Comments first draft report *Pyridine*

Dr. Hartmut Höke sent the following comments by e-mail on February 12th of 2023

Dr. Hartmut Höke **Weinheim, 11 Feb. 2023**

Comment on

DECOS 2022: Pyridine: Evaluation of the carcinogenicity and genotoxicity, DRAFT. *Version date: 10 Nov. 2022.* Subcommittee on the Classification of Carcinogenic Substances of the Dutch Expert Committee on Occupational Safety, a Committee of the Health Council of the Netherlands

My main request is that You re-consider the results of the NTP-mouse study and to re-discuss their value for classification to Carc. 1B. I doubt that Your "Weight of Evidence" is robust and sound enough as to offer a firm scientific justification for doing so. And to be somewhat pathetic, this allocation eventually fails to meet the high criteria of Section 1.5 You like to comply with:

> *"The classification systems on mutagenicity and carcinogenicity are based on a weight of evidence assessment, in which more weight is given to evidence obtained from human data than to evidence obtained from animal studies or laboratory data. Furthermore, the weight of evidence depends on the number of reliable studies that show clear associations between exposure and the occurrence of genotoxicity or carcinogenicity. This implies that studies with significant shortcomings contribute to a lesser extent to the overall weight of evidence."*

I am wondering most about the common judgement of "clear evidence" of carcinogenesis in mice, which is also supported by DECOS. The final proposal for Carc. Cat 1B seems to be mainly driven by the increases in the malignant tumour rates observed in either rodent species for different tumour types.

Evaluations in an Overview:

The starting point seems to be IARC´s evaluation; however this turn out to be variable over time, in 2000 the substance allocated to IARC-Group 3, then in 2019 to IARC-Group 2B, although a new pertinent data-base hasn´t fundamentally grown since 2000 as to justify this change in mind, neither the latest IARC-monography on pyridine does explain this fact.

- **IARC 2000:** Conclusion based on the NTP studies (2000)
  Pyridine is *not classifiable as to its carcinogenicity to humans (Group 3)*.

- **IARC 2019:** Conclusion based on the NTP studies (2000):
  Pyridine is *possibly carcinogenic to humans (Group 2B)*, saying "There is sufficient evidence in experimental animals for the carcinogenicity of pyridine").

The DECOS draft-evaluation joins the recent IARC conclusion, at least using the same wording (DECOS., 4.5, p.25)

> *"Classification in category 1B (presumed to be carcinogenic in humans) requires a causal relationship between the substance and an increased incidence of malignant neoplasm*

in two or more animal species. In a well performed study by the National Toxicology Program (NTP), sufficient evidence for carcinogenicity of pyridine in rats (renal tubule adenomas and carcinomas, testicular adenomas and mononuclear cell leukaemia) and mice (hepatoblastoma and hepatocellular adenomas and carcinomas) was found. Therefore, the Committee recommends classifying the substance as presumed to be carcinogenic in humans, and recommends classifying the substance in category 1B."

The conclusions given in the underlying NTP-report TR 470 (p.71) are somewhat more distinctive and differentiated: Instead of "sufficient" evidence, "some" and "equivocal" evidence was preferred for three results in rat

"**Pyridine, NTP TR 470 (p.71)**

CONCLUSIONS

Under the conditions of these 2-year drinking water studies, there was some evidence of carcinogenic activity of pyridine in male F344/N rats based on increased incidences of renal tubule neoplasms. There was equivocal evidence of carcinogenic activity of pyridine in female F344/N rats based on increased incidences of mononuclear cell leukemia. There was equivocal evidence of carcinogenic activity in male Wistar rats based on an increased incidence of interstitial cell adenoma of the testis. There was clear evidence of carcinogenic activity of pyridine in male and female B6C3F$_1$ mice based on increased incidences of malignant hepatocellular neoplasms.*

The following results I can sum up from the drinking-water studies (NTP 2000):

In rats (F344/N): There is evidence of a positive trend of kidney cancer in males, with a significant dose-response only at the highest dose, this tumor type not found in females but instead a slight positive trend in leukemia was observed, dose–related, but against a high background rate.

In the mice strain (B6C3F$_1$): a general high (inherent) prevalence of liver cancer was striking! That means, the background tumor rates (negative controls) were very high.

In male and female mice, there was evidence of a slight (statistically significant) increase in the number of (malign) carcinomas and (benign) adenomas. However, a dose-relationship was missing with the doses increasing, i.e. within the dose-range applied, there was no increase in tumor rate.

Other point to be taken into account:

- Pyridine can be accepted as not being genotoxic.

