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COMMENT

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Comment on "Why there is no evidence that pyridine killed the English crabs" by A. T. Ford, M. F. Fitzsimons and C. Halsall, *Environ. Sci.: Adv.*, 2024, 3, 1385, DOI: https://doi.org/10.1039/D4VA00006D

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The risks to ecosystems that are posed by chemicals present in the environment need to be properly understood in order to ensure that they are both properly managed during their life cycle, and to understand the potential causes of serious ecological impacts. A mass mortality event which occurred off the North East coast of England in late 2021 affecting crabs and lobsters was an occasion when chemical risk assessment was used to help understand the possible causes. The environmental risk assessment of chemicals typically considers both the exposure to the chemical in question and the hazard posed by it to quantitatively evaluate the level of potential harm posed. There are established procedures for evaluating the relevance and reliability of both hazard and exposure data for chemicals, and their use within risk assessment provides traceability and clearly documents any limitations associated with the data which helps to ensure that they are not used inappropriately. The additional transparency surrounding the limitations and uncertainties associated with the data used can enhance the scientific credibility of assessments which are complex or politicized. This comment considers the quality of the evidence available for understanding whether pyridine may have been the cause of the mass crustacean mortality event which occurred off the North East coast of England in late 2021.

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Environmental significance

This comment aims to promote best practice in conducting environmental risk assessments of chemicals. Formalised evaluations of the relevance and reliability of the data and methods used in environmental risk assessments of chemicals facilitates the development of transparent and robust risk assessments, and assists decision makers in understanding the uncertainties and limitations that are associated with them. Ultimately this ensures that the findings can be used to ensure that important ecosystems are adequately protected, without imposing an unreasonable burden on society through requiring remediation works that would deliver no clear benefit.

A mass mortality event occurred off the North East coast of England in late 2021 affecting crabs and lobsters,[1] and several parties attempted to identify the probable cause of the mortality. The various parties involved have put forward differing arguments regarding the most probable cause of the mortality event.[2,3] Ford, Fitzsimons, and Halsall[4] identified a number of issues that raise significant doubts about whether the chemical pyridine could have been the cause of this toxicity event. This comment relates to the methodological approaches used in the assessment presented by Ford, Fitzsimons, and Halsall[4] and is not intended to challenge the conclusions drawn.

The issues raised by Ford, Fitzsimons, and Halsall[4] provide an opportunity to reflect upon best practice in performing environmental risk assessment for chemicals, and particularly whether this has been followed in collating the evidence that the conclusions have been based on. If assessments such as these are under-protective of ecosystems then there could be serious implications for commercial fisheries, as well as potential effects on the local ecosystems. On the other hand, if assessments are over-protective and result in decisions to undertake remedial action, such as the removal of contaminated sediment, there could be a considerable cost to society to carry out the works but without any benefit to the local environment and its associated ecosystems.

An important aspect of ensuring that environmental risk assessments of chemicals are sufficiently robust to support decision making is in ensuring that the data used as the basis

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for them are both relevant to addressing the issues, and also that the data used are reliable. There are a variety of different kinds of data that need to be assessed including chemical analysis and ecotoxicity test data, as well as information on environmental fate and the results of predictive modelling.

Procedures for evaluating the reliability of ecotoxicity testing have been available, and applied in regulatory assessments, for many years.[5] Klimisch and co-authors[5] proposed the use of four different categories (or scores) to which data could be assigned. These are as follows:

1. Reliable without restrictions.
2. Reliable with restrictions.
3. Not reliable.
4. Not assignable.

A fifth category was also proposed for data that have not been subject to the assessment approach, and it was suggested that this might be applied to other special studies such as those evaluating modes of action. The four main categories have been adopted and applied within many regulatory assessment schemes. The fifth category has not been used widely, although this is likely to be due to the fact that other studies also need to be assessed as reliable, but lie outwith the scope of the assessment approach used.

