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ICH M7(R2): Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk
2nd Addendum

Step 4 document – to be implemented

16 May 2023

International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use

Legal Notice

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Background

- The second revision of ICH M7(R2) was developed based on the Concept Paper (19 September 2018)
- ICH M7(R2) includes a 2nd Addendum to ICH M7 complementing the first Addendum published in ICH M7(R1). The 2nd Addendum was signed off as a *Step 2* document (6 October 2021) to be issued by the ICH Regulatory Members for public consultation
- The 2nd Addendum was developed to include additional monographs for 7 mutagenic impurities and derive Acceptable Intakes (AIs) for them
- Additionally an update was made in the main M7 guideline text changing the HIV treatment duration from 1 - 10 years to > 10 years - lifetime
- After public consultation and EWG discussions, this document has been signed off as a *Step 4* document (3 April 2023) to be implemented by the ICH Regulatory Members

Key Principles

- This presentation provides a summary of the second addendum and revisions of ICH M7(R1)
- This presentation explains the merger of the first addendum with this second addendum
- The addendum is now a separate document to the core guideline and both documents will be linked on the ICH website

Guideline Objectives

- **Objectives remain the same as indicated in ICH M7 guideline finalized June 23rd 2014**
- **The guideline revision (R2) focuses on selecting additional relevant mutagenic impurities and develop monographs with compound specific AIs to include in the Addendum**
- **Revise the main guideline**
  - changing the HIV treatment duration from 1-10 years to > 10 years - lifetime

Table of Contents

List of Abbreviations used in this presentation

- AI – Acceptable Intake
- API – Active Pharmaceutical Ingredient
- ATSDR – Agency for Toxic Substances and Disease Registry
- CPDB – Carcinogenicity Potency Database
- EWG – Expert Working Group
- HC – Health Canada, Canada
- HPRT – Hypoxanthine-Guanine-Phosphoribosyltransferase
- NTP-TR – National Toxicology Program-Technical Report
- PDE – Permissible Daily Exposure
- TD_{50} – Tumor Dose 50 (dose with 50 % tumor bearing animals)
- US-EPA – United States Environmental Protection Agency
- WHO-IPCS – World Health Organisation-International Program on Chemical Safety

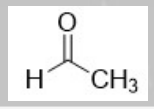
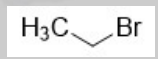
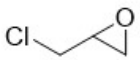
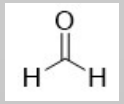
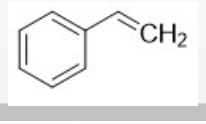
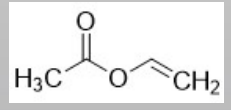
Summary of Guideline Content

- **The addendum explains the development of chemical specific AIs or PDEs (when appropriate)**
- **Methods used to develop AIs or PDEs and in which cases an AI and in which cases a PDE should be developed**
- **21 monographs developed for individual chemicals according to methodology explained in the addendum**
- **Change of HIV treatment duration from 1 - 10 years to >10 years - lifetime in Note 7, table 4**

Results of Public Consultation

- **Addition of Note 3 to the addendum explaining how to apply the limit for formaldehyde in products applied via inhalation of 215 ppb or 8 mg/day whichever is lower**
- **Addition of supporting references to some of the monographs, e.g. EFSA 2020 (Ref 29) and ECHA 2021 (Ref 30) in styrene monograph**

Mutagenic impurities added to the addendum

Compound	CAS#	Chemical Structure	Lifetime Limit AI and/or PDE (µg/d)
Acetaldehyde	75-07-0		PDE (oral) 2,000 AI 185 (all other routes)
1,2-dibromoethane	106-93-4		AI 2
Ethyl bromide	74-96-4		AI 32
Epichlorohydrin	106-89-8		AI 3
Formaldehyde	50-00-0		PDE (all other routes 10,000) AI (inhalation) 8,000 or 215 ppb, whichever is lower
Styrene	100-42-5		AI 154
Vinyl acetate	108-05-4		PDE (oral) 2,000 AI 758 (all other routes)

