation of dose/concentration-response for human health

27. Chapter R.8 of the REACH guidance describes a process and steps to be followed in order to characterise dose-response, and includes sections which relate to skin sensitisation. The guidance recommends, in general, deriving a DNEL (Derived No-Effect Level), that is, the level of exposure above which humans should not be exposed, for threshold effects, or, when no DNEL can be derived, deriving a DMEL (Derived Minimal Effects Level). It is recognised, however, that for some substances, although they exert their effects by a threshold mode of action, the available data do not allow reliable identification of the threshold, and that this might be the case for the endpoint of sensitisation.

Four steps involved in characterising dose-response

28. The first step involves gathering typical dose descriptors, or other information on potency. It is recognised that, for skin sensitisation, there is no straight-forward derivation of a DNEL, and that a more qualitative approach may need to be followed. The second step requires deciding on a mode of action, whether it is threshold or non-threshold. For skin sensitising substances, the chemical is believed to exert its effect by a threshold mode of action, but available data do not allow reliable identification of that threshold. Therefore, the third step is to follow a more qualitative approach. In the case of skin sensitisation, if LLNA data are available, the potency will need to be categorised based on the EC3 value. Step four of the process involves selecting the leading health effect, taking into account the corresponding qualitative description. The Risk Characterisation for endpoints with no derived DNEL is based on the qualitative description of potency from the third step.

Appendix R.8-10 of the REACH guidance concerning skin sensitisation

29. This Appendix sets out various approaches to categorising potency of sensitisers, and describes how to derive an induction specific DNEL for skin sensitisation.

Induction and elicitation of immune response

30. Sensitisation, as described in paragraph 6 above, usually involves two phases, induction and elicitation. Both induction and elicitation show a dose-response relationship and have a threshold. The threshold for induction is defined as the highest level of exposure that fails to induce sensitisation (ECB 2003). The threshold for elicitation is defined as the highest level of exposure that fails to elicit a reaction in a previously sensitised subject (Basketter 2005b).

31. The relationship between sensitising potency and elicitation has not been well characterised. The dose-response relationship for induction and elicitation differs, as the dose needed to induce sensitisation in a non-sensitised subject is usually greater than the dose needed to elicit a reaction in a previously sensitised subject. However, elicitation thresholds appear to correlate poorly with induction potency (Basketter 2005b). There is a lot of variation in elicitation thresholds between individuals, depending on the sensitising potency of the substance, the duration, site and extent of the exposure, and also the extent to which sensitisation has been acquired. Therefore, because of the difficulty in deriving thresholds for elicitation, the derivation of DNELs and the quantitative and qualitative approaches to defining potency of sensitisers is focused only on induction.

Potency categorisation using animal data

32. As described in paragraphs 10 to 13 above, of the available animal tests, the LLNA provides the most informative dose-response data that can be used to derive an EC3 (Effect Concentration 3) value as a basis for potency categorisation. The EC3 value, in $\mu g/cm^2$, can be used for quantitative assessment to derive no-effect levels, including a DNEL, providing that relevant assessment factors are applied. Examples of quantitative assessments for specific skin sensitisers in defined exposure situations are available in the published literature, but in these cases a

Weight of Evidence approach, using human as well as the LLNA animal data, has usually been applied (for example, in Gerberick 2001).

Potency categorisation using human data

33. Testing for induction of sensitisation in humans is no longer conducted on ethical grounds, but in some cases data from historical predictive testing is available to inform on potency. For instance, data from the human repeat insult patch test (HRIPT), or from the human maximisation test (HMT) may provide information on potency for induction. Data from reliable historical human predictive tests can be used in combination with LLNA data in a Weight of Evidence approach to calculate a NOAEL (No Observed Adverse Effect Level) or LOAEL for induction of sensitisation. However, in a recent publication (Basketter 2014), the authors have used only human data to characterise 6 categories of human sensitising potency, and have provided human NOELs (No Observed Effect Level) where sufficient data were available. This type of data is only available for a limited number of chemicals (131 in the 2014 paper), and expert judgement has been relied upon to categorise substances, so the authors state that the outcome should be taken as their considered view. Nevertheless, the REACH guidance does point out that this kind of potency categorisation can be used in qualitative assessment, and to recommend appropriate risk management measures (RMMs).

34. Testing individuals with a pre-existing contact allergy, on the other hand, is performed widely as part of clinical examinations, to determine the person's sensitisation to a particular substance or substances. Such testing demonstrates previous induction of sensitisation to that substance, but is not designed to determine an elicitation threshold. However, clinical data can be used in qualitative assessment, as, for instance, the sensitising potency of a chemical could be evaluated by comparison of the incidence of skin sensitisation in a human population with exposure information, if that is known. Thus, if a high incidence of contact allergy is observed in an exposed population to a certain substance, and there is a relatively low degree of exposure, this could be considered as an indication that the substance is a strong sensitiser (European Commission, 2000).

