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Exemptions:

WATCH COMMITTEE

Interpretation of results from *in vitro* dermal penetration studies

Issue

1. How to use skin penetration data from *in vitro* studies to assess potential body burdens from skin exposure to substances.

Timing Considerations

2. No particular timing issues.

Recommendation

3. WATCH is invited to consider the issues noted in this cover paper and to respond to the question in paragraph 17.

Background

4. One of the principal routes of exposure to chemicals is via the skin. In conducting a risk assessment for a substance, in the manner required by various EU and international regulatory programmes, it is often necessary to calculate the body burden of a substance that could arise via exposure of and uptake via the skin. This requires an assessment of the extent to which a substance can be expected to pass through the skin. A position on the extent of dermal penetration can be arrived at by prediction, by default assumptions, or by measurement in test systems, either *in vivo* or *in vitro*. In this context, recently adopted Organisation for Economic Development and Cooperation (OECD) test guidelines, both for *in vivo* and *in vitro* skin absorption studies, now represent the most appropriate methods to use for regulatory purposes.

5. Since adoption by the OECD (in April 2004), the *in vitro* test guideline has usually been the method chosen by those commissioning studies, for the assessment of dermal penetration and an increasing number of such studies are being submitted to regulatory authorities, including HSE, within data packages under a number of EU regulatory programmes.

6. The currently acceptable *in vitro* protocol is set out in OECD TG 428 (Annex 1) and the strengths and weaknesses of this TG are discussed in an associated OECD Guidance Document (Annex 2). Briefly, test substance is applied to a skin preparation suspended over suitable liquid medium (the receptor fluid) and the amount of test substance translocated into the skin, and across the skin preparation into the receptor fluid is monitored.

7. The TG does not stipulate the exact conditions; but allows for the use of full thickness skin, split skin or epidermal membranes, a test period of up to 24 hours (see Annex 2, para 66, for further discussion and relevance to

occupational exposure scenarios) with flow-through or static conditions, a physiological receptor fluid or one modified for lipophilic substances and full reconciliation of all test material.

8. For the *in vitro* test method, it can be envisaged that at any point in time the substance originally applied will reside in one of four places – remaining at the point of application, on the skin surface; within the epidermis (approximately, the stratum corneum); within the dermis, or in the receptor fluid, having passed completely through the skin layers. The principal barrier to skin penetration is the stratum corneum.

9. Of course, *in vitro* studies of skin absorption have been conducted in the past, before the introduction of the relevant OECD test guidelines, and have been seen and used by HSE in its work on chemicals. When assessing findings from *in vitro* dermal penetration studies seen in the past, it has been usual practice within HSE to consider that the percentage skin absorption over any period of time (eg 24 hours) comprises the percentage of material that had translocated into the receptor fluid plus that within the dermis (ie having crossed the stratum corneum barrier); material residing in the stratum corneum has not been included.

10. Such an approach was followed in the assessment of two major substances that some members may remember coming to WATCH in the last few years - pentabromodiphenyl ether (WATCH/9/98) and styrene (WATCH/05/2001) – within the context of the EU Existing Substances Regulation (ESR). However, currently there is a debate in the EU for another ESR substance, medium chain chlorinated paraffins (MCCP), regarding a recently conducted *in vitro* skin absorption study and whether or not material within the stratum corneum should be included within the overall dermal penetration value. In this instance, inclusion of the stratum corneum component would result in a doubling of the dermal penetration value (within the designated reference time period), compared with if it were excluded. To date, HSE has argued against this inclusion. A similar controversy has also now arisen for a specific biocidal substance being reviewed by HSE under the EU Biocidal Products Directive (BPD).

11. Within the EU, there is a Technical Guidance Document (EC 2002) which provides generic guidance on risk characterisation undertaken within the New Substances (“7th Amendment”), ESR and BPD legislation. Annex 3 to this paper contains an extract from the TGD relevant to *in vitro* dermal absorption studies. This text does indicate (see second block of highlighted text) that for “very lipophilic” substances there is a problem with substance solubility in receptor fluids, producing a tendency for substance to remain within the skin layers, such that there is a justification for the inclusion of material residing within the skin layers in the overall (conservative) estimate of skin absorption for such substances. However, more generally, HSE feels that the text is not particularly clear or consistent; the third piece of highlighted text in Annex 3 implies that *in vitro* – *in vivo* comparison can be made, but the first piece of highlighted text suggests that making such comparisons from the data currently available is “difficult”. To sum up, with the appearance of the recent

OECD and EU TGD documentation, it is now apparent that there are differing views amongst interested parties about how to consider the stratum corneum component in the determination of dermal absorption in *in vitro* studies. **In some cases, inclusion or exclusion of this component can have dramatically different consequences for the conclusions reached in a risk assessment and for the regulatory action thereby suggested; hence this is not a trivial issue.**

Argument

12. In risk assessment, skin absorption information is usually required in the form of the percentage of substance to which the skin could be exposed that would be absorbed into the body in a defined period of time; often, the time period is 24 hours, ie a “daily” intake. HSE’s current approach in seeking to identify the extent of skin penetration within a defined period of time, using an *in vitro* study, is to disregard any test substance still residing in the stratum corneum as contributing to the overall penetration value; the argument being that if the substance hasn’t crossed the stratum corneum in that time period (eg 24 hours), then it has not been “absorbed” within that same time period.

13. As indicted above, others are now arguing for a different position, that of inclusion of the stratum corneum component. HSE considers that this represents an unrealistic situation; at least some substance lodged in the superficial layers of the stratum corneum would be lost to the outside via the natural process of skin cell sloughing. Furthermore, substances can react chemically with components of the stratum corneum, and thus not be available for systemic uptake. There is also the argument that material that has not crossed the skin in 24 hours is not part of the “daily” intake of substance for that 24-hour period. In the context of this debate, HSE has sought the opinion of Professor Faith Williams from the University of Newcastle, a renowned expert in the field of skin absorption. Faith has kindly agreed to attend the October WATCH meeting, as an ad hoc member for this item. In preliminary discussions, she has suggested a possible pragmatic resolution of this issue might be to assume that, for any substance, 50% of the material lodged in the stratum corneum will be available for systemic uptake and the remainder lost via sloughing.

Link to HSC Strategy

14. This is an important general issue within the Statutory Responsibilities element of the HSC Strategy.

Consultation

15. No wider consultation on this paper, beyond HSE has been undertaken at this stage.

European Context

16. This is a generic issue for risk characterisation that applies to HSE’s work within several regulatory programmes including ESR and BPD.

Action

17. WATCH is asked to help develop, with HSE, a clear position on how to deal with test substance retained in the stratum corneum, in interpreting *in vitro* skin penetration studies and deriving an overall dermal penetration value to be taken forward into risk assessment (risk characterisation) work.

Contact:

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References/Attachments

- Annex 1 OECD (2004) Guideline for the testing of chemicals. Skin absorption: *in vitro* method, (TG 428).
- Annex 2 OECD (2002) Guidance Document for the conduct of skin absorption studies, (No 28).
- Annex 3 Extract from: EC 2002. EC (European Commission) (2002). Technical Guidance Document on Risk Assessment in support of Commission Directive 93/67/EEC on Risk Assessment for new notified substances and Commission Regulation (EC) No 1488/94 on Risk Assessment for existing substances, and Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market. Parts 1-4. 2nd edition. Publication No. EUR 20418/EN/1, EUR 20418/EN/2, EUR 20418/EN/3, EUR 20418/4. Office for Official Publications of the EC, Luxembourg. [Internet publication at <http://ecb.jrc.it/>]