Acetaldehyde

- **Mutagenic in Hypoxantine-Phosphoribosyl-Transferase (HPRT) assay in mammalian cells, while negative in the Ames test**
- **Route-specific differences in carcinogenic response**
- **Oral exposure – no relevant carcinogenic response in rats**
  - Highest doses: males 246 mg/kg/day, females 260 mg/kg/day
- **Inhalation exposure – carcinogenic in rat**
  - Nasal adenocarcinoma significantly increased at all doses tested
  - Effect of detoxification in nasal mucosa is unclear
  - Carcinogenic effect possibly limited to site of contact
  - Threshold mode of action is unclear for inhalation
- **Significant human exposure**
  - Endogenously formed as metabolite of carbohydrates
  - Exposure via food
  - Efficiently detoxified by aldehyde dehydrogenase
  - Threshold assumed

Acetaldehyde - PDE (oral) 2 mg/day – AI (all other routes) 185 µg/day

- **Oral PDE of 2 mg/day was determined**
  - Based on average daily intake from food (Uebelacker & Lachenmeier, 2011)
- **For all other routes the AI is 185 µg/day**
  - 28 month inhalation study in rat (Woutersen et al. 1986) considered the most relevant study
  - Relevant outcome: nasal adenocarcinoma
  - TD_{50} calculated by CPDB = 185 mg/kg/day
  - AI = (185 mg/kg/d / 50,000) x 50 kg = 185 µg/day

1,2 Dibromoethane – AI 2 µg/day

- **Mutagenic in Ames test and HPRT test in Chinese Hamster Ovary (CHO), cells**
- **Carcinogenic in mouse and rat**
  - By oral exposure – most sensitive forestomach
  - By inhalation – most sensitive lung and nasal cavity
- **Most robust study for derivation of AI**
  - National Toxicology Program (NTP) inhalation study in F344 rats (NTP TR-210)
  - TD_{50} calculated by CPDB = 2.33 mg/kg/day; – similar to TD_{50} of most robust oral study in mice
  - AI = (2.33 mg/kg/day / 50,000) x 50 kg = 2.33 µg/day
  - AI rounded to 2 µg/day
  - One AI for all routes justified due to similar TD_{50} values for inhalation and oral application

Epichlorohydrin – AI 3 µg/day

- **Mutagenic in Ames test and mouse lymphoma test**
- **Carcinogenic at site of contact**
  - Forestomach and oral cavity tumors for oral administration
  - Nasal tumors for inhalation
  - Injection site sarcomas for subcutaneous injection
- **Most robust study for derivation of AI**
  - Oral study in rat (Wester et al., 1985)
  - TD_{50} calculated by CPDB = 2.55 mg/kg/day
  - AI = (2.55 mg/kg/day / 50,000) x 50 kg = 2.55 µg/day
  - AI rounded to 3 µg/day

Ethyl Bromide

- **Ethyl bromide is an alkylating agent and mutagenic in Ames as a gas**
- **Carcinogenic in NTP inhalation studies in mice and rats (NTP-TR 363)**
  - Target organs were: uterus, adrenal gland in males and liver in both sexes
  - Most sensitive endpoint was adrenal gland pheochromocytomas in male rat – significant increase at all doses compared to control
  - Lack of dose-dependent increase, trend analysis negative, TD_{50} calculated by CPDB not statistically significant and not considered appropriate
  - The Expert Working Group (EWG) calculated TD_{50} values for each dose separately. All calculated values were statistically significant

Ethyl Bromide – AI 32 µg/day

- **Derivation of the AI**
  - NTP inhalation study (NTP-TR 363)
  - TD_{50} calculated by CPDB not considered appropriate
  - The EWG calculated a TD_{50} for each dose (see Note 2 in the addendum for reference)
  - The EWG chose the most senstitive TD_{50} = 32.2 mg/kg/day for calculating the AI
  - AI = (32.2 mg/kg/day / 50,000) x 50 kg = 32.2 µg/day
  - AI rounded to 32 µg/day

Note 2

- **Note 2 demonstrates the calculation of TD_{50} values for each of the ethyl bromide dose levels in the NTP-TR 363 study:**

$$TD_{50\ low\ dose} = \frac{0.693}{0.0215055234}$$

$$TD_{50\ mid\ dose} = \frac{0.693}{0.0059671034}$$

$$TD_{50\ high\ dose} = \frac{0.693}{0.0042161616}$$

- **The low dose provides the most conservative TD_{50}**
- **TD_{50} = 0.693 / 0.0215055234 = 32.2 mg/kg/day**

