This document includes:

- **Comments received on AOP 202 following a request for endorsement by written procedure sent by the OECD Secretariat to the WNT and WPHA with the deadline of 10 June 2022,**
- **Responses from AOP 202 authors**

Expert affiliation/ Country	Page #	Expert Comments	Responses from AOP author
DE/BfR	GC[1]	This AOP is well-written, and we support the endorsement of this AOP for declassification and publication. We just have a few general comments to the authors for future consideration: 1. Currently, it seems like a "big jump" from the key event MLL chromosomal translocation to the adverse outcome infant leukaemia. Even