The reliability scoring approach has been updated for ecotoxicity data[6] to improve consistency between different assessors, and to improve the recording of the restrictions that apply to studies. Similar assessment approaches are also applied in some specific regions for certain regulatory purposes.[7]

Risk assessment practitioners identified a potential imbalance between the data used for the hazard and exposure aspects of environmental risk assessments of chemicals, and proposed a conceptually comparable assessment approach for evaluating the reliability and relevance of environmental exposure datasets (Criteria for Reporting and Evaluating Exposure Datasets CREED).[8] Notably, because the different applications to which environmental exposure datasets may be put have different kinds of data requirements, the CREED approach included an explicit and documented evaluation of the purpose for which the exposure datasets are being assessed.[9]

The authors of CREED argue that an unambiguous statement of the assessment purpose ensures that the data used are appropriate, and that an explicit identification of the requirements focuses the attention of the assessor to specify the most appropriate information that is required.[9] Some examples of cases where the explicit evaluation of the data requirements may have focused both the questions asked, and therefore also the data requirements, for the assessment of whether or not pyridine could have been the cause of the mass crustacean mortality event are identified below.

Data that are identified as relevant for the assessment purpose must be evaluated to ensure that they are also reliable, and to identify any limitations that may be associated with the use of the data.[10] The CREED evaluation approach[11] also requires any limitations of the dataset, in terms of either its reliability or relevance to the assessment, to be recorded. This is to improve consistency and traceability across assessments. Ford, Fitzsimons, and Halsall[4] raised five questions, and we consider what information is relevant to addressing them, and how the reliability of the data used could have been assessed.

A possible limitation of the CRED and CREED assessments is that they are focused on specific areas, with CRED being particularly focused on evaluating the suitability of ecotoxicity data for deriving regulatory thresholds such as Environmental Quality Standards. Although the CREED assessment is suitable for applying to a wide range of potential applications it is focused on environmental exposure data for chemicals, and is not suitable for application to evaluating environmental fate data or model predictions. Similarly, neither of the approaches can be applied to data from microcosms, mesocosms, or other field based ecological community data. These formalised assessments may also require additional time to be committed to the evaluation of information, although this is generally considered to be reasonable for the increased transparency and traceability that they provide.

Key questions addressed.

1. How strong is the evidence that pyridine was found in high concentrations in crab tissues?
2. Is pyridine 'exceptionally' toxic to crustaceans?
3. Has pyridine ever been recorded at concentrations likely to cause acute toxicity?
4. Does pyridine adsorb to sediments?
5. Could pyridine hang around long enough, and at sufficient concentrations, to cause acute mortality across 70 km of coastline?

1. How strong is the evidence that pyridine was found in high concentrations in crab tissues?

The information that is relevant to addressing this question is biota monitoring data for pyridine concentrations in crabs that were sampled from the time and location of the incident. Additionally, data for pyridine concentrations in crabs from uncontaminated reference locations is also relevant for comparison. The reliability of such data can be evaluated following the CREED approach (see SI S1).

Ford, Fitzsimons, and Halsall[4] refer to, and present data from, samples that were analysed after the incident following the development of a method suitable for the analysis of pyridine in biota samples.[12] These data have been assessed following the CREED approach, and found to be reliable with restrictions and relevant without restrictions at the silver assessment level, with the limitation on the reliability that only sampling dates were reported, but the sampling times were not reported. The data are not usable at the gold assessment level due to some information being missing from the report, particularly in relation to the sample collection (which was not covered by the analytical report), a lack of information about the effect of sample storage, and limited information being available about the analysis of method blanks due to the standard addition approach having been used.

Based on the analyses reported[12] biota samples from the area impacted by the incident did not generally contain higher concentrations of pyridine than samples collected from

reference locations or commercially available samples. One sample collected from Bram Sands Tees, which was not analysed by the Environment Agency, did have a higher concentration of pyridine of 2.36 mg kg^{-1} wet weight, the next highest concentration was over an order of magnitude lower and was not for a sample from the impacted area.

2. Is pyridine 'exceptionally' toxic to crustaceans?

This is a case where an explicit assessment of the purpose may have influenced what data are relevant to the assessment. The distinction here is whether the assessment is focused on the assessment of the generic level of acute toxicity to crustaceans or marine crustaceans, without consideration of species, habitat, or exposure duration, or specifically on acute toxicity to the Brown Crab *Cancer pagurus* (L.)? These different kinds of information might all be considered appropriate to the assessment, but with differing levels of relevance to the assessment. For example, data on the acute toxicity of pyridine to *C. pagurus* would be the most relevant data, but an assessor may be required to use data for either other marine crustacean species and, in the absence of data for other marine species, may also be forced to use data for freshwater species. Both of which have implications on the level of certainty associated with which any conclusions are drawn.

