

# Dermal absorption of PT 21 active substances

Agreed at Human Health Working Group meeting WG-V-2016

## 1. Introduction

ECHA organised in cooperation with experts from Member State Competent Authorities (MSCAs) and Associated Stakeholder Organisations (ASOs) a workshop entitled “*Dermal absorption from antifouling products and other matrices that form a dry film during testing*” on 19 May 2016 in Berlin, hosted by BfR. The workshop was organised to identify practical ways forward in performing and interpreting dermal absorption studies on antifouling products providing short-term and long-term recommendations. A report of the workshop has been published on the ECHA website<sup>1</sup>.

Based on the discussions at the workshop, the current document contains SECR proposals, agreed at WG-V-2016, on how to perform dermal absorption testing and how to interpret the results of dermal absorption studies performed on PT 21 products.

## 2. Proposals

The relevant text of the workshop report are presented in text boxes, and the proposals, now agreed by the WG, are indicated below these quotations.

### 2.1. Adapting the study protocols

In general, the protocols as given in OECD guidelines<sup>2</sup> 427 (*in vivo*) and 428 (*in vitro*), supported by OECD Guidance Document<sup>3</sup> No. 28 for the conduct of skin absorption studies, are considered appropriate.

However, some of the recommended procedures relating e.g. to duration of exposure and terminal fractionation are difficult to apply to film-forming antifouling paints. The chapters below give some considerations relevant specifically to PT 21 dermal absorption testing.

#### 2.1.1. Application volume

*Berlin workshop report:*

*The OECD guidelines 427 and 428 recommend an application rate of up to 10 µL for liquids. This was considered appropriate, noting however that the application rate should primarily relate to the exposure situation.*

#### Proposal #1

<sup>1</sup> [https://echa.europa.eu/documents/10162/4221979/workshop\\_report\\_pt-21\\_dermal\\_absorption\\_en.pdf/d4125915-3bc4-46a2-89b0-a9e88ea4fd5d](https://echa.europa.eu/documents/10162/4221979/workshop_report_pt-21_dermal_absorption_en.pdf/d4125915-3bc4-46a2-89b0-a9e88ea4fd5d)

<sup>2</sup> [http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects\\_20745788](http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788)

<sup>3</sup> <http://www.oecd.org/env/ehs/testing/seriesontestingandassessmenttestingforhumanhealth.htm>

It is recommended to follow the OECD guidelines 427 and 428 in applying up to 10 µL/cm<sup>2</sup> for liquids.

### 2.1.2. Washing/removing the paint

*Berlin workshop report:*

*For an antifouling paint, the washing step at the end of the exposure period was considered in general unnecessary because the paint would not be removed by washing. An attempt to remove the paint using other means such as scrubbing would give concern for animal welfare, and it might enhance dermal absorption due to rupturing of the surface of the skin.*

*It was however pointed out that removing the paint by scrubbing in in vivo studies might best reflect the real situation where exposed people would make an effort to remove the paint from skin. For in vitro studies, scrubbing should not be performed as it would damage the skin membrane and invalidate the test and its interpretation.*

*If washing is performed, it should preferably follow the label recommendations of the product, if the procedure is applicable to an in vitro test method or acceptable in vivo from the animal welfare point of view. Consideration should be given to whether it is at all possible to remove the test substance by washing, and the washing step could be skipped if the paint is expected to remain on the skin.*

#### Proposal #2

*In vivo*

*In vivo* studies are in general not expected to be performed, as the dermal absorption studies should preferably be performed *in vitro* (see proposal #7). The following advice would be relevant if an *in vivo* study is nevertheless performed.

The washing step at the end of the exposure period should normally be skipped as the paint is expected to remain on the skin. If in an exceptional case washing is attempted, it should preferably follow the label recommendations of the product, if the procedure is acceptable from the animal welfare point of view.

Removing the paint using other means such as scrubbing should not be attempted, as it could give concern for animal welfare. It might also enhance dermal absorption due to rupturing of the surface of the skin.

Note: One might consider removing the paint by scrubbing to reflect the real situation where exposed people would make an effort to remove the paint from skin, but the procedure would be unreliable if not standardised.

#### Proposal #3

*In vitro*

If washing is performed, it should preferably follow the label recommendations of the product, if the procedure is applicable to an *in vitro* test method. Consideration should be given to whether it is at all possible to remove the test substance by washing, and the washing step could be skipped if the paint is expected to remain on the skin.