Formaldehyde

- **Formaldehyde is mutagenic in the Ames test and in the HPRT test in mammalian (TK6) cells**
- **Carcinogenicity**
  - Carcinogenic in animals by inhalation route; tumors in the nasal cavity
  - Was not considered carcinogenic in studies via the oral route
    - One out of three studies was positive for leukemia/lymphosarcoma, however inappropriate and deficient study design and analyses invalidated the use of this study
- **Carcinogenic mode of action**
  - Formaldehyde is considered to be a site-of-contact carcinogen acting mainly by cytolethality/regenerative cellular proliferation
  - Formation of DNA-protein crosslinks by formaldehyde is involved in cytolethality however, this may not be the primary mode of action
  - Conolly et al. (2004) described a model to calculate human cancer risk of formaldehyde inhalation using non-linear-based and linear-based mechanisms

Formaldehyde

- **Significant human exposure**
  - Endogenously formed – body turn over is up to 50 g/day
  - Component of many foods – daily oral intake range 1.5-14 mg/day
- **Regulatory limits**
  - US-EPA, WHO-IPCS, ATSDR, HC limit formaldehyde via oral exposure to 0.2 mg/kg/day or 10 mg/day for a 50 kg person based on non-cancer endpoint
  - For inhalation HC recommends a limit of 100 ppb in air as a 1 hour average and WHO recommends 77 ppb in air as a 30 min average to protect humans from local irritation and sensitization effects

Formaldehyde AI (inhalation) = 8 mg/day or 215 ppb whichever is lower

- **EWG considered the Conolly et al. (2004) model as the most suitable to derive an AI**
- **The AI is derived by calculating the cumulative daily formaldehyde dose inhaled at the formaldehyde air concentration associated with 1:100,000 cancer risk. This cummulative daily dose is 8.2 mg/day, rounded to 8.0 mg/day and represents an upper limit over a 24 hour period. AI = 8 mg/day**
- **However, inhaling 8 mg formaldehyde with one breath e.g. with an inhalation drug taken once per day via an inhaler, is not considered appropriate.**

Formaldehyde AI (inhalation) = 8 mg/day or 215 ppb whichever is lower

- **To protect patients from local irritation and sensitization effects of formaldehyde the EWG calculated a concentration limit for inhalation of formaldehyde in drug products**
- **The concentration limit was derived by calculating the formaldehyde concentration in air when inhaling 8 mg over a 24h breathing period.**
- **With an average human breathing volume of 28.8 m^3 /day and weight of air of 1293 g/m^3 the concentration is calculated:**
  215 ppb = (0.008g/day / 28.8 m^3/day) x 1/1293 g/m^3

Note 3 - Application of formaldehyde AI (inhalation) = 8 mg/day or 215 ppb whichever is lower

- **For formaldehyde the limit is 215 ppb or 8 mg/day whichever is lower**
- **Example 1: albuterol actuator with formaldhyde as impurity in API**
  - delivers 90µg API/actuator.
  - Tidal volume inhaled with each actuator is 400 - 500 ml (female/male adult)
  - Calculation of formaldehyde limit in API:
    - 215 ppb of formaldehyde in 400 ml tidal volume: 0.215 x 30 g/mol / 24.45 = 0.263 mg/m^3. 0.263 mg/m^3 x 1000 L x 0.4 L = 0.105 µg formaldehyde. Formaldehyde in API: 0.105 µg formaldehyde / 90 µg API = 0.12 %
- **Example 2: Albuterol sulfate actuator with formaldehyde as a drug product impurity**
  - delivers 35 mg drug product/actuator
  - Calculation of formaldehyde limit in drug product:
    - with the 215 ppb in air limit, the allowed formaldehyde amount is 0.105 µg
    - 0.105 µg formaldehyde / 35 mg drug product = 3 ppm