Understanding the likelihood of a mass mortality event being caused by pyridine would be much more certain if based directly on the results of high reliability acute toxicity tests on *C. pagarus* than it would if the assessment was based on information about the chronic toxicity of pyridine to the small freshwater crustacean *Daphnia magna*, although this is a standard test species. Information on the acute toxicity of pyridine to *C. pagarus* in laboratory tests would be limited only in that the tests were performed in a laboratory, rather than in a field setting, whereas data on the chronic toxicity of pyridine to *D. magna* would have limitations in terms of the test species, the exposure duration, and the endpoint, in addition to any uncertainties associated with extrapolation between the laboratory and field conditions.

There are two ways in which the issue of "exceptional toxicity to crustaceans" could be evaluated that are of relevance to the example of the potential for pyridine to have caused the mass crustacean mortality event. These are the absolute sensitivity of *C. pagarus* to pyridine, and the sensitivity of *C. pagarus*, and other marine crustaceans relative to other marine species that could also have been affected by exposure to elevated levels of pyridine. The reliability of any relevant ecotoxicity data can be evaluated in accordance with the CRED approach.

Ford, Fitzsimons, and Halsall[4] considered an ecotoxicity test conducted on *C. pagarus*,[13] and also considered data for pyridine ecotoxicity from the US EPA Ecotoxicology Knowledgebase (**https://cfpub.epa.gov/ecotox/**). Data was presented from the US EPA Ecotoxicology Knowledgebase that indicate annelids as potentially being more acutely sensitive to pyridine than crustaceans. Furthermore, Ford *et al.* concluded that crustaceans were likely to be less sensitive to pyridine than many other industrial chemicals, although no data was presented to support that assertion. The authors also identified a number of serious concerns about the *C. pagarus* toxicity test, with low levels of replication in the experiment being specifically identified as a problem, although did not draw a clear conclusion about the reliability of the study.

The critical study from the US EPA Ecotoxicology Knowledgebase[14] on annelids (see SI S2), and the acute toxicity test on *C. pagarus*[13] (see SI S3) have both been reviewed for their reliability and relevance in accordance with the CRED approach. Both of these studies serve different purposes in this assessment. The annelid study[14] provides an indication of the sensitivity of another taxonomic group against which crustacean toxicity could be compared, whereas the *C. pagarus*[13] study provides an indication of the sensitivity of a relevant marine crustacean to pyridine.

The annelid toxicity study tested the toxicity of pyridine to the annelid *Tubifex tubifex* in a soil slurry over 21 days, and did not follow a standardised test guideline. Test validity criteria were reported for mortality only, and there were significant limitations associated with the statistical approaches used for deriving effect concentrations. The only mortality endpoint that was within the range of the tested concentrations was the 21 days LC10 of 0.85 ($\pm$0.08) mg L^{-1} pyridine. Overall the study was found to be reliable with restrictions, and also relevant with restrictions. Both the exposure duration and method of exposure limit the extent to which direct comparisons can be made between the results of this test and acute toxicity data for crustaceans.

Data for the acute toxicity of pyridine to crustaceans in standardised and GLP compliant toxicity tests following OECD Guideline 202 (ref. 15) is reported on the ECHA Chemicals Database (**https://chem.echa.europa.eu/**). A 48 hours EC50 for Daphnia magna of 320 mg L^{-1} is reported for pyridine and methylpyridines. Although this value is much higher than the 21 days LC10 of 0.85 for *T. tubifex* it is for a higher effect level and much shorter duration, both of which limit the comparability of the two studies. Furthermore, it is not clear whether the *D. magna* data relates to pyridine or is read-across from a structurally similar methylpyridine substance.

Several limitations were identified in the review of the acute toxicity test on *C. pagarus*, many of which have already been identified.[4] These limitations were to do with the lack of any validity criteria, and insufficient numbers of replicates both in the control and exposures. There were also significant limitations associated with the statistical analysis, although these are likely to be due to the insufficient replication in both the control and exposures to allow a robust statistical analysis to be performed, and a lack of sufficient SI being available to allow the calculation of endpoints and validity criteria to be checked. Overall the study was found to be unreliable, despite being highly relevant.

Overall, it is not possible to draw firm conclusions about the relative sensitivity of annelids and crustaceans to pyridine without a more detailed review of the toxicity of pyridine to aquatic organisms due to limitations associated with all of the sources of data, and particularly the fact that the most relevant study, on the acute toxicity of pyridine to the marine crustacean *C. pagarus*, is unreliable.

