|  |  |
| --- | --- |
| **Action items** | 1) Include Background section addressing the context of the heme synthesis pathway and other relevant pathways/modulators. |
| **Intended changes** | * Add more context to background by describing where UROD lies within the heme biosynthesis pathway (similar to KE369 page) * Consider using wording from comment # 4 in Annex 2 (clinical vs. toxicological view) |
| **Changes** | Background section added. See text 1.1. |

**Text 1.1 Background section**

Heme is a cyclic tetrapyrrole cofactor containing Fe2+ porphyrin-containing ferroprotein that forms various hemoproteins such as hemoglobin, cytochromes and catalases [[62]](#cite_note-Thunell2000-4). Its biosynthesis mostly occurs in the liver and involves 8 separate steps. Porphyria is a disorder in which the disturbance of any of the steps of heme biosynthesis results in accumulation and excretion of porphyrins[[2]](https://aopwiki.org/events/369#cite_note-Kennedy1990-1). A variety of porphyrias exist depending on which enzyme in the pathway is deficient. This AOP describes a situation in which the 5th step of heme biosynthesis, uroporphyrinogen decarboxylase (UROD), which converts uroporphyrinogen to coproporphyrinogen, is inhibited.

Hepatic uroporphyria is viewed somewhat differently by clinicians and toxicologists. **For the former** it is mostly a sporadic disease (porphyria cutanea tarda; PCT) occurring sometimes in patients exposed to a variety of insults such as alcohol, estrogens, hepatitis viruses, HIV and on dialysis. Importantly, very early on it was found that lowering body iron stores by bleeding or now chelators causes remission [[61]](#cite_note-Thunell2000-4). In some northern European and US patients, carrying the hemochromatosis mutation is a risk factor but in other patients other iron susceptibility genes may contribute. Carrying a UROD mutation (lowering activity) is also a risk factor but still dependent on other susceptibility factors to see porphyria. To reproduce these findings experimentally has proved challenging but now possible. **For toxicologists** hepatic uroporphyria has mostly been seen as a toxic, but unique and curious endpoint of polychlorinated ligands of the AHR.  Experimentally, TCDD in mice is the most potent agent consistent with AHR mode of action but is more difficult in rats and other organisms. Hexachlorobenzene (HCB) has been greatly studied for its porphyria-inducing abilities and a large incident of porphyria in some young people in Turkey 60 years ago was ascribed to susceptible individuals who had consumed HCB. It is controversial whether HCB is a weak AHR ligand. Evidence of porphyria in people exposed accidentally or occupationally to accepted AHR ligands such as TCDD and PCBs is thin. Importantly, iron status can profoundly modify experimental uroporphyria induced by these chemicals especially in mice. In fact iron overload alone of mice will eventually produce a strong hepatic uroporphyria which is markedly genetically determined and toxicity can be ameliorated by chelators resembling PCT. Thus hepatic porphyria could alternatively be viewed as an iron AOP. At an overall level hepatic uroporphyria in animals and patients is the outcome of complex genetic traits and external stimuli in which in some traditional toxicological circumstances binding of a chemical to the AHR may have a major contribution[[67]](#cite_note-Thunell2000-4) but in others may not.

|  |  |
| --- | --- |
| **Action items** | 2) Examine the evidence demonstrating that AhR induced uroporphyria is modulated by iron, other cellular pathways (e.g. estrogen activation, other oxidative stress pathways) and antioxidants (ascorbic acid). |
| **Intended changes** | * Remove iron as a stressor and add as a modulating factor in KER868 * Include other potential modulating factors in discussion   + Levels of ascorbic acid negatively correlated with CYP1A2 induction (/activity?) (see text from comment #28)🡪 may help-explain diff. in strain susceptibility   + antioxidant capacity within a hepatic cell may be a confounding factor as to why some animals and humans are susceptible or resistant to ArH inducers * General increase in oxidative stress [i.e. lipid peroxidation, reactive oxygen species (ROS) and oxidized glutathione] due to TCDD is not dependent on functional CYP1A2 (Comment 28d), and may potentiate UROX. |
| **Changes** | * Iron was removed from the stressor list on the AOP main page and added to KER868 as a modulator. Ascorbic acid was also added. See text 2.1 * No relevant litterature was found to support the benefic effect of antioxidant on uroporphyria development. However, the involvement of cellular oxidative stress in UROX was added to the uncertainties of KER868. See text 2.2. |