35. There are some diagnostic tests, such as the patch test dose-response, or the Repeated Open Application Test (ROAT), which provide human elicitation threshold data. However, the potency of induction cannot be derived directly from these, other than the suggestion that a low elicitation threshold could indicate high potency, and vice versa (European Commission, 2000).

Potency categorisation using in vitro data

36. There are no officially adopted EU-OECD in vitro tests for skin sensitisation currently available. A few assays are under development, in the areas of dermal bioavailability, chemical reactivity, and cell-based assays, but at present such tests can only be used as supporting evidence in combination with other kinds of data.

Potency categorisation using non-testing data

37. Non-testing methods for skin sensitisation include grouping of chemicals (using read-across and chemical categories), chemistry considerations and (Q)SARs ((quantitative) structure-activity relationships). A number of (Q)SARs have been

reported in the published literature concerning skin sensitisation, though most are suitable only for hazard identification using a Weight of Evidence approach. With some QSARs the potency and the EC3 value can be estimated, but their validity and adequacy need to be established.

38. If experimental data are lacking on a given substance, it is possible to perform read-across if information is available for substances that are closely related structurally. Mechanistic read-across is thought to be useful, in which a substance is assigned to an appropriate reaction applicability domain, and its reactivity and/or hydrophobicity are quantified relative to known sensitisers in the same domain, for which experimental information is available. It is then possible to predict the likely sensitisation potential and potency within a range. The assumption behind mechanistic read-across is that sensitisation potential is related to a combination of reactivity and hydrophobicity.

Assessment factors

39. Depending on the method used, assessment factors for inter- and intra-species variation, for dose response uncertainties, and for uncertainties related to the quality of the database need to be considered and applied where necessary. Thus, if the vehicle or matrix in which the sensitiser is contained differs significantly from that used to determine the NOAEL,LOAEL or EC3, the application of an additional assessment factor, of 1 to 10-fold, may need to be considered. A 1 to 10-fold assessment factor may be required to account for specific exposure conditions, for instance when the experimental set up differs from actual human exposure conditions. The effect of repeated exposure may also need to be considered and accounted for. The application of skin sensitisation-specific assessment factors is decided by expert judgement, and justified on a case by case basis.

Derivation of induction specific DNEL for skin sensitisation

40. If the derivation of the DNEL is based on LLNA data alone, the EC3 value, expressed in dose/unit area of exposed skin, μg/cm^2, can be considered as the LOAEL for induction, and hence an additional assessment factor of between 3 and 10 is require to extrapolate to a NOAEL. By application of relevant assessment factors, a DNEL can be derived expressed in μg/cm^2/day. Assessment factors for inter-species variation also need to be used, but can be lowered to less than 10 on a case by case basis.

41. For substances where both the LLNA and historical human predictive test data of good quality are available, the DNEL can be derived by a Weight of Evidence approach. A reliable NOAEL from a well conducted human repeat insult patch test (HRIPT) would have precedence over the LLNA EC3 value. Assessment factors may need to be applied also.

42. It may be possible to derive a DNEL for a substance based on read-across from structurally related substances for which experimental data are available. Assessment factors will need to be considered, and, on a case by case basis, an additional factor to account for the uncertainty related to the use of read-across may be required.

Risk characterisation

43. In risk characterisation, the derived induction specific DNEL is compared with the estimated exposure, both expressed in $\mu g/cm^2/day$. Such information is required for all exposure scenarios concerning sensitisers, unless there is confidence that a scenario for which data are available is more conservative than the one being considered. It also has to be taken into account that the exposure might occur more than once a day, or repeatedly over a longer period of time, and might lead to accumulation of the substance on the same site on the skin. If exposure is less than the DNEL, it can be assumed that, at the specific exposure, no induction in a non-sensitised person would occur. However, even at this exposure level, a reaction in a previously sensitised person could occur.

The skin sensitisation Adverse Outcome Pathway (AOP)

44. The OECD has recently issued a report entitled "The adverse outcome pathway for skin sensitisation initiated by covalent binding to proteins" (OECD 2012). The report provides a mechanistic description of the events leading to skin sensitisation. The key events in this pathway are described as follows: sensitising chemicals penetrate the skin and bind covalently to skin proteins, a process called haptenation. Binding occurs either directly, or after prior oxidation or metabolism to form reactive species. The chemical reactivity is thought to trigger activation of epidermal keratinocytes and dendritic cells in the skin. The dendritic cells present haptenated proteins to hapten-specific T cells in draining lymph nodes. If these cells interact, T cell proliferation occurs in the lymph node, resulting in an increased number of hapten-specific effector and memory T cells in the person. The process is repeated on subsequent re-exposures to the sensitising chemical, and, when an unknown threshold number of hapten-specific T cells is reached, the person is said to be sensitised, and a T cell mediated skin reaction (allergic contact dermatitis) will be elicited at the site of the re-exposure.

