

“Dermal absorption from antifouling products and other matrices that form a dry film during testing”

Report of workshop held in Berlin (BfR) 19 May 2016

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1. Introduction

Antifouling products are biocidal products applied to the hull of a ship or boat to slow the growth of subaquatic organisms attaching to the hull. They are designed to harden on the surface of the vessel and remain there for years in contact with water, slowly releasing the active substance.

The properties of antifouling products complicate the conduct of dermal absorption studies according to OECD test guidelines as well as the interpretation of such studies because the paint will form a hard layer on the skin. This layer remains attached for the duration of the test and fractionation of the skin at study termination is often not possible when using established techniques based on tape stripping. It is expected that material transfer from the paint layer to the stratum corneum is reduced once the paint has dried, noting that antifouling paints are designed to provide very slow release of the active substance. The currently available guidance or guidelines give limited advice on either testing such products or interpreting the results of tests performed with such products.

The aim of the workshop was to identify practical ways forward in performing and interpreting dermal absorption studies on antifouling products providing short-term and long-term recommendations and identifying possible research needs.

The workshop was organised by ECHA and supported by an organising committee consisting of experts from Germany, UK, Netherlands, CEPE and CEFIC.

The workshop was hosted by the Federal Institute for Risk Assessment (BfR), Berlin, Germany.

2. Content of the workshop

The workshop comprised of an introductory session with presentations from experts on dermal absorption of antifouling products from testing houses, industry, academia, competent authorities, EFSA and ECHA. This was followed by breakout group discussions involving all attendees.

The workshop was expected to provide advice on the following questions, which were also the topics of the three break-out groups:

a. Performing new studies

How can dermal absorption studies be performed to obtain reliable information for antifouling formulations and other matrices that form a dry film during testing? What are the specific challenges?

b. Interpreting existing studies

What are the limitations of existing studies performed for the assessment of dermal absorption for antifouling paints? How should these be addressed in data evaluation?

c. Read-across/extrapolation

Not all antifouling products will be tested for dermal absorption. For extrapolation of dermal absorption to another antifouling product, what principles should be applied?

How would these principles allow for grouping of formulations that form similar types of matrices? Would it be possible to set default values for antifouling formulations?

Brief summaries of the presentations are provided in Annex 3.

The input from the break-out groups is provided in Annexes 4-6. These break-out group conclusions were presented in a final session where they were further discussed.

The discussion at the final session is reflected below in section 3. *Conclusions*.

3. Conclusions

The following conclusions reflect the discussion in the final session, where proposals of the break-out groups were presented and discussed.

3.1. Adapting the study protocols

The protocols as given in OECD guidelines 427 (in vivo) and 428 (in vitro) were considered appropriate, and only some minor modifications to these protocols were suggested.

Application volume

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.

Washing/removing the paint

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.

Exposure duration

These considerations concern the duration of exposure, which in a regular dermal absorption study lasts until the test substance is washed off from the skin after approximately 8 h of exposure.

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.

Skin fractionation

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.

Type of study

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.

New methodologies

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.

3.2. Interpreting study results

Stratum corneum

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 guidance¹ which is applicable to biocides, the first two tape strips could be excluded. For several antifouling substances in the biocides

¹ http://ec.europa.eu/food/plant/docs/pesticides_ppp_app-proc_guide_tox_dermal-absorp-2012.pdf

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 $\mu\text{L}/\text{cm}^2$, 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.

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

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.

Information from humans

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 B² (e.g. chapter 1.3.2.9).

3.3. Read-across to (other) formulations

Information on active substance in other formulations

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.

Information on active substance in solution

If information is available on the absorption of the dissolved active substance in solution (i.e.

² https://echa.europa.eu/documents/10162/15623299/biocides_guidance_human_health_ra_iii_partb_en.pdf

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.

4. Follow-up

During the final discussions of the workshop, industry participants emphasised the urgent need of guidance development on studying dermal absorption of antifouling products. Based on the discussions, ECHA will prepare a document that is expected to be discussed at the BPC Human Health Working Group meeting in September 2016. The document will aim at harmonising the principles used in interpreting existing dermal absorption studies and providing guidance on issues to be taken into account when performing new studies.