- In rat and mouse, the target organs are different. But also sex differences occur in the rat species: **in male rats**, kidney tumors are addressed, and by the way almost all (benign) adenomas, only two (malign) carcinomas (*) can be derived from the results overview, and this relates to the low dose group (see DECOS, Tab.3a, p.21: (*) The comparison of line 1 with 2 reveals no difference between both, but only for the low dose, the difference showing two tumours in line 2,

which were identified as malignant: 5 vs 3 tumours). That means in males the tumour increase is dominated by benign types rather than malignant ones.

In **female rats**, surprisingly no kidney tumors are reported, however a dose-related increase in leukemia is described but before a high background of spontaneous leukemia (24%) (DECOS, Tab.3b, p.21). In F344/N rats, leukemia is said to be a common neoplasm, while not in Wistar rats *(NTP TR 470, 2000, 69)*. A previous study having used Wistar (Dieter et al. 1989 cited) was included in NTP´s evaluation: There were no increases in leukemia following pyridine treatment, and this behind a low spontaneous tumor rate *(NTP TR 470, 2000, 69).*

- **Mouse model (B6C3F$_1$)** is known to have a high spontaneous liver-tumor rate (NTP TR470, p.69). Therefore, it´s a questionable use for this endpoint, in particular with respect of the toxicological relevance to humans.
  The lack of a dose-related tumor response was attributable to the fact that overall the dose selection proves to be suboptimal (in principle too high), resulting already in a high percentage of tumor-bearing animals of more than >80% to 90% in the first dose-groups, while the neg. controls were at 75% to >80% (male and female, DECOS, Tab. 6a and 6b, resp.).
  The NTP report (TR 470, p.69) gives the following opinion: The liver neoplasms from cellular adenomas to cellular carcinomas, along with the hepatoblastoma, represent a biological and morphological continuum in the progression of proliferative lesions, primarily and frequent in mice. The hepatoblastomas, which exhibit a low spontaneous background rate, are regarded as a possibly discrete variant of a malignant liver neoplasm. It is considered adequate to use the combined incidences for the interpretation of a carcinogenic potential.

  This raises doubts that pyridine acts as a typical primary liver carcinogen (initiator), since furthermore, it is not genotoxic. Instead, it might act as a promotor, i.e. pyridine may promote the growth of spontaneous liver tumors that in the mouse strain develop at high age to a high degree, anyway.

- Overall, rat and mouse, neither showing convincing carcinogenic potential. The rat model seems to be more relevant, whereas the mouse is a weak or even unsuitable model that fails to confirm more reliable findings in the rat, while probably delivering false-positive data.

- For more details, several factors have to be considered, the age of the animals (2 years), the time to tumour (early before control, at very high age, tumour-related interim mortality), potential non-neoplastic pre-stages before tumor development (hyperplasia, cirrhotic changes?), impairing the health status of the animals?.

- Metabolism may be taken into account, because key metabolites are known (also in humans). Are the metabolites known to be mutagenic of carcinogenic?

NOTE: A typo – in the headline of Table 6b “female” must be added.

The results tables are fine, displayed in a well-readable and transparent manner. But survival data (only qualitatively addressed in the annexes) are

missing. May have a certain relevance, since the survival was low throughout, also in the control groups.

References

- **DECOS 2022: Pyridine:** Evaluation of the carcinogenicity and genotoxicity, DRAFT. *Version date: 10 Nov. 2022.* Subcommittee on the Classification of Carcinogenic Substances of the Dutch Expert Committee on Occupational Safety, a Committee of the Health Council of the Netherlands

- **NTP 2000:** Toxicology and Carcinogenesis Studies of Pyridine (CAS No. 110-86-1) in F344/N Rats, Wistar Rats, and B6C3F1 Mice (Drinking Water Studies). Natl Toxicol Program Tech Rep Ser 2000; 470: 1-330.

- **IARC (2019):** Some Chemicals That Cause Tumours Of The Urinary Tract In Rodents. IARC monographs on the evaluation of carcinogenic risks to humans, Volume 119, International Agency for Research on Cancer.

- **IARC (2000):** Pyridine summary & evaluation. IARC IPCS Inchem, 77, 503, International Agency for Research on Cancer

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Not cited:

- Chen W; Zijtveld, D. (2021): Pyridine - An overview of available data on mutagenicity and carcinogenicity. RIVM letter report 2021-0191, RIVM

- SCOEL 2004: Recommendations from the Scientific Committee on Occupational Exposure Limits for Pyridine. SCOEL/SUM/106, European Commission

The NIOSH sent the following comments by e-mail on February 27th of 2023

Thank you for the opportunity to provide review comments on the DECOS draft advisory report on Pyridine.