45. Recent work on obtaining sensitiser potency information has turned to modelling the skin sensitisation AOP, and has been described in several recent papers (for example, in the reviews by Adler 2011 and Maxwell 2011). A recent publication (Maxwell 2014) describes the development of two mathematical models to try to provide predictions of the magnitude of the T cell response to a sensitising chemical, with comparisons made to data from clinical datasets, such as human diagnostic patch test results, instead of animal test data.

46. Maxwell 2014 describes that the group are working on two linked mathematical models. The first is a model of "total haptenated protein" (tHP), and the second of "$CD8^+$ T cell response" ($CD8^+$ T model). The aim of the two models is to provide a dose-response prediction for skin sensitisation.

47. The tHP model involves using non-animal skin diffusion and protein binding datasets as the model input parameters. It aims to predict the total amount of haptenated protein that would be generated in the epidermis and dermis, the viable layers of the skin, as a function of time, for a given skin exposure scenario. It starts with the concentration of available sensitising chemical in the epidermis and dermis, and attempts to predict the total amount of modified protein that would be generated

in these layers of the skin. Pharmacokinetic models, based on experiments with radio-labelled chemicals applied to *ex vivo* human skin (Davies 2011) are used as input. Less data are available on the rate of protein haptenation however, and experiments to measure reactivity kinetics are required.

48. The $CD8^+$ T model aims to predict the numbers of hapten-specific human $CD8^+$ central memory T cells that might be generated following repeated skin exposures to a chemical sensitiser, using the previous tHP prediction to determine the amount of specific antigen in the draining lymph node. Human data are available for measurements taken in blood in the absence of antigen, for such parameters as death, proliferation and differentiation rates of the types of T cells (Mclean and Michie, 1995), but there is a lack of data on hapten-specific immune responses. The current model has been developed using human-relevant literature on pathogen infections to predict the $CD8^+$ T cell immune response that might be induced after skin exposure to antigenic stimuli. However, the authors of the Maxwell 2014 paper report that there is now a research programme under way that compares $CD8^+$ T model predictions with results from clinical tests for thresholds of skin sensitisation, such as the human diagnostic patch test, or the human repeat insult patch test, in patients recently diagnosed with allergic contact dermatitis.

49. The overall aim of the modelling approach is to quantify the relationship between the dose of sensitiser applied to the skin and the extent of the hapten-specific T cell response that might result. The authors of the Maxwell 2014 paper suggest that, by benchmarking their mathematical model predictions against clinical datasets, they should be able to predict whether or not a given skin exposure to sensitiser will generate enough hapten-specific T cells to cause an adverse immune response in humans if they are re-exposed to a sensitising chemical. If the predictions were reliable, risk assessment decisions for skin sensitisation could potentially be made without the use of animal test data.

Questions for the Committee

1. The BRAWG Report recommends that research should be conducted on the extent to which current or new formulations may change the ability of chemicals to act as sensitisers; would Members consider it useful for a research project to be conducted which could make comparisons between the sensitising potency of an active ingredient, and the potency of the formulation/s containing it? Such a project could include reviewing the data held by HSE (and possibly others).
2. Do Members believe that further work is necessary to characterise relative sensitising potency more precisely? If so,do they have any recommendations as to the work that should be undertaken?
3. Would Members agree that there is a need to develop classification of preparations based on the concentration and sensitising potency of ingredients within the preparation? How could this be achieved?
4. There is a question as to whether the trigger value of 1% for skin sensitisers is adequately cautious to protect bystanders and residents if they are exposed to a sensitising formulation. Would Members consider there would be value in comparing chemical-specific DNELs with the trigger values currently used by

ECHA? Do Members have any other suggestions on how the trigger values could be “validated”?

5. What are Members’ opinions on the modelling approach to skin sensitisation, and on the research programme outlined in paragraphs 48 to 49 above? Is there scope for further work in this area? Do Members have any view on the likely reliability of predicting potency for elicitation based on modelling induction of sensitisation?

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Appendix 1 to TOX/2014/30

COMMITTEE ON THE TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Report of the Joint Working Group on Bystander Risk Assessment

This report is available at:

http://cot.food.gov.uk/cotreports/cotjointreps/brawgreport

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Government response to recommendations of the report of the Joint Working Group on Bystander Risk Assessment (BRAWG)

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