3. Has pyridine ever been recorded at concentrations likely to cause acute toxicity?

The most relevant information for addressing this question is aquatic monitoring data for pyridine, from the time and location of the incident. This is because any acute toxicity to crabs caused by pyridine is expected to have occurred *via* exposure to contaminated water.[13] Model predictions of exposure concentrations would be relevant with limitations in relation to uncertainties associated with the magnitude and location of the source, and the movement, dispersal, and dilution of the plume. Information on pyridine levels at reference locations may also be useful for comparison. The reliability of measured data for pyridine, such as that identified by Ford, Fitzsimons, and Halsall[4] should be evaluated in accordance with the CREED approach, and alternative approaches would be required for the evaluation of modelled concentration data.

Some data on the levels of pyridine in sediments collected from around the impacted area was reported as part of the study that reported pyridine levels in crabs.[12] The analysis has been evaluated as reliable, although an additional relevance assessment is required for the new purpose of the assessment. In this case water samples are identified as the appropriate medium for sampling, but samples from other media are acceptable if no water samples are available, but the data from them would have significant limitations associated with it (see SI S4).

Information on the concentrations of pyridine in sediments could be used in conjunction with information on the partitioning of pyridine between water and sediments to provide estimates of pyridine exposure concentrations in the water, but such estimates would be subject to further limitations associated with the method used to derive them such as the reliability of the partition coefficients, their relevance to the sediments in question, and uncertainties about the sediment properties.

4. Does pyridine adsorb to sediments?

The relevant information for addressing this question is environmental partitioning data for pyridine, and data for pyridine partitioning to local sediments.

The information that is required for addressing this question, and the following one, is not currently covered by any formalised procedures for the evaluation of reliability because the relevant environmental fate information does not fall entirely within the scope of either CREED or CRED, although it has similarities with both. The organic carbon normalised partition coefficient (K_{OC}) is normally used for evaluating the partitioning of organic chemicals, and may be determined according to standardised procedures based on empirical studies with soils and sewage sludges, an estimation method based on High Performance Liquid Chromatography, or Quantitative Structure Activity Relationships (QSAR).

The environmental fate studies must also be evaluated for their reliability, and although there are no formalised systems for such an assessment the same general principles of CRED and CREED can be applied. Where studies are performed following standardised testing methods, such as OECD 106,[16] they can be assessed for compliance with the relevant test guideline, and many non-standard tests will be based on similar principles to the standardised methods. Similarly, the use of any calculation-based methods, such as QSAR predictions, or conversion between particulate and dissolved concentrations can be performed according to standardised methods for chemical risk assessment,[17] whereas the use of more refined approaches would require more detailed information to be provided to demonstrate its reliability.

Ford, Fitzsimons, and Halsall[4] reported a $\log K_{OC}$ value for pyridine of 0.84,[18] however this value is derived by calculation from the $\log K_{OW}$ value. The Quantitative Structure–Activity Relationship (QSAR) programme EPI Suite 4.11 (**https://www.epa.gov/tsca-screening-tools/download-epi-suitetm-estimation-program-interface-v411**) database reports an experimental $\log K_{OC}$ value for pyridine of 1.6, and provides a predicted $\log K_{OC}$ value of 1.86 (K_{OC} 71.7 L kg^{-1}) calculated from the molecular connectivity index, and a predicted $\log K_{OC}$ value of 1.46 (K_{OC} 28.9 L kg^{-1}) calculated from $\log K_{OW}$, for which a measured $\log K_{OW}$ value of 0.65 was used. The REACH registration for pyridine reported on the ECHA Chemicals Database also uses the QSAR predicted K_{OC} value of 71.7 L kg^{-1}. Guidance on the assessment of QSAR predictions is available from the OECD,[19] although a single prediction for a single substance based on a commonly used model would not typically require the result checklist to be completed.

There does not appear to be any empirical data for the partitioning of pyridine to either soils, sediments, or sewage sludges available other than that used in the development of the EPI Suite QSAR.[20] The original source of this data has not been identified for review. However, the information was evidently reviewed for inclusion in the study, and was used in the development of QSAR models for the prediction of K_{OC}. Although there is considerable uncertainty associated with the information on the environmental partitioning of pyridine, due to the limited experimental data and variation between different QSAR predictions, it is unlikely that pyridine undergoes any considerable degree of adsorption to sediments. The collection of empirical partitioning data for pyridine to soils or sediments, for example according to the OECD 106[16] guideline, would resolve this issue rather than relying on predicted data. Empirical evidence of soil and sediment partitioning would also account for any partitioning mechanisms that are not driven by apolar partitioning to organic matter, which is the focus of the K_{OC} parameter.