5. Annexes

Annex 1. Workshop programme

Draft programme

Dermal absorption from antifouling products and other matrices that form a dry film during testing

19 May 2016

Hosted by Federal Institute for Risk Assessment (BfR), Berlin, Germany

Introductory session I

Room D146

Chair: Antero Airaksinen (ECHA)

8.30	Registration	
9:00	Welcome and background – setting the scene	Carsten Kneuer (BfR) and Antero Airaksinen (ECHA)
9:15	Understanding and interpreting dermal penetration studies of copper-based antifouling paints – a case study	Gordon Fern (Institute of Occupational Medicine, Edinburgh, UK)
9:40	Information on guidance development – EFSA and OECD	Arianna Chiusolo (EFSA)
10:00	What are PT 21 antifouling paints?	Eivind Berg (Chairman of the CEPE antifouling working group)

10:25 – 10:50 Coffee break

Introductory session II

Room D146

Chair: Carsten Kneuer (BfR)

10:50	<i>In vivo</i> assessment of Dermal Penetration – Benefits and Drawbacks	Craig Poland (IOM)
11:15	<i>In vitro</i> dermal absorption studies with antifouling products	Clive Roper
11:40	New methods to determining distributions of the test material in different layers of the skin – alternatives to tape stripping	Maike Windbergs (Saarland University; Helmholtz Institute for Pharmaceutical Research)
12:05	Explanation and distribution of break-out groups	Carsten Kneuer (BfR)

12:15 – 13:15 Lunch

Break-out groups

Rooms D145, D146 and D150

13:15 **Break-out group a)**

How can dermal absorption studies be performed to obtain reliable information for antifouling formulations and other matrices that form a dry film during testing?

Break-out group b)

What are the limitations of existing studies performed for the assessment of dermal absorption for antifouling paints? How should these be addressed in data evaluation?

Break-out group c)

Not all antifouling products will be tested for dermal absorption. For extrapolation of dermal absorption to another antifouling product, what kind of principles should be applied?

15:15 – 15:45 Coffee break

Final session

Room D146

Chair: Susy Brescia (Health and Safety Executive, UK)

- 15:45 Input from break-out group a
- 16:00 Input from break-out group b
- 16:15 Input from break-out group c
- 16:30 Panel discussion on proposals from the break-out groups
- 17:30 Wrapping up the proposals of the workshop

17:40 End of the workshop

Annex 2. Organising committee

Antero Airaksinen (Chair)	European Chemicals Agency (ECHA)
Susy Brescia	Health & Safety Executive, UK
Coen Graven	National Institute for Public Health and the Environment (RIVM), Netherlands
Carsten Kneuer	Federal Institute for Risk Assessment (BfR), Germany
Carol Mackie	Regulatory Compliance Limited (RCL); representing CEPE
Kirsi Myöhänen	European Chemicals Agency (ECHA)
Jack Poppleton	Arch Timber Protection, Lonza; representing CEFIC

Annex 3. Presentation summaries

Welcome and background – setting the scene

Antero Airaksinen, ECHA

During dermal absorption testing, antifouling products harden rapidly into a hard paint film, which is difficult to remove by washing and is mostly not available for absorption. Standard methods for deriving dermal absorption values may not be suitable for antifouling products due to the strong matrix effect that directly results from the fact that antifouling paints are designed to stay on the hull of the vessel for years in contact with (sea) water, slowly releasing the active substance.

Based on the experience, ECHA had drafted a proposal for temporary measures in assessing dermal absorption from antifouling products. The proposal was not agreed in the Human Health Working Group, and consequently ECHA considered it necessary to organise a workshop among all parties, including Member States, industry and testing houses.

In the Review Programme of biocidal active substances, ten substances have been finalised to date. In assessing dermal absorption, for five substances 2-5 tape strips were excluded from the absorbed dose and for three substances the whole stratum corneum was excluded because absorption to receptor fluid was essentially complete and more than 75 % of total absorption occurred within half of the study duration. The agreement to exclude five tape strips was not based on guidance but on expert judgment citing e.g. the matrix effect.

Information on dermal absorption is of crucial importance to the human health risk assessment of antifouling substances. Clarity on the interpretation of existing studies and on the principles for new testing are needed.