**Text 2.1. Modulating factor of KER868**

Iron

Iron status can profoundly modify the level of uroporphyrin accumulation especially in mice. In fact iron overload alone of mice will eventually produce a strong hepatic uroporphyria which is markedly genetically determined and toxicity can be ameliorated by chelators [[15-16]](https://aopwiki.org/relationships/868#13-0). In human suffering from uroporphyrin accumulation, it was found that lowering body iron stores by bleeding or now chelators causes remission [[17]](https://aopwiki.org/relationships/868#13-0).

Cycling between the ferrous (Fe2+) and ferric (Fe3+) redox states allows Fe to catalyze the Haber-Weiss reaction, in which highly reactive •OH is generated from H2O2 and O2•−. Thus, by catalyzing the formation of reactive oxygen species, it is suggested that iron can increase the rate at which uroporphyrinogen is oxidized to uroporphyrin and therefore enhance uroporphyrin formation [[18]](https://aopwiki.org/relationships/868#13-0).

Ascorbic acid

Ascorbic acid (AA) can prevent uroporphyrin accumulation experimental uroporphyria, but only when hepatic iron stores are normal or mildly elevated [[19]](https://aopwiki.org/relationships/868#13-0). It was shown in chick embryo liver cells that AA could prevent uroporphyrin accumulation caused by treatment with 3,3',4,4'-tetrachlorobiphenyl and 5-aminole-vulinate by competitively inhibiting microsomal CYP1A2-catalyzed oxidation of uroporphyrinogen[[20]](https://aopwiki.org/relationships/868#13-0). Oppositely, in a spontaneous mutant rat that requires dietary AA, hepatic uroporphyrin accumulation caused by treatment with 3-methylcholanthrene or hexachlorobenzene was found to be enhanced when the animals were maintained on a very low AA dietary intake[[21]](https://aopwiki.org/relationships/868#13-0).

**Text 2.2. Involvement of ROS in UROX**

In mice, TCDD can elicit AhR-dependent, CYP1A1/A2-independent mitochondrial ROS production suggesting that general oxidative stress induced independently of CYP1A2 induction may contribute to the resulting overall UROX by TCDD [[14]](https://aopwiki.org/relationships/868#13-0).

|  |  |
| --- | --- |
| **Action items** | 3) Present a summary of the significant uncertainties and inconsistencies for this AOP on the main AOP131 specific page. |
| **Intended changes** | * Include summary of uncertainties on main AOP page that combines issues mentioned throughout KERs (specifically CYP1A2 essentiality/induction in humans, UROD inhibition in birds and potential alternate pathways)   + These will remain on individual KER pages, and simply summarized on the AOP main page. * Add PAHs to main AOP page as stressor * Add more details about stressors including characteristics of strong vs low affinity AHR agonists |
| **Changes** | * A summary of uncertainties was added to the main AOP page in the Evidence Assessment section. See text 3.1 * PAH was added to the main AOP page as stressor. |

**Text 3.1. Uncertainties**

Uncertainties

CYPs other than CYP1A2 are able of catalyzing uroporphyrinogen oxidation, raising doubts on the essentiality of CYP1A2 for this pathway. For instance, Phillips et al.[35] were able to generate mild uroporphyria in a Cyp1A2-/- mouse model that is genetically predisposed (Hfe-/-, Urod-/+) to develop porphyria.

The essentiality of CYP1A2 induction in human porphyria cutanea tarda is unclear. UROX activity in human liver microsomes was not correlated with CYP1A2 content[66]. Furthermore, there is contradictory evidence regarding the association between CYP1A2 polymorphism and susceptibility to porphyria cutanea tarda [63-64]. It may be possible that in patients with a genetic variation in UROD causing an inherent reduction in activity, the activity of CYP1A2 is less important.