5. Could pyridine hang around long enough and at sufficient concentrations to cause acute mortality across 70 km of coastline?

There are several ways in which this question may be considered. This could consider the potential for degradation of

pyridine, or the rate of removal, or partitioning, of pyridine from the dissolved phase to sediments, the overall rate of removal of pyridine taking account of both degradation and partitioning, or by also taking the transport, dilution, and dispersion of pyridine into account. All of these types of information may be considered as relevant to addressing this question.

Information on the fate of pyridine in the environment, such as information on biodegradation, and removal from sediment water systems may be obtained from the results of standardised testing methods, such as OECD TG 308.[21] As noted above, these standardised tests can be evaluated for consistency against the guideline for the evaluation of their reliability.

Hydrological modelling of the flows, topography, and pyridine inputs also needs to be evaluated for its reliability. Although there are numerous hydrological and dispersion models available in order for them to be suitable for application to a specific area, such as the region of interest for this incident, they must be parameterised for the local bathymetry, tides, and currents. Parameterisation of the model for the local conditions would therefore be an important aspect in the evaluation of the relevance of its outputs to the location of the incident. Evaluating the reliability of modelled data requires an evaluation of the reliability of the models used to make the predictions, and also any required input parameters. The most robust way of evaluating the reliability of model predictions is through the use of independent validation, *i.e.* using the model to predict other information for which the true result is known. Predictive models can be used to provide predictions of the fate of substances for which data are available to demonstrate their suitability for the purpose. This kind of evaluation would be most appropriate if performed for substances with comparable fate characteristics to the substance that is the focus of the assessment. Alternatively, sensitivity analyses can be used to identify the most important parameters affecting the uncertainties associated with the predictions. Models for which the reliability is assessed only through sensitivity analyses would be treated as being of lower reliability than models that have been demonstrated as reliable based on the accuracy of predictions of independent validation data.

Estabrook *et al.*[13] modelled the dispersion of a numerical passive tracer, to represent a hypothetical release of pyridine, from dredged areas along the North East coast. The most robust way of evaluating the reliability of the model predictions would be to compare them against measured data. Given that the model is principally being used to predict the dispersion of a contaminant plume this could be done for a different substance for which the release quantities are known and monitoring data in the relevant area is available. The reliability of the model predictions made by Estabrook *et al.*[13] is unknown, and there are a number of potential sources of uncertainty associated with them, including the mass of pyridine assumed to have been released, the rate of removal of pyridine due to degradation, and the rate of removal of pyridine due to partitioning to sediment, in addition to any uncertainties associated with the dispersion of the contaminant plume. However, it must be recognised that the model predictions made by Estabrook *et al.*[13] are one of the only sources information available regarding the possible levels of pyridine that crabs may have been exposed to. Consequently, although there may be significant limitations associated with the modelled exposure concentrations of pyridine it remains a relevant piece of information.

Conclusion

Formalised evaluations of the relevance and reliability of data and methods used in environmental risk assessments of chemicals facilitates the development of transparent and robust risk assessments, and assists decision makers in understanding the uncertainties and limitations that are associated with them. Ultimately this ensures that the findings can be used to ensure that important ecosystems are adequately protected, without imposing an unreasonable burden on society through requiring remediation works that would deliver no clear benefit. Furthermore, there is additional value in the application of these methodologies in enhancing the scientific credibility of assessments which are related to complex or politicized environmental events, such as the mass mortality event affecting crabs and lobsters which occurred off the North East coast of England in late 2021.[1] Taking account of both the reliability and relevance of all of the information that is used for risk assessment is essential to reliably evaluating potential risks. Furthermore, a clear understanding of the limitations of the evidence for different purposes enables both the assessor, and any reviewers, to ensure that all of the information is used appropriately.

Conflicts of interest

There are no conflicts of interest to declare.

Data availability

All data used in the preparation of this comment is publicly available.

Supplementary information: results of CREED and CRED assessments of the data used in this comment. See DOI: **https://doi.org/10.1039/d4va00420e**.

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