Removing paint from the surface by washing or tape stripping could be acceptable if it can be demonstrated that the skin membrane is not damaged and that no parts of the stratum corneum are removed.

Removing the paint by scrubbing should not be attempted as it would damage the skin membrane and invalidate the test and its interpretation.

### 2.1.3. Exposure duration

#### *Berlin workshop report:*

*For antifouling paints, it was noted that the exposure period is usually 24 h in an in vitro study and 48 or 72 h in an in vivo study due to the inability to remove the substance by washing. It was suggested that the difference in exposure duration between e.g. 8 and 24 h would have little impact on the results because the paint will dry during the first hour, after which much less transfer of the active substance to the skin would be expected. On the other hand, some of the available information on tests on antifouling paints shows an increase in the concentration of the active substance in the receptor fluid until the end of the study. It was noted that transfer to the skin from dried films may be very different depending on the type of active substance (e.g. granular metal vs. small organic molecules) and the exact composition of the film.*

*The opinion of the group was divided on whether or not a longer exposure time would generally result in a more conservative absorption estimate. It would be useful to investigate whether there is a significant difference in absorption with exposure times of 8 and 24 h.*

*It is not possible to extend the duration of an in vitro dermal absorption study much above 24 h because the skin sample loses its integrity. In contrast, groups of animals with termination time points later than 24 h are commonly included in in vivo dermal absorption studies. Comparison of information from groups with different termination time points can help understanding the fate of substance contained in the hardened paint layer and skin residues.*

#### Proposal #4

It has been argued that an extended contact time, which usually cannot be avoided in the testing of antifouling products, results in an inherently conservative approach. Due to the matrix effect it is however usually not possible to estimate how much material is transferred from the paint layer to the skin after drying. The results should therefore be taken as they are and this should not be used as an argument to reduce conservatism elsewhere.

If information is available on the kinetics of absorption until the end of the study, this information can be used in the overall considerations (See EFSA 2012<sup>4</sup> and OECD Guidance notes 156, 2011<sup>5</sup>).

#### Proposal #5

##### *In vivo*

If *in vivo* studies are performed, the recommendation of OECD Test Guideline 427 to use groups of animals with termination time points later than 24 h (e.g. 48 and 72 h) should be followed to enable comparison of information from groups with different termination time points. This can help understanding the fate of substance contained in the hardened paint layer and skin residues.

#### Proposal #6

##### *In vitro*

Due to skin sample losing its integrity after 24 h, it is not necessary to extend *in vitro* dermal absorption studies beyond this time.

It is however noted that according to OECD guideline 428 "for test substances that penetrate slowly, longer times may be required."

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<sup>4</sup> Guidance on dermal absorption; EFSA Journal 2012;10(4):2665.

<https://www.efsa.europa.eu/en/efsajournal/pub/2665>

<sup>5</sup> Guidance notes on dermal absorption; OECD Series on Testing and Assessment No. 156, ENV/JM/MONO(2011)36. <https://www.oecd.org/chemicalsafety/testing/48532204.pdf>

#### 2.1.4. Skin fractionation

*Berlin workshop report:*

*No clear recommendation was made regarding the usefulness of skin fractionation. If performed, it would only be relevant to separate the stratum corneum from the epidermis. It was mentioned that apart from tape stripping, other techniques are available that can provide the desired information on the distribution of the test substance within the skin; see e.g. paragraph 72 of OECD Guidance document 28.*

No proposals are made in addition to those presented in Chapter 2.2.1. *Stratum corneum*.

#### 2.1.5. Type of study

*Berlin workshop report:*

*In vivo studies are no longer the preferred method for studying dermal absorption due to animal welfare reasons and because in vitro methods are considered sufficiently reliable. Most testing laboratories offer mainly in vitro studies to investigate dermal absorption.*

##### Proposal #7

Dermal absorption studies should preferably be performed *in vitro*. This follows from the general principle of reducing animal testing, as indicated in e.g. ECHA Guidance Vol III Part A: Information requirements and EFSA Guidance on dermal absorption (2012)<sup>4</sup>.