UROD inhibition is not always observed and/or is less pronounced in avian models of porphyria, mainly in quail [47]. It is suggested that a mechanism other than UROD inhibition explain the extent of porphyrin accumulation in birds. Therefore, the applicability of this AOP to avian remains uncertain.

The characterization of the UROD inhibitor isolated by Phillips [38] has been criticized by Danton[66]. Therefore, UROD inhibitor has yet to be identified.

AhR binding stressors under certain conditions do not lead to adverse effect in particular mammalian strains[25].

|  |  |
| --- | --- |
| **Action items** | 4) Revisit the interpretation of the observations of porphyria in AhR-null mice in the context of KER868 and send a proposed modified text to reviewers before the end of this review. Discussion on iron as a modulating factor would also be useful in this context. |
| **Intended changes** | (KER868=CYP1A2 Induc🡪UROX)   * Elaborate on conditions of experiment in which mild porphyria observed in AHR-null mice (what were the potential driving forces?)   + Iron over-load + genetic predisposition in UROD gene (lower inherent enzyme activity) * Re-word to clarify that the CYP1A2 enzyme is necessary, but its induction is not. (i.e. not alternate CYP1A2-independent pathway, but alternate pathway independent if CYP1A2 induction🡪 may not be an alternate pathway at all, but the same pathway potentiated by iron; which will make more sense once iron is added as a modulating factor) |
| **Changes** | * I added some precisions about the effect of the triple knock-out. I also specified that this knock-out can induce UROX leading to porphyria. This precision (“leading to porphyria”) is important since UROX was repeatidly shown to occur in absence of CYP1A2. However, except for this specific triple knock-out experiment, CYP1A2 is required for the development of porphyria. See bold text 4.1. * The fact that UROX can occur in absence of CYP1A2 is detailed in the Uncertainties section of KER868. See text 4.2. |

**Text 4.1 Precision on conditions of experiment**

Phillips et al.[[11]](https://aopwiki.org/relationships/868#cite_note-Phillips2011-11) were able to generate uroporphyria in a Cyp1A2-/- mouse model that is genetically predisposed **(Hfe-/-, Urod-/+, which translates into intrinsic iron-overload and reduced UROD activity)** to develop porphyria in the absence of external stimuli; CYP1A2 knockout alone prevented porphyrin accumulation, but with the addition of iron and ALA to the triple knockout, modest porphyria was observed. Therefore, under extreme porphyric conditions, **UROX leading to porphyria** can occur in the absence of the CYP1A2 enzyme.

**Text 4.2. Uncertainties for KER868**

It is worth noting that Cyp1a2(-/-) knockout mice have up to 40% of the UROX activity of Cyp1a2(+/+) mice[[7]](https://aopwiki.org/relationships/868), suggesting that some UROX activity is CYP1A2-independent. Likewise, transfection of human Cyp1a1, Cyp3a4, Cyp3a5, or Cyp2e1 in insect cells resulted in UROX activity[[10]](https://aopwiki.org/relationships/868), suggesting that UROX can be catalyzed by other CYPs than CYP1A2 both in mouse and human. Additionally, iron overload or other induced pathways can potentially induce UROX[[13]](https://aopwiki.org/relationships/868#13-0). However, it was shown in mice that only CYP1A2-dependent UROX activity is associated with UROD inhibition[[7]](https://aopwiki.org/relationships/868). No such experiment was conducted in human; therefore, uncertainties remain for that species.

In mice, TCDD can elicit AhR-dependent, CYP1A1/A2-independent mitochondrial ROS production suggesting that general oxidative stress induced independently of CYP1A2 induction may contribute to the resulting overall UROX by TCDD [[14]](https://aopwiki.org/relationships/868#13-0).