Formaldehyde PDE (all other routes) = 10 mg/day

- **For all other routes, a PDE of 10 mg/day is recommended**
  - Based on the limit set by US-EPA, WHO-IPCS, HC and ATSDR
  - Considered broadly accepted and justified by environmental exposure ranges
  - Carcinogenicity of formaldehyde is considered specific to the inhalation route

Styrene

- **Styrene is mutagenic in the Ames test only with metabolic activation and mutagenic in vivo in lymphocytes of exposed workers**
  - Metabolite styrene 7,8-oxide is considered the mutagenic compound
  - Styrene 7,8-oxide forms covalent adducts with DNA, adducts identified in vitro, in vivo and, in exposed humans
- **Carcinogenic potential**
  - Carcinogenic in mice via oral and inhalation routes
  - Carcinogenic in rats only in a single study via inhalation (relevance questionable due to study limitations) but negative in another inhalation study and all oral studies
  - Styrene 7,8-oxide is carcinogenic in mice by oral and dermal application and, in rat via oral and transplacental exposure

Styrene – AI 154 µg/day

- **Carcinogenic mode of action**
  - Metabolite styrene 7,8-oxide considered the main mutagenic agent
  - Styrene induced oxidative stress, immunosuppression and chronic inflammation are the potential contributing factors
- **Derivation of the AI**
  - Mouse lung tumors are considered the most relevant tumors
  - The most sensitive and robust study (Cruzan et al. 2001) provides a TD_{50} calculated by CPDB of 154 mg/kg/day
  - AI = (154 mg/kg/day / 50,000) x 50 kg = 154 µg/day

Vinyl Acetate

- **Genotoxic potential**
  - Vinyl acetate is not mutagenic in Ames test, but genotoxic causing chromosomal damage in human lymphocytes
  - Extensive evidence that genotoxicity is mediated via its metabolite, acetaldehyde
- **Carcinogenicity**
  - Four studies are listed in CPDB
    - Two mouse studies, one with oral one with inhalation
    - Two rat studies, one with oral one with inhalation
    - Tumors observed were uterine, espophageal and forestomach ~~tumors~~ in mouse and liver, thyroid, uterine and nasal tumors in rat
  - In other published oral studies in mice and rat tumors observed in the oral cavity, esophagus and forestomach
  - Vinyl acetate was negative in an inhalation study in mice, and positive for nasal tumors in rats at the high dose

Vinyl Acetate PDE (oral) 2mg/day – AI 758 µg/day for all other routes

- **PDE (oral) – Vinyl acetate undergoes rapid hydrolysis to form acetic acid and acetaldehyde. Based on the same consideration as for acetaldehyde the PDE for oral route is set to 2 mg/day**
- **AI (all other routes): The 2 year inhalation study in rats listed in CPDB (Bogdanffy et al. 1994) was considered the most robust and appropriate for derivation of an AI. TD_{50} calculated by CPDB was 758 mg/kg/day**
  AI = (758 mg/kg/day / 50,000) x 50 kg = 758 µg/day

Implementation of the change of the HIV treatment duration from 1-10 years to > 10 years - lifetime

For regulatory submissions 18 months after the date that the M7(R2) reached *Step 4* (3 April 2023), the 1.5 µg/day or other appropriate acceptable intake would be applied in situations that are explained in detail in the ICH M7(R2) Q&As document in Q&A 7.4

Considerations

- **ICH M7 2nd addendum should be read in conjunction with the main guideline ICH M7(R2), the ICH M7 Q&A document and the M7(R1) Training material video presentation**

Conclusions

- **ICH M7(R2) has revised the format of M7 into two linked separate documents**
  - First document is the core guideline including a table of all monographs, that are included in the addendum and hyperlinked to the second document
  - Second document is the addendum containing all monographs of mutagenic impurities assessed by the EWG
- **HIV treatment duration changed from 1 - 10 years to > 10 years - lifetime in main M7 guideline text**
- **Seven new monographs for mutagenic impurities added plus two additional Notes (Note 2 and 3) to explain derivation of AI for ethyl bromide and use of concentration/exposure limits for formaldehyde in drugs for inhalation**
- **List of Compounds (Appendix 3) updated to include all monographs**
- **Grammatical editing and formatting e.g. updating URLs**

Contact

- **For any questions please contact the ICH Secretariat:**

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