#### 2.1.6. New methodologies

*Berlin workshop report:*

*Innovative new methodologies were presented during the workshop to study the distribution of the active substance within skin and between skin and adherent film. One such methodology involves vertical sectioning and histology followed by scanning electron microscopy (SEM) and energy dispersive X-ray (EDX) spectroscopy. Another methodology involves confocal Raman microscopy, and does not require prior vertical sectioning, to visualize where the test material is located in the skin membranes.*

*Promising results have been achieved using these new methodologies that in the long term might be used in dermal absorption studies. Industry experts proposed that such new methodologies should not be used routinely in regulatory dermal absorption studies for antifouling products especially because they are currently not included in the OECD test guidelines or guidance. Industry experts indicated that experimental work employing these new techniques has been conducted mainly to provide evidence and illustrate the fate of an active substance in antifouling paints in in vitro dermal absorption studies. Results from SEM-EDX studies examining the distribution of copper between the antifouling paint and the skin have however already been used in regulatory decision making.*

##### Proposal #8

Dermal absorption studies using alternative analytical methodologies could be provided by the applicant on a voluntary basis to provide evidence and illustrate the fate and distribution of the active substance in dermal absorption studies. Non-guideline studies should not replace guideline studies, but could be provided to supplement them.

Note: Results of studies examining the distribution of copper (particles) between the antifouling paint and the skin were used in the approval of copper containing active substances.

## 2.2. Interpreting study results

### 2.2.1. Stratum corneum

*Berlin workshop report:*

*The key question for the interpretation of the study results for antifouling paints is whether, and to which extent, the material remaining in the stratum corneum should be considered as absorbed.*

*When performing tape stripping, the major part of the test material is included in the first tape strips that will mostly contain material from the hardened paint layer on top of the skin. There was however no agreement on the principles to be used in deciding how many tape strips could be excluded from the absorbed dose. According to EFSA guidance<sup>4</sup> which is applicable to biocides, the first two tape strips could be excluded. For several antifouling substances in the biocides review programme, the first five tape strips were excluded. One of the speakers however presented information showing that there can still be some paint on top of the skin sample after ten tape strips. On the other hand, the paint is not a consistent layer and tape strips would consequently contain both paint and parts of the stratum corneum. Hence, separation of the two is currently not possible using tape stripping. It was also emphasised that the number of tape strips needed to remove the paint depends on the properties of both the tape and the paint and should therefore be considered on a case by case basis. In principle it may even be possible to cut small pieces of tape to remove the remaining visible paint without removing the stratum corneum around it.*

*One of the speakers showed that in a paint formulation containing particulate active substances, the hardened paint layer had an equal distribution of active substance particles. The interface between the paint layer and the skin contained mostly of the inert binder, and there was thus very little direct contact between the active substance particles and the skin. Another speaker demonstrated that the particles did not enter the skin but remained in the paint layer.*

*The participants agreed that the material in the paint layer should be considered as non-absorbed, while recognising that there is currently no method to separate the paint layer and the stratum corneum.*

*The possibility of excluding the whole stratum corneum from the absorbed dose was discussed. It was pointed out that little material would be expected to transfer from the dried paint layer to the stratum corneum, although this might also depend on the properties of the active substance and the formulation. Since the material hardens during the first hour and the exposure duration is usually 24 h in vitro and 48 or 72 h in vivo, the results of the experiment would be expected to already reflect the situation where little further systemic exposure takes place in practice after the first hour. Dermal absorption takes place by diffusion, where the driving force is the concentration gradient which would diminish over time due to drying of the paint layer. This would diminish the transfer from paint to stratum corneum, as well as from stratum corneum to the epidermis. While this logic as such was not questioned, it was pointed out that, in at least some dermal absorption studies on antifouling products, the amount of test material in the receptor fluid did increase until the end of the study. The kinetics of the test material transfer during the exposure time should be further investigated to allow solid conclusions to be made on the extent to which transfer takes place from the dried paint layer to the skin.*

*It was considered likely that in a real situation, following any major incidents of direct skin exposure to the paint, the exposed person would wipe off the excess paint. This would result in a thinner layer of paint and fast drying, all of which would contribute to lowering the amount of antifouling paint penetrating through human skin. Such conditions may on the other hand be similar to typical testing conditions with a maximum dose of 10 µL/cm<sup>2</sup>, corresponding to a maximal wet film thickness of 100 µm. On the other hand, scrubbing of the skin following direct exposure might also enhance the dermal absorption of any remaining paint.*