Phillips et al.[[11]](https://aopwiki.org/relationships/868#cite_note-Phillips2011-11) were able to generate uroporphyria in a Cyp1A2-/- mouse model that is genetically predisposed (Hfe-/-, Urod-/+, which translates into intrinsic iron-overload and reduced UROD activity) to develop porphyria in the absence of external stimuli; CYP1A2 knockout alone prevented porphyrin accumulation, but with the addition of iron and ALA to the triple knockout, modest porphyria was observed. Therefore, under extreme porphyric conditions, UROX leading to porphyria can occur in the absence of the CYP1A2 enzyme.

Altogether, these results indicate that while CYP1A2 is a major catalysis of UROX activity, other CYPs and/or modulating factors are involved in the pathway.

|  |  |
| --- | --- |
| **Action items** | 5) Authors to propose changes in the Biological Plausibility section of KER865 that would include the uncertainty regarding in vivo evidence for uroporphyrinogen oxidation leading to UROD inhibition. |
| **Intended changes** | (865=UROX🡪UROD inhib.)   * Expand on uncertainties   + Not clear whether CYP1A2 directly or indirectly produces an UROD inhibitor via uroporphyrinogen oxidation, or reactive oxygen species generated from iron overload or other induced pathways can also potentially induce UROX. * Lack of evidence in vivo, in which other pathways may be more relevant |
| **Changes** | * Uncertainties were modified. See text 5.1. |

**Text 5.1. Uncertainties on KER865**

The precise mechanism of UROD inhibition has yet to be identified. It could be a direct or indirect inhibition via an oxidized uroporphyrinogen generated by CYP1A2 or reactive oxygen species derived from iron overload, or other induced pathways.

The characterization of the inhibitor isolated by Phillips et al.[[1]](https://aopwiki.org/relationships/865#cite_note-Phillips2007-1) has been criticized by Danton and Lim[[8]](https://aopwiki.org/relationships/865#cite_note-Danton2007-8). Namely, they claim that the high-performance liquid chromatography/electrospray ionization tandem mass spectrometry results were interpreted incorrectly. They analyzed the fragmentation pattern themselves, and concluded that the compound is not a tetrapyrrole or an uroporphyrinogen or uroporphyrin related molecule, but rather a poly(ethylene glycol) structure. The expected chemical instability of the inhibitor – a partially oxidized porphyrinogens that bear unsubstituted methylene group(s) at the meso position – might play an important role in the difficulty to characterize it [[9]](https://aopwiki.org/relationships/865#cite_note-Phillips2007-1).

Porphodimethene inhibitor 16 (PI-16), a synthetic inhibitor of UROD, was developed based on its similarity to coproporphyrinogen, uroporphyrinogen, and the previously suggested endogenous inhibitor of UROD [9]. This molecule directly interacts with UROD to specifically and effectively inhibit its activity. PI-16 structural similarity to an oxidized uroporphyrinogen including the suggested endogenous inhibitor supports the hypothesis of an oxidized uroporphyrinogen as endogenous UROD inhibitor.

|  |  |
| --- | --- |
| **Action items** | 6) Revisit the literature relevant to applicability of KER1070 (UROD inhib.🡪 HCP acc.) in various avian species and modify the relevant sections accordingly. |
| **Intended changes** | * Change WOE call for chicken to low or medium (revisit literature to determine) * Expand on text about possibility that this KER is not applicable to Quail (and potentially other birds) based on in Lambrecht study * Also consider re-visiting overall WOE call for birds on AOP main page (and expand in taxonomic applicability text). |
| **Changes** | * The WOE call for chicken was changed to moderate. * The uncertainties were modified to include of possible explaination for the lack on consistency in UROD inhibition tested in avian. See text 6.1. * The overall WOE call for birds on AOP main page was modified for the Japanese quail that was set to Low. The other avain species are described as Moderate. The uncertainties regarding avians were added to the uncertainty section on the main AOP page. |

