Dear Danish Working Environment Authority,

Thank you for the opportunity to comment on the evaluation from the Quality Committee of the report: Pyridine: Scientific basis for setting a health-based occupational exposure limit.

Based on the comment from the Quality Committee, we assume that the Committee agrees with the major decisions in our risk assessment, including selection of critical effects, and the consideration of threshold effects for nose lesions and cancer.

Below, we respond to the points raised by the Quality Committee. Each rebuttal is provided in italics just below each comment from the Quality Committee.

Yours sincerely Anne Thoustrup Saber, Alicja Mortensen, Pernille Danielsen, Niels Hadrup and Ulla Vogel

NRCWE's response to the points raised by the Quality Committee

Short report from Danish Working Environment Authority´s (AT) Occupational exposure limit quality committee. Evaluation of the report: Pyridine Scientific basis for setting a health-based occupational exposure limit. Report is from 2023.

Members of the quality committee: Frederikke Juul Tidselholdt (Environmental Protection Agency); Anoop Kumar Sharma (Technical University of Denmark-National Food Institute); Zara Ann Stokholm (Aarhus University - Department of Clinical Medicine); Lisbeth E Knudsen (University of Copenhagen - Department of Public Health).

This report is based on a meeting 27th January 2025 arranged by The Danish Working Environment Authority, where the results from the report were discussed after the authors from the working group at NRCWE (Anne Thoustrup Saber and Ulla Vogel) presented the content of the report. After the presentation, the members of the quality committee asked questions to the authors.

This report reviews data relevant to assessing the hazards of pyridine in humans and in animals. Toxicokinetics is briefly described, and previous risk assessments pyridine are summarized. The scientific basis for setting an occupational exposure limits (OEL) are presented. The current Danish OEL (TWA 8h) for pyridine is 15 mg/m^3. The working group evaluated the relevant literature on pyridine from both epidemiological and animal studies. However, since no suitable human studies were identified, endpoints were based on animal studies. The working group regards pyridine-induced liver cancer and lesions in the nose as critical effects. The evidence for a non-threshold mechanism of action for cancer is weak. However, since a non-threshold mechanism-of-action for pyridine-induced cancer cannot be excluded, the present working group considers pyridine-induced cancer in mice as non-threshold mechanism. The current working group calculated an excess of cancer incidences for 1:1,000 at 57 µg/m^3 and 1:10,000 at 5.7 µg/m^3. The working group considers pyridine-induced nose lesions in rats as a threshold effect. A DNEL was calculated, based on a 4-day inhalation study in rats. This results in a DNEL

at 11 µg/m^3 for olfactory mucosal lesions. The working group notes that the study on changes in the nose mucosa is based inhalation exposure, which is the occupationally relevant exposure route while the cancer risk estimates are based on oral exposure of mice. This means that the risk estimates based on the cancer study come with additional uncertainties due to 1) the use of a different exposure route than what is relevant in an occupational setting, and 2) an unclear mechanism-of-action for genotoxic effects. On that background the present working group is of the opinion that both risk estimates should be taken into account.

Editorial and minor comments

In the Introduction: Literature research: will be performed change to was performed.

Response: The suggested change has been implemented.

Chemical and physical properties: Include the acid dissociation constant of pyridine.

Response: The dissociation constant (pKa) has been included under the 'Substance identification' section.

Manufacture and use: mention the production volume of pyridine in Denmark. Mention the odour threshold also.

Response: Information from The Danish Working Environment Authority on the yearly Danish production volume of pyridine has been included under the 'Manufacture and Use' section.

The odour threshold previously stated under 'Chemical and physical properties' has now also been included in the 'Manufacture and use' section.

Furthermore, the following sentence has been included in the 'Executive summary': "*The working group notes that the odour threshold of pyridine (0.2 ppm (650 µg/m^3) (SCOEL, 2004)) is above the suggested risk estimates and DNEL".*

The reference to REACH should be more precise and the reference should be mentioned in the reference list.