Understanding and interpreting dermal penetration studies of copper-based antifouling paints – a case study

Gordon Fern, Institute of Occupational Medicine, Edinburgh, UK

Under the Biocidal Products Regulation (BPR), organisations are required to submit data on active substances from dermal penetration studies conducted to OECD test guidelines.

Initial work carried out with copper compounds identified that tape stripping data could be unduly influenced by dried paint films - due to strong adhesion to the surface of the skin. This issue has the potential to result in the overestimation of the dermal penetration potential of the PT21 antifouling product.

It was concluded and agreed that only copper detected in the receptor fluid should be used as a determination of copper dermal penetration for the purposes of active substance approval.

Issues however were noted for copper thiocyanate where a lack of data on thiocyanate penetration resulted in an assigned default value of 5%, irrespective of assessed copper penetration of 0.04% for paints containing copper thiocyanate.

Work was therefore undertaken by the IOM to identify and implement suitable techniques that could be used to identify and locate copper thiocyanate particles within a paint film and to investigate any potential particulate migration into the underlying skin.

A combination of transactional histology, scanning electron microscopy and energy dispersive X-ray spectroscopy was successfully used to identify the distribution of active components within the paint film and to demonstrate that there was no evidence to suggest migration of copper thiocyanate particles from the paint layer into the underlying skin.

The evidence suggested that the dermal penetration of thiocyanate during the dermal

penetration study would be equal to that of the copper and significantly lower than the default 5% value taken for the risk assessment.

Information on guidance development – EFSA and OECD

Arianna Chiusolo, EFSA

EFSA guidance on dermal absorption was issued in 2012 to assist on critical aspects relating to the setting of dermal absorption values to be used in risk assessments of chemical plant protection products. In 2015, the European Commission asked EFSA to assess in first instance the scientific quality of new human in vitro dermal absorption studies made available from industry and public institutions and compile a comprehensive dataset of dermal absorption studies. Subsequently, based on the evaluation of the new studies, EFSA is asked to consider whether the current guidance on dermal absorption should be revised. The EFSA Scientific Report on the assessment of new dermal absorption studies, published on November 2015, indicated that their scientific quality complies with regulatory standards (OECD) and that the new data provide sufficient information for the revision of the current EFSA guidance on dermal absorption. Among the activities undertaken by the EFSA working group on Dermal Absorption, discrepancies/deviations among the dermal absorption guidance and guideline documents (e.g. EFSA, OECD, SCCS, EMA, ECETOC, EPA) are being collected with the intention to submit a Project Proposal (SPSF) to the OECD Working Group of National Coordinators of Test Guidelines (WNT) and ask for updating guidelines.

What are PT 21 antifouling paints?

Eivind Berg, Chairman of the CEPE antifouling working group

Purpose and use of antifouling paints

The hulls of ships and boats must be kept as free from fouling organisms as possible. This is important for reasons of safety, economy and the environment. Antifouling products are used both by amateurs and professionals, but their application technique is normally different.

Composition and properties of antifouling paints

Antifouling paints consist of three groups of ingredients: Biocides, binders and others. Before the paint film has dried, it also contains solvents.

- The biocides are copper (as either copper oxide or copper thiocyanate) and organic co-biocides. Formulations may contain a high share of copper by weight, but by volume copper constitutes less than one sixth of the paint and co-biocides far less.
- The binder is the film-forming component – it moulds all the solid ingredients. Today's self-polishing antifouling paints are based on acrylic polymer binders. The side-chains of the acrylic backbone differ and may have molecules such as copper or silicone. Abietinic acid from pine trees (also called rosin) is a frequently used co-binder. Antifouling binders have to be stable in order for less than half a millimetre to last for years exposed to constant friction against seawater. Therefore, the binder forms a continuous matrix to prevent water penetration. Antifouling paints have enough binder to so-called "wet" and incase all the other dry constituents. That means that particles of biocides, pigments and other ingredients are completely enveloped within the binder.
- The other ingredients are colour pigment, fillers, additives and – until dried – also solvents. Colour pigments are when present, used in small amounts. A filler in almost all formulations is zinc oxide since it is used to fine-tune the polishing determined by the binder. Thixotropic agents are necessary additives to prevent the paint from

running/sagging from a vertical surface. Both during and after drying an antifouling paint film is homogenous. Biocides and other ingredients do not stratify or agglomerate but are evenly distributed throughout the paint film. The common solvents are xylene (always containing some ethyl benzene) and solvent naphtha.