**Text 6.1. Uncertainties on KER1070**

Uroporphyrin accumulation in avian models is less consistently accompanied by decreased UROD activity, and when it does occur, it is less marked than in mammals[[13]](https://aopwiki.org/relationships/1070#cite_note-James1989-13)[[14]](https://aopwiki.org/relationships/1070#cite_note-Lambrecht1988-14). Although numerous studies show both a decrease in UROD activity and porphyrin accumulation in avian species, Lambrecht et al.[[14]](https://aopwiki.org/relationships/1070#cite_note-Lambrecht1988-14) reported the accumulation of porphyrins in chicken embryo hepatocytes and Japanese quail liver without a decrease in UROD activity. They also note that the modest reduction in UROD activity (often less than 50%) is not enough to explain the extent of porphyrin accumulation observed and suggests there may be another mechanism at play. Alternatively, the difference between avian and mammals in regard to UROD inhibition may lie in the time-course of the response rather than its mechanism (Lambrecht et al., 1990).

|  |  |
| --- | --- |
| **Action items** | 7) Reviewer to provide authors with the reference(s): (a) for description of HPLC methods for measurement of UROD activity, and (b) to support the change in the current paragraph that porphyrins are responsible for neuropsychiatric symptoms of porphyria. Authors to include the info in the AOP as appropriate. |
| **Intended changes** | * HPLC ref. received 🡪 add to methods section (UROD activity)   Francis and Smith (1983) Analytical Biochemistry 138:404-410   * Statement linking porphyrins to neuropsychiatric symptoms has been removed from KER866 (HCP accumulation🡪uroporphyria) and references updated accordingly. |
| **Changes** | * The method and its appropriate reference were added to KE845. See text 7.1. * Statement linking porphyrins to neuropsychiatric symptoms has been removed from KER866 (HCP accumulation🡪uroporphyria) and references updated accordingly |

**Text 7.1 Method to measure UROD activity**

Another method based on reverse-phase HPLC was developed[[15]](#cite_note-Jones2003-9). This assay system uses either uroporphyrinogen III or pentacarboxyporphyrinogen I as substrate and liver homogenate in sucrose treated with a suspension of cellulose phosphate as enzyme source.

|  |  |
| --- | --- |
| **Action items** | 8) Authors to consider including references provided by reviewers that would help support evidence throughout the AOP and provide feedback to reviewers before the end of review to ensure all critical aspects have been sufficiently covered. |
| **Intended changes** | * References have been received from reviewers * Francis and Smith (1984) Analytical biochemistry, **138**(2), 404-410. * Urquhart et al (1988) Biochem J. 253: 357-362 * Smith and Chernova (2006) Disruption of heme synthesis by polyhalogenated aromatics. Advances in Molecular toxicology Vol 3 Ch 6. * CYP Induction EURL ECVAM Validation project report * 🡪 Upon reading these references, it will be decided if and where they should be included throughout the AOP. |
| **Changes** | * Francis and Smith (1985) was cited in the method section of KE845. See reference 15 on the KE page. * Smith and Chernova (2006) was cited at the end of the background section. See reference 67 in text 1.1 * EURL ECVAM Validation project report was cited in the method section of KE 850. The method was described. See text 8.1. |

**Text 8.1 Measurement of CYP1A2 induction**

### LC/MS-MS

The European Union Reference Laboratory for Alternatives to Animal Testing (EURL-ECVAM) is working on a human hepatic in vitro metabolically competent test systems to evaluate CYPs induction. Cryopreserved human HepaRG® or cryopreserved human primary hepatocytes are incubated in presence of a potential CYP1A2 inducer and the identity and abundance of CYP1A2 product is evaluated using analytical HPLC (High Performance Liquid Chromatography) coupled with mass spectrometry (MS). HPLC is applied for concentration and purification of the product to be detected, whereas MS is applied for its specific quantification [[31]](https://aopwiki.org/events/850#cite_note-Garrison1996-29).