*The majority of the participants were in favour of excluding the material in the stratum corneum from the absorbed dose, provided that the results at 24 h (in vitro) or 48/72 h (in vivo) are used. Exclusion of stratum corneum is acceptable and in accordance with current guidance when absorption has in effect ceased by the end of the experiment. Some participants however argued that this has not been shown to be the case generally and would need to be demonstrated for each product in future dermal absorption studies. Excluding the material in the stratum corneum could be supported by information on absorption kinetics in the dermal absorption study, as well as by information on the dermal absorption of the active substance in a solvent or the active substance from a different formulation type (other than paint). The participants agreed that dermal absorption of the active substance in a solvent should be considered as a worst-case over the dermal absorption of the active substance from an antifouling formulation.*

*It was suggested that any new dermal absorption studies on antifouling products should include in the report photographic evidence of the tape strips. This will help in evaluating the amount of antifouling paint remaining in each tape strip. This suggestion was widely supported.*

*It was also considered that any additional information concerning e.g. absorption kinetics, or histology showing the distribution of the active substance (not only particles) across the skin, would enable a more accurate assessment of the results. In this context, one participant criticised the recommendation in the EFSA guidance to disregard the material in stratum corneum solely based on the relative amount in the receptor fluid at half of the study duration. Adequate use should always be made on the kinetic information obtained in accordance with OECD test guidelines 427 and 428.*

#### Proposal #9

The number of tape strips needed to remove the paint depends on the properties of the tape and the paint formulation and should be considered on a case by case basis. For the studies already performed, it is at least in most cases not possible to differentiate between the active substance present in the paint layer and in the stratum corneum. In this case, for these existing studies it is considered reasonable to exclude the first five tape strips, as was agreed for several active substances in the Review Programme. If the information is not available on first five tape strips, then the available information needs to be considered on a case by case basis. Taking into account the absorption kinetics and other available information, consideration can be given to the possibility of excluding all tape strips from the absorbed dose, provided that the results at 24 h (*in vitro*) or 48/72 h (*in vivo*) are used.

With regard to antifouling paints, overall the EFSA Guidance on dermal absorption should be considered as indicative, and all available information should be used in the specific context of PT 21 testing. Overall, therefore, the decision on whether or not to exclude some or all material associated with tape strips should be taken on a case-by-case basis, taking into account the following information:

- Information on absorption kinetics in the dermal absorption study, e.g. whether absorption is practically complete at the end of the observation period and the amount associated with tape strips can be excluded completely (provided that the results at 24 h [*in vitro*] or 48/72 h [*in vivo*] are used).
- Information on dermal absorption of the active substance in a solvent or a different formulation type. Dermal absorption of the active substance in a solvent should be considered as a worst-case over the dermal absorption of the active substance from an antifouling formulation.
- Histology or other appropriate techniques showing the distribution of the active substance (not only particles) across the skin.

#### Proposal #10

*New studies to be performed*

Any new dermal absorption studies on antifouling products should be performed according to OECD Guidelines and should additionally include in the report photographic evidence of the tape strips. This will help in evaluating the amount of antifouling paint remaining in each tape strip and concluding on the number of tape strips that should be excluded from the absorbed dose.

The material in the paint layer should be considered as non-absorbed, but this conclusion is difficult to put into practice as there is currently no method to separate the paint layer and the stratum corneum. In the Berlin workshop it was suggested that the visible paint layer could be removed by small pieces of tape without removing the stratum corneum around it. Currently this procedure could be recommended only as a supplementary examination in addition to the normal tape stripping because there is no experience and because it is not possible to exclude the possibility of complicating the interpretation of the results if used as the sole tape stripping method.

### 2.2.2. Using the limit of detection/quantification (LoD, LoQ)

*Berlin workshop report:*

*It has been argued that where no test material can be measured in any of the compartments due to being close to the detection limit, the value of LoD/LoQ should be used instead. This is mostly relevant for studies using non-radioactive material.*

*Although the participants accepted the principle, it could be problematic if it involves adding up measurements at e.g. different time points, potentially resulting in an overly conservative absorption estimation. Therefore, in principle, LoD/LoQ could indeed be used but always taking into account the context to avoid over-conservatism.*

#### Proposal #11

Where no test material can be detected in any of the compartments due to being below the detection limit, the value of LoD should be used. Where test material is detected but cannot be quantified in any of the compartments due to being below the quantification limit, the value of LoQ should be used. Care should be taken not to add up measurements at e.g. different time points, which could potentially result in an overly conservative absorption estimation.

Note: This is mostly relevant for studies using non-radioactive material.