Response: The specific reference has been included in the text and in the reference list.

Human exposure:
The concentration of pyridine in orange juice by D'Souza et al., 1980; Damani et al., 1982 is different:

i. In a very small oral study with two male volunteers receiving 3.4 mg [14C]-pyridine in orange juice (approximately 0.05 mg/kg), about 65% and 68% was recovered in the urine 24 hours after exposure, mostly in the form of the metabolite pyridine-N-oxide (D'Souza et al., 1980; Damani et al., 1982).

ii. In a very small oral study, two male volunteers received pyridine at a dose of 3.4 mg of [14C]-labelled pyridine (~0.04 mg/kg bw) in orange juice (D'Souza et al., 1980; Damani et al., 1982). Twenty-four hours after exposure, 65% and 68% of the dose was recovered in urine of the two volunteers. Two main metabolites were identified: pyridine N-oxide, which accounted for 32% of the dose, and N-methylpyridinium ion, accounting for 6 and 12% of the dose, respectively, for the two volunteers. Approximately 25% of the dose was not characterized (D'Souza et al., 1980; Damani et al., 1982).

iii. Two volunteers received 3.4 mg [14C]-pyridine in orange juice (approximately 0.05 mg/kg) and after 24 hours N-methylpyridinium ion (approx. 5.5% and 12% of the dose) was identified in urine (D'Souza et al., 1980). Pyridine-N-oxide was also detected, accounting for 32% of the administered dose (Damani et al., 1982)).

The reported concentration of pyridine in orange juice should be the same throughout the report and use the same unit.

Response: The reported concentration of pyridine in orange juice has been double-checked and corrected accordingly.

Occupational exposure levels: A wide range in change to "A wide range of… "

Response: The suggested change has been implemented.

Cancer studies: "increasing to 2.1 after 15 years", include the 95% CI for the SMR value of 2.1.

Response: Paddle et al (1991) only state that the increased lung cancer risk at 2.1 after 15 years was statistically significant at the 5% level, but did not provide 95% CI. This has now been added in the report.

Animal studies: Long-term studies: Change to long-term exposure and this is a header and should be in bold.

Response: The heading has been changed and made bold.

Non-neoplastic effects: "An overview of the non-neoplastic lesions are presented in Table x", write the table number.

Response: Table x has been corrected to Table 2.

Previous evaluations of pyridine: "Although the authors identified one human long-term exposure study, the data could not be used for hazard assessment because the doses were too high and the participants were epileptic patients who used pyridine as drug for anticonvulsant treatment" and the reference is missing.

Response: The reference has been included

Comments on the OEL

In the executive summary, the quality committee, suggest mentioning that no human studies were identified on reproductive and developmental effects.

Response: The sentence 'The present working group furthermore notes that no human studies on reproductive and developmental effects were identified' has been included in the executive summary.

At the meeting, it was decided that the quality committee would run QSAR modelling (Danish (Q)SAR Database, https://qsar.food.dtu.dk) to get information of the prediction of genotoxicity data of pyridine, because the experimental evidence of pyridine genotoxicity is extremely weak. The QSAR modelling show that both *in vitro* and *in vivo* genotoxicity endpoint predictions are by far mostly negative.

Response: Following the comments from the quality Committee, the working group repeated the QSAR analyses of pyridine genotoxicity. Data from the Danish (Q)SAR Database show that pyridine is negative in domain in the Ames test for mutagenicity. In addition, the Database also reports that experimental data for Ames test are negative.

A sentence on the QSAR results has been added to the 'Cancer' paragraph in the 'Mechanisms of toxicity' section.

Therefore, the quality committee suggests using the threshold effect for cancer, DNEL value of 40 µg/m^3 together with the DNEL at 11 µg/m^3 for olfactory mucosal lesions as occupational exposure limits.

Response: The NFA working group is of the opinion that the evidence for lack of genotoxicity is not strong enough to be able to rule out a non-threshold mechanism of action. Therefore, no change was made.