|  |  |
| --- | --- |
| **Action items** | 9) Abstract will be modified to better reflect the wider context of porphyrias and the enzymes involved. |
| **Intended changes** | * Include summary statement on context of UROD within heme biosynthesis pathway in Abstract. |
| **Changes** | * The abstract was modified to specify that uroporphyrinogen is uniquely induced by homozygous mutation in UROD or chemical exposure leading to UROD activity inhibition. See bold text in Text 9.1. A more descriptive link with the heme biosynthesis pathway was included in the background section. |

**Text 9.1 Abstract on AOP main page**

Hepatic uroporphyria is a disorder where the disturbance of heme biosynthesis results in accumulation and excretion of uroporphyrin, heptacarboxyl- and hexacarboxyl porphyrin: collectively referred to as highly carboxylated porphyrins (HCPs)[[1]](#cite_note-Fox1988-1)[[2]](#cite_note-Kennedy1990-2)[[3]](#cite_note-Kennedy1998-3). **The disorder is due to a homozygous mutation in uroporphyrinogen decarboxylase (UROD), an enzyme involved in the heme biosynthesis pathway** [**[4]**](#cite_note-Thunell2000-4)**, or may be chemically induced, which involves the inhibition of UROD.** This adverse outcome pathway (AOP) describes the linkages leading to chemically induced porphyria through the activation of the aryl hydrocarbon receptor (AHR), a ligand-activated transcription factor.  AHR activation leads to the induction of cytochrome P450 1A2, a phase I metabolizing enzyme, which in turn results in excessive oxidation of uroporphyrinogen.  This oxidation produces a UROD inhibitor, preventing the conversion of uroporphyrinogen to coprouroporphyrinogen and increasing the synthesis of the UROD inhibitor in a positive feedback loop.  The accumulation of uroporphyrinogen leads to its preferential oxidation and accumulation of HCP in various organs (Uroporphyria).  This AOP was developed in accordance with OECD guidelines and demonstrates a high degree of confidence as a qualitative AOP. The quantitative understanding of this AOP however is not yet complete, preventing the accurate prediction of uroporphyria from lower level key events.

|  |  |
| --- | --- |
| **Action items** | 10) Authors to modify the Regulatory Applicability section to reflect the discussion under agenda item 10. |
| **Intended changes** | * Uncertainties in relevance for human risk assessment (CYP1A2 involvement, sig. drop in UROD activity required to view clinical symptoms (70%)…inherent activity could play a large role dictating how much inhibition necessary) * Consider on-going efforts within EURL-ECVAM on tests for measurement receptor-mediated cyp1a2 induction.   + Report sent by reviewer to primary author   + Decision to include pending review of document |
| **Changes** | * The various uncertainties regarding CYP1A2 involvement in human were clearly stated in the uncertainty section. See text 3.1 and 4.2. * The EURL-ECVAM report on measurement of receptor-mediated cyp1a2 induction was included in KE850. See text 8.1. |

|  |  |
| --- | --- |
| **Action items** | 11) EAGMSTG to consider the future formatting of AOPs for external review. |
| **Intended changes** | * None |
| **Changes** | * None |

|  |  |
| --- | --- |
| **Action items** | 12) EAGMSTG and Wiki developers to consider including information on NON-applicability to particular species. |
| **Intended changes** | * None |
| **Changes** | * None |

|  |  |
| --- | --- |
| **Comment Annexe #2** | 11a) Abstract: Second sentence is incorrect. There are 8 enzymes of the pathway in liver and hepatic uroporphyria is really the consequence of only inhibition of UROD |
| **Intended changes** | * Corrected by addressing TC action items 1 and 9. |
| **Changes** | * Corrected by addressing TC action items 1 and 9. |

|  |  |
| --- | --- |
| **Comment Annexe #2** | 14) Table 3, Temporal concordance: Davies at al. 2008 results depicted incorrectly |
| **Intended changes** | * Add the corrected CYP1A2 values and include the relative porphyrin levels in the last column (0, 56, 677).   + The 20 and 270 refer to absolute values of porphyrins (from Figure 1A) and the text states that this is a 56 and 677-fold change, so effectively give the same information. * The UROD column will be left blank. |
| **Changes** | * The table was modified. See Table 14.1 |