- Antifouling paints are chemically stable in the normal definition of water solubility. At skin temperature the paint is touch dry in less than half an hour. After a normal working day, just a fraction of the original solvent will still be present in the paint film.

Because biocides cannot migrate within the quickly drying paint film and because they exist as homogeneously distributed particles – each inside a stable non water soluble binder casing, the potential for skin-biocide contact is very low.

In vivo assessment of Dermal Penetration – Benefits and Drawbacks

Craig Poland, Institute of Occupational Medicine, Edinburgh, UK

This talk seeks to present the rationale for in vivo test approaches to assessing dermal absorption and introduce the relevant guidelines surrounding this test. The principle of the test as well as the procedures for the application of test substances, duration and sampling as well as determination of absorption will be discussed with a focus on the issues that occur when performing such in vivo tests. The aim of the talk is to provide a succinct overview of the test methods but with a focus on the challenges faced, in particular with the model and adherence to the test approach such as sample removal. The culmination of the presentation is the discussion of the relative advantages in disadvantages of the in vivo approach to determining dermal absorption and its use in the assessment of PT21 products.

In vitro dermal absorption studies with antifouling products

Clive Roper, Charles River, Edinburgh, UK

This presentation will overview the biology of the skin, the physics of absorption (diffusion), skin penetration study with an antifouling paint and how the data is used in a risk assessment. Examples of biocidal antifouling paint skin pen data will be examined. Proposals for future tests and interpretation are also discussed.

New methods to determining distributions of the test material in different layers of the skin – alternatives to tape stripping

Maike Windbergs, Saarland University; Helmholtz Institute for Pharmaceutical Research

Risk assessment as well as dermal drug delivery require knowledge of rate and extent of substance penetration into the human skin. However, current analytical procedures are destructive, labor intense and lack accurate spatial resolution.

As a novel analytical technique, Raman microscopy recently gained a lot of attention in the field of skin research. Based on characteristics like chemical selectivity and three-dimensional non-invasive measurements, the technique bares the potential to overcome current limitations in skin absorption testing and depth profiling.

The talk intends to introduce Raman microscopy and its potential for skin absorption testing. Different research studies will be presented including the analysis of transferability of results obtained from different skin donors and substance quantification in skin tissue. One major drawback for direct assignment of Raman peak intensity to substance concentration is Raman signal attenuation in deeper skin layers. One study will show direct quantification of a drug

within human skin based on a mathematical algorithm derived from an artificial skin surrogate. Furthermore, relative drug depth profiling will be presented correlating the Raman peaks of the drug with endogenous Raman skin peaks. Comparing drug depth profiles in human skin by confocal Raman microscopy to depth profiles acquired by destructive skin segmentation, extraction and drug quantification by HPLC revealed similar variability for both methods, thus confirming the suitability for non-invasive skin depth profiles by confocal Raman microscopy as valuable alternative to destructive state-of-the-art techniques.

Annex 4. Outcome of break-out group a) Performing new studies

How can dermal absorption studies be performed to obtain reliable information for antifouling formulations and other matrices that form a dry film during testing? What are the specific challenges?