### 2.2.3. Information from humans

*Berlin workshop report:*

*It was pointed out that any available information on humans should be taken into account in the risk assessment as indicated in the Guidance on the BPR Vol III Part B (e.g. chapter 1.3.2.9).*

#### Proposal #12

Available information on humans should be taken into account in the risk assessment as indicated in the ECHA Guidance Vol III Part B<sup>6</sup> (e.g. chapter 1.3.2.9).

<sup>6</sup> [https://echa.europa.eu/documents/10162/15623299/biocides\\_guidance\\_human\\_health\\_ra\\_iii\\_partb\\_en.pdf](https://echa.europa.eu/documents/10162/15623299/biocides_guidance_human_health_ra_iii_partb_en.pdf)

## 2.3. Read-across from (other) formulations

### 2.3.1. Information on active substance in other formulations

*Berlin workshop report:*

*In order to minimise unnecessary testing, it is necessary to understand how results obtained on one antifouling product could be used in the assessment of another product. There were no general agreements on criteria, as the question will need to be assessed on a case by case basis. The workshop however identified several aspects that need to be considered when performing read-across.*

*To enable read-across, the two products would need to have similar physical-chemical characteristics, including the rapidity of drying where vapour pressure and boiling point need to be considered. The polarity of the solvent, as well as the solubility of the active substance in the solvent are also relevant. The relevance of rheological and thixotropic properties of the product need to be considered.*

*The compositions of the products need to be similar in terms of active substance content, excess of binder and other dry material, solvent system and whether the active substance is particulate or dissolved in the formulation. If the active substance is particulate, then also the particle size is relevant. A formulation where the active substance is dissolved would be a worst case compared to a formulation containing a particulate active substance.*

*The participants did not consider it possible to establish principles for identifying a worst-case matrix for testing.*

#### Proposal #13

Read-across between formulations will need to be assessed on a case by case basis. General principles relating to data gap filling for dermal absorption are described in the OECD Guidance Notes<sup>5</sup> No. 156 on Dermal Absorption (2011) and the EFSA Guidance on Dermal Absorption (2012)<sup>4</sup>.

In addition, regarding substances rather than formulations, guidance is available on the principles for grouping and read-across for chemicals in the ECHA Read-Across Assessment Framework<sup>7</sup> (RAAF, 2015), REACH Guidance chapter R.6.2<sup>8</sup> and OECD Guidance No. 194 (2014)<sup>9</sup>.

The following properties, amongst others, need to be considered when evaluating acceptability of grouping and read-across between antifouling formulations:

- Similar physical-chemical characteristics, including the rate of drying where vapour pressure and boiling point need to be considered
- Similar polarity of the solvents
- Similar solubility of the active substance in the solvents (a formulation where the active substance is dissolved would be a worst case compared to a formulation containing a particulate active substance)
- Similar active substance content
- Similar type and amount (see EFSA 2012<sup>4</sup>) of binder and other suspended material
- Similar solvent system

<sup>7</sup> [https://echa.europa.eu/documents/10162/13628/raaf\\_en.pdf](https://echa.europa.eu/documents/10162/13628/raaf_en.pdf)

<sup>8</sup> [https://echa.europa.eu/documents/10162/13632/information\\_requirements\\_r6\\_en.pdf](https://echa.europa.eu/documents/10162/13632/information_requirements_r6_en.pdf)

<sup>9</sup> [http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/im/mono\(2014\)4&doclanguage=en](http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/im/mono(2014)4&doclanguage=en)



- Both products contain particulate active substance or both contain dissolved active substance
- Similar particle size if the active substance is particulate (a formulation with smaller particle size would be the worst case)

### 2.3.2. Information on active substance in solution

*Berlin workshop report:*

*If information is available on the absorption of the dissolved active substance in solution (i.e. not in a formulation), this could be used as a worst case value for the product, especially if the solvent used was similar to that of the antifouling formulation. Dermal absorption would generally be expected to be higher for the dissolved active substance than for the active substance in an antifouling paint formulation due to the matrix effect. The evaluation would always need to be made case by case, taking into consideration the test substance and the formulation of the antifouling product, as well as the study protocol used.*

#### Proposal #14

The dermal absorption of the dissolved active substance from the solution may be used as a worst case value for the active substance in the product, if the solvent used was similar to that of the antifouling formulation. Due to the matrix effect, dermal absorption from an antifouling paint formulation might be lower than from dissolved active substance. However, case by case consideration is always needed, taking into account the test material and the formulation of the antifouling product.

The relevance of the study protocol used has to be considered.