**Table 14.1**



|  |  |
| --- | --- |
| **Comment Annexe #2** | 28) What is unclear from cited studies is how CYP1A2 induction and UROX ultimately lead to UROD inhibition? Is this a direct or indirect effect? |
| **Intended changes** | * Describe positive feedback loop in AOP main page (maybe in abstract, or in separate section)   + Add qualitative positive feedback loop in graphic representation of AOP (simply for explanatory purposes; will not involve the addition of any new KERs)   + Careful to mention uncertainty in UROD inhib for birds…ie. CYP1A2 induction enough to drive UROX. * Also include discussion of positive feedback look in appropriate KERs under the new section titled “Known Feedforward/Feedback loops influencing this KER”   + Mainly under KER1070 (UROD inhib.🡪 HCP acc.) since this isn’t a direct relationship * Potentially under KER865 and 868 |
| **Changes** | * A short description of a positive feedback loop was inserted in the abstract of the AOP. See text 28.1. * Uncertainties regarding UROD inhibition in avian was added to the main AOP page. See text 3.1. * A description of the positive feedback loop was added to KER865. See text 28.1. |

**Text 28.1 Positive feedback loop in the abstract**

Hepatic uroporphyria is a disorder where the disturbance of heme biosynthesis results in accumulation and excretion of uroporphyrin, heptacarboxyl- and hexacarboxyl porphyrin: collectively referred to as highly carboxylated porphyrins (HCPs)[[1]](#cite_note-Fox1988-1)[[2]](#cite_note-Kennedy1990-2)[[3]](#cite_note-Kennedy1998-3). The disorder is due to a homozygous mutation in uroporphyrinogen decarboxylase (UROD), an enzyme involved in the heme biosynthesis pathway [[4]](#cite_note-Thunell2000-4), or may be chemically induced, which involves the inhibition of UROD. This adverse outcome pathway (AOP) describes the linkages leading to chemically induced porphyria through the activation of the aryl hydrocarbon receptor (AHR), a ligand-activated transcription factor.  AHR activation leads to the induction of cytochrome P450 1A2, a phase I metabolizing enzyme, which in turn results in excessive oxidation of uroporphyrinogen.  **This oxidation produces a UROD inhibitor, preventing the conversion of uroporphyrinogen to coprouroporphyrinogen and increasing the synthesis of the UROD inhibitor in a positive feedback loop.** The accumulation of uroporphyrinogen leads to its preferential oxidation and accumulation of HCP in various organs (Uroporphyria).  This AOP was developed in accordance with OECD guidelines and demonstrates a high degree of confidence as a qualitative AOP. The quantitative understanding of this AOP however is not yet complete, preventing the accurate prediction of uroporphyria from lower level key events.

**Text 28.2 Positive feedback loop under KER865**

Induction of CYP1A2 increases its availability and consequently its ability to compete with UROD to oxidize uroporphyrinogen. At least one of these oxidation products is believed to be a competitive inhibitor of UROD. Therefore, UROD inhibition potentiates the oxidation of uroporphyrinogens by CYP1A2 to porphyrins leading to increased porphyrin accumulation and in turn UROD inhibition.

|  |  |
| --- | --- |
| **Comment Annexe #2** | 28) TCDD did elicit AhR-dependent, CYP1A1/A2-independent mitochondrial ROS production in mice suggesting that general oxidative stress induced independently of CYP1A2 induction may contribute to the resulting overall UROX by TCDD (Senft et al., 2002). |
| **Intended changes** | * Add this possibility under uncertainties/inconsistencies section in KER868 |
| **Changes** | * The text was added to the uncertainties in KER865. See text 28.2. |

**Text 28.2 Uncertainties in KER865**

In mice, TCDD can elicit AhR-dependent, CYP1A1/A2-independent mitochondrial ROS production suggesting that general oxidative stress induced independently of CYP1A2 induction may contribute to the resulting overall UROX by TCDD [[14]](https://aopwiki.org/relationships/868#13-0).