Question	Input from the group
<p>Application rate is 1-5 mg or up to 10 µl/cm². Could the loading dose be decreased to facilitate the washing procedure?</p>	<p>A: Rather not. Its probably the reverse, as a thick layer may come off easier in one piece. 10 µl/cm² is considered a suitable volume.</p> <p>Remarks:</p> <p>Generally, the amount applied should primarily relate to the exposure situation. As close as you can.</p> <p>However, homogeneity of dose and application across the skin surface should be ensured in the interest of accuracy / quality of the experiment.</p> <p>When dosing / preparing and storing dosing solution, take care of solvent evaporation / hardening / settling etc.</p>
<p>The recommended <u>exposure time</u> in the OECD guideline is shorter than the entire duration of the experiment. Is this valid for antifouling formulations and if not, what would be the appropriate exposure time? Or should exposure be extended to 24 h such that the stratum corneum material, which is difficult to measure, could be discounted?</p>	<p>A: Total observation time should remain at 24h. It was proposed “not to bother” about washing at 8h (6-10h) but leave the film on for 24h. However, washing at 8 (6-10) h may be justified and would be in line with TG428.</p> <p>R: Exposure should mimic real life situation.</p> <p>Not much difference expected between 8 and 24h for a dry AF paint film (but data to support this might be collected).</p> <p>Non-professional and professional will try to remove the paint (after 8h latest). Its not clear whether this may increase absorption (e.g. foreseeable misuse of solvents).</p> <p>Removal during the test may compromise the experiment as the result of the manipulations.</p>
<p>Following the exposure time, the <u>removal procedure</u> depends on the expected use condition. What are the appropriate washing procedures, if any, for antifouling formulations?</p>	<p>A: For in vivo, rinsing is not recommended for practical and animal welfare reasons.</p> <p>For in vitro, if washing is performed, it should – as stated in TG428 – depend on the expected use conditions. Washing was not considered mandatory. This may preferentially follow the product label recommendations.</p> <p>R: There was considerable discussion around the most appropriate washing procedure and not general</p>

Question	Input from the group
	<p>agreements (mentioned ware: tape stripping, soap solution, scrubbing, solvent, etc.)</p>
<p>Which tape strips should be excluded from the calculations? Under which conditions and on what scientific basis? Are there alternatives to tape stripping?</p>	<p>A: It does not seem possible to define a certain number of tapes, as the material removed depends at least on tape, operator and paint.</p> <p>Two tapes may or may not be appropriate. Enough tape strips to take away the paint should be taken.</p> <p>The challenge is to separate paint film and skin / stratum corneum. Other methods than / in addition to tape stripping may be useful in this context, but may be difficult to apply on a routine basis.</p> <p>The decision on how many tape strips to exclude can currently not be standardised but will depend on the paint properties. A proper justification and supporting data needs to be provided with the study report.</p> <p>There was no general agreement on whether the amount in stratum corneum should be included in the calculation. Reference was made to existing guideline (TG428) and guidance (EFSA 2012) on this issue.</p>
<p>Upon termination, the skin may be fractionated. Does this also apply to antifouling formulations? If so, which fractions should be obtained and how should these be obtained without compromising the reliability of the results?</p>	<p>A: In any case, a full mass balance should be done.</p> <p>Skin fractionation may augment analytical challenges (for non-radioactive methods).</p> <p>It is not essential to fractionate epidermis from dermis (as both will be considered absorbable).</p> <p>Fractionation of stratum corneum from living epidermis was discussed on the last slide.</p>

Annex 5. Outcome of break-out group b) Interpreting existing studies

What are the limitations of existing studies performed for the assessment of dermal absorption for antifouling paints? How should these be addressed in data evaluation?

When discussing the main questions, the group was asked to consider also:

- Do you have concrete proposals that could be used at product authorisation or active substance approval?
- What changes would be necessary to existing guidance documents to take into account important suggestions/recommendations from the group?
- Are there specific research needs?

Question	Input from the group
<p>1. How should existing in vitro and in vivo studies be interpreted?</p> <ul style="list-style-type: none"> • If stratum corneum could not be separated from the dry paint on the surface, should the material in the stratum corneum be ignored in determining the overall absorption value? Under which conditions could this be done? Always? For in vitro and in vivo studies? • Can a longer exposure time (e.g. 24 vs. 6 h) be used to compensate for other limitations in the available studies? • How should limitations resulting from very low level of absorption close to the LoD/LoQ be handled? This is especially important in running studies with non-radioactive material and where there is a naturally occurring background of the substance in the skin. 	<p>For antifouling paints, the material in the stratum corneum should be excluded from the absorbed dose provided that the results at 24 h (in vitro) or 48/72 h (in vivo) are used. This recommendation concerns both in vitro and in vivo studies and is based on the following arguments:</p> <ul style="list-style-type: none"> • Antifouling paints generally form a dry matrix on top of the skin. • Less material is expected to be available to enter stratum corneum when the paint has dried (after approximately 30 min). • Under real life condition, it is expected that the skin exposed to antifouling paint is washed and scrubbed clean. • The longer exposure time does not compensate for other limitations. The inability to remove the substance might not result in a significantly more conservative estimate as little absorption is expected once the paint has dried. <p>Using LoD/LoQ could be problematic if it involves adding up measurements at e.g. different time points, potentially resulting in an overly conservative absorption estimation.</p> <p><u>Research need:</u> To what extent is retention of the active substance in the paint matrix relevant to active substances that are added to the paint formulation in liquid form?</p>
<p>2. Which features of existing studies are essential for such studies to be used?</p>	<p>At least the following information was considered necessary for in vitro studies:</p>

