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

2nd Addendum

Step 2 document – for comments

6 October 2021

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

Legal Notice

Outline

- Background
- 2nd Addendum Objectives and Work process
- 2nd Addendum monographs
- Conclusions

Background

- **The second revision of ICH M7(R2) includes the addition of 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 Oct 2021) to be issued by the ICH Regulatory Members for public consultation**
- **The 2nd Addendum was developed to provide 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 to lifetime
- **A Concept Paper (Sep 19th 2018) has been developed to guide the development of the 2nd Addendum**
- **The EWG anticipates to finalize as a *Step 4* document Jun / 2022**

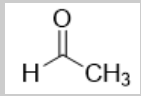
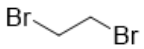
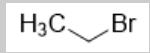
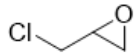
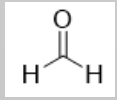
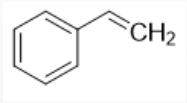
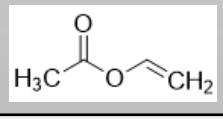
2nd Addendum Objectives

- **Selecting relevant mutagenic impurities to include in the Addendum**
- **Develop AIs or Permissible Daily Exposures (PDEs) for selected mutagenic impurities based on published data**
- **Write monographs describing the data and process for deriving the substance specific AIs or PDEs**

Work process

- Stakeholders survey was performed for additional mutagenic impurities to include in the 2nd Addendum to ICH M7
- Seven mutagenic impurities commonly used in pharmaceutical manufacturing were selected for monograph development
- AIs or PDEs were derived based on the principles outlined in ICH M7
- Due to the growing length of the ICH M7 document the EWG decided to separate ICH M7 into a main guideline document and an addendum document interlinked on the ICH webpage

Mutagenic impurities included in the 2nd addendum

Compound	CAS#	Chemical Structure
Acetaldehyde	75-07-0	
1,2-dibromoethane	106-93-4	
Ethyl bromide	74-96-4	
Epichlorohydrin	106-89-8	
Formaldehyde	50-00-0	
Styrene	100-42-5	
Vinyl acetate	108-05-4	

Acetaldehyde

- **Mutagenic in Hypoxantine-Phosphoribosyl-Transferase (HPRT) assay in mammalian cells, while negative in Ames**
- **Route specific differences in carcinogenic response**
- **Oral exposure – no relevant carcinogenic response in rats**
  - Highest doses males 246 mg/kg/d, females 260 mg/kg/d
- **Significant human exposure**
  - Endogenously formed as metabolite of carbohydrates
  - Exposure via food
  - Efficiently detoxified by aldehyde dehydrogenase
  - Threshold assumed
- **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

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

- **Oral PDE of 2 mg/day 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
  - Tumor Dose $(TD)_{50}$ calculated by Carcinogenicity Potency Database (CPDB) = 185 mg/kg/d
  - AI = (185 mg/kg/d / 50,000) x 50 kg = 185 µg/d

1,2 Dibromoethane – AI 2 µg/day

- **Mutagenic in Ames and HPRT assay in 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/d – similar to TD_{50} of most robust oral study in mice
  - AI = (2.33 mg/kg/d / 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 and mammalian cells**
- **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/d
  - AI = (2.55 mg/kg/d / 50,000) x 50 kg = 2.55 µg/day
  - AI rounded to 3 µg/day

Ethylbromide

- **Ethylbromide 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 analyses negative and TD_{50} calculated by CPDB not statistically significant
  - TD_{50} values were calculated for each single dose by EWG and were statistically significant

Ethylbromide – AI 32 µg/day

- **Derivation of the AI**
  - NTP inhalation study (NTP-TR 363)
  - TD_{50} calculated by CPDB not statistically significant
  - TD_{50} therefore calculated by EWG for each single dose (see Note 2 at the end of the step 2 document for reference)
  - The EWG chose the most senstitive TD_{50} = 32.2 mg/kg/d for calculating of the AI
  - AI = (32.2 mg/kg/d / 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 the ethylbromide 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} = 32.2 mg/kg/d**

Formaldehyde

- **Formaldehyde is mutagenic in the Ames assay and in mammalian cells**
- **Carcinogenicity**
  - Formaldehyde was carcinogenic in animals by inhalation route for tumors in the nasal cavity
  - Formaldehyde was considered not to be 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
  - Componant of many foods – daily oral intake range 1.5-14 mg/day
- **Regulatory limits**
  - US-EPA, WHO-IPCS, ATSDR, Health Canada, Canada limit formaldehyde via oral exposure to 0.2 mg/kg/d 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
All other routes PDE = 10 mg/day

- **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 cumulative 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
All other routes PDE = 10 mg/day

- **To protect patients from local irritation and sensitization effects of formaldehyde the EWG calculated a concentration limit for inhalation of formaldehyde with 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/d / 28.8 m^3/d) x 1/1293 g/m^3)

Formaldehyde AI (inhalation) = 8 mg/day or 215 ppb whichever is lower
All other routes PDE = 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, Health Canada, Canada and ATSDR
  - Considered broadly accepted and justified by environmental exposure ranges
  - Carcinogenicity of formaldehyde is considered specific to the inhalation route

Styrene – AI 154 µg/day

- **Styrene is mutagenic in the Ames assay only with metabolic activation and mutagenic in vivo**
  - 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**
  - Styrene is carcinogenic in mice via oral and inhalation routes
  - Styrene is 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 induces 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/d
  - AI = (154 mg/kg/d / 50,000) x 50 kg = 154 µg/day

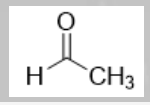
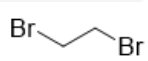
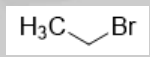
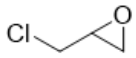
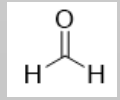
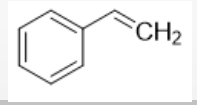
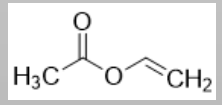
Vinyl Acetate

- **Genotoxic potential**
  - Vinyl acetate is not mutagenic in Ames, but genotoxic causing chromosomal damage
  - 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
    - Effects 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 oral cavity, esophagus and forestomach and oral cavity and lips in rats were targets
  - 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/d**
  AI = (758 mg/kg/d / 50,000) x 50 kg = 758 µg/day

Mutagenic impurities and AIs and/or PDEs included in the 2nd 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)

Conclusions

- **ICH M7(R2) restructures the format of the guideline by dividing it into two linked documents**
  - First document is the main guideline including a table of all compounds, including the seven new mutagenic impurities with monographs 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 to lifetime in main M7 guideline text**
- **Seven new monographs for mutagenic impurities added plus one Note (Note 2) to explain derivation of AI for ethyl bromide**
- **Table with monographed mutagenic impurities edited to include new monographs**
- **Grammatical editing and formatting e.g. updating URLs**

List of abbreviations

- **AI – Acceptable Intake**
- **ATSDR - Agency for Toxic Substances and Disease Control**
- **CPDB – Carcinogenicity Potency Database**
- **HPRT – Hypoxanthine-Guanine-Phosphoribosyltransferase**
- **NTP-TR – National Toxicology Program-Technical Report**
- **PDE – Permitted 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**

Contact

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

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