Attached you will find review comments from Barbara Alexander, Shirisha Chittiboyina, and Patti Erdely.

Comments on DECOS draft document on 02162023

By: Barbara M. Alexander, Senior Service Fellow
NIOSH/Division of Field Studies and Engineering
Cincinnati, Ohio, USA

PAGE NUMBER, LINE NUMBER	COMMENT
General Comments	The Committee's recommendations are appropriate.
Specific Comments	
Page 9, lines 10-13	The same sentence is repeated twice.
Page 21, line 9	"Historical control data" is consistently misspelled as "Historial control data" in the headers of tables starting with Table 3a.
Page 21, line 19	Did not find the label "d" anywhere in the table above.
Page 22, line 14	The expression "weight gain loss" sounds contradictory. It would be clearer to express it as a "reduction in weight gain" or similar wording.

Comments on DECOS draft document on Pyridine

By: Shirisha Chittiboyina, MS, PhD, Senior Service Fellow/Toxicologist
NIOSH/Division of Science Integration
Cincinnati, Ohio, USA

PAGE NUMBER, LINE NUMBER	COMMENT
General Comments	Overall document seems to provide sufficient information to classify Pyridine as "presumed to be carcinogenic to humans." There is adequate reference to the IARC monograph (2019). It was not clear whether any new evidence has been added from 2019 to 2021 (when this document has been updated). Also, there is inconsistency across the document on how the references are cited (numbered in most places and et al., in certain sections). This is an editorial comment, but this is also very critical as it is confusing for the reader to follow the citations in the text with a different format in each section.
Specific Comments	
Page 22, line 12, section 4.3.2. Supplemental chronic study in rats	The document does not clearly state the rationale of including this particular study in detail as it does not corroborate the National Toxicology Program (NTP) study or add to the final conclusion by the committee.
Page 21, lines 1-2, Section 4.3.1 Rats	Consider rewriting this sentence from "tumours in this study occurred in the absence of alpha-2u-globulin, alpha 2u-globulin nephropathy is excluded as possible underlying mechanism" to "tumour incidence was observed even in the absence of alpha 2u-globulin hence eliminating its possible role in the reported nephropathy." The IARC monograph (2019) on pyridine does mention that pyridine satisfies 4 of its 7 criteria for alpha 2u-globulin mediated nephropathy. The fact that female rats showed renal adenomas is to be highlighted for advocating that tumor incidence might not be correlated with alpha 2u-globulin.

Comments on DECOS draft document on Pyridine
By: Patti Erdely, Research Biologist
NIOSH/Health Effects Laboratory Division
Morgantown, West Virginia, USA

PAGE NUMBER, LINE NUMBER	COMMENT
General Comments	The Committee's recommendations are appropriate.
Specific Comments	
Page 9, lines 9-13	The sentence starting with "In the case of pyridine,…" is repeated in the text.
Page 10, line 12	The extra space after "action ." needs to be deleted.
Page 11, line 22	"…volunteers received and oral dose…" should be "…an oral dose..."
Page 15, line 28	Add a parenthesis after "intervals."

Comments second draft report *Pyridine*

The NIOSH sent the following comments by e-mail on May 17th of 2023

Thank you for the opportunity to provide review comments on the DECOS revised draft advisory report on Pyridine.

Attached you will find review comments from Shirisha Chittiboyina, and Patti Erdely.

Comments on DECOS draft document on PYRIDINE

By: Shirisha Chittiboyina, MS, PhD, Senior Service Fellow/Toxicologist
NIOSH/Division of Science Integration
Cincinnati, Ohio, USA

PAGE NUMBER, LINE NUMBER	COMMENT
General Comments	Overall document seems to provide sufficient information to classify Pyridine as "suspected to be carcinogenic to humans." There is adequate reference to the IARC monograph (2019). The authors have corrected the reference citations based on the previous comments.
Specific Comments	
Page 21, line 16, section 4.3.2. Supplemental chronic study in rats	The document does not clearly state the rationale of including this particular study in detail as it does not corroborate the NTP study or add to the final conclusion by the committee. This comment was provided previously but there was no response reflecting this in the document.

Comments on DECOS draft document on Pyridine
By: Patti Erdely, Research Biologist
NIOSH/Health Effects Laboratory Division
Morgantown, West Virginia, USA

PAGE NUMBER, LINE NUMBER	COMMENT
General Comments	The Committee's recommendations are appropriate.
Specific Comments	
	No additional revisions needed.