Question	Input from the group
	<ul style="list-style-type: none"> • Material in the receptor fluid and epidermis/dermis • Experiment duration of 24 h • Information on LoD/LoQ if relevant <p>At least the following information was considered necessary for in vivo studies:</p> <ul style="list-style-type: none"> • Material in the epidermis/dermis, excreta, blood and the carcass • Experiment duration of at least 24 h • Information on LoD/LoQ if relevant
<p>3. Can information on the active substance or other formulations be used?</p> <ul style="list-style-type: none"> • Can dermal absorption studies conducted on the pure active substance or on the active substance in other formulation types be used to derive a sufficiently reliable dermal absorption value for the active substance in the antifouling paint? If so, under which conditions? Can this be scientifically justified to avoid using highly conservative default values? 	<p>Information on other formulations could be acceptable on a case by case basis, as the worst case value.</p> <p>Dermal absorption of pure active substances is expected to be higher than from antifouling products due to the matrix and could be used as a worst case value.</p>

Annex 6. Outcome of break-out group c) Read-across / extrapolation

Not all antifouling products will be tested for dermal absorption. For extrapolation of dermal absorption to another antifouling product, what principles should be applied?

How would these principles allow for grouping of formulations that form similar types of matrices? Would it be possible to set default values for antifouling formulations?

When discussing the main questions above, please consider also:

- Do you have concrete proposals that could be used at product authorisation or active substance approval?
- What changes would be necessary to existing guidance documents to take into account important suggestions/recommendations from the group?
- Are there specific research needs?

Question	Input from the group
<p>How similar would two products need to be to perform read-across?</p> <ul style="list-style-type: none"> • How to define “similar” antifouling products for extrapolation purposes? • In addition, is it necessary to have information on concentration dependence of dermal absorption in order to perform an extrapolation to different active substance concentrations? What principles should be used if such information is not available? 	<p>Similar definition:</p> <p>Quick drying or vapour pressure/boiling point/polarity of solvent and solubility of active substance in solvent</p> <p>Excess of binder vs active substance(s) and other dry substances</p> <p>Active substance enclosed in the binder</p> <p>Particulate material present – same active substance(s)</p> <p>Data required: Rheology/thixotropic/particle size profile of paint</p> <p>Description of binder characteristics</p> <p>Evidence of similarity: SEM</p> <p>Note: Small amount of substance on surface of skin and available for dermal penetration</p> <p>Note: In antifouling paints is there a concentration dependency within the matrix?</p>
<p>What can be said about the effect of different co-formulants on dermal absorption?</p> <p>What about differences in the physico-chemical properties of the products?</p>	<ul style="list-style-type: none"> • Solvent system • Binder • Active substances

Question	Input from the group
<ul style="list-style-type: none"> • Are there key co-formulants in antifouling paints which have to be similar or even identical to achieve similarity? • How can the physico-chemical properties of two or more different products contribute to defining similarity? 	
<p>How should different paints/matrices containing the same active substance be handled during testing for read-across purposes?</p> <ul style="list-style-type: none"> • Can the worst-case matrix be identified/predicted to allow for a conservative read-across approach between antifouling paints? 	<p>We decided that the group did not have sufficient data to provide an answer at this time.</p>

Annex 7. List of participants

Name	Organisation
Airaksinen Antero	European Chemicals Agency (ECHA)
Baehr Carsten	Lonza Ltd., Switzerland
Berg Eivind	CEPE antifouling working group
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Graven Coen	RIVM, NL
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Long Kevin	Regulatory Compliance Limited, UK
Lori Julia	ANSES, France
Lütte Steffen	BfR (Federal Institute for Risk Assessment), Germany
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Mackie Carol	RCL, Edinburgh, UK
McGurk Catherine	AkzoNobel / CEPE
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Pieper Christina	BfR (Federal Institute for Risk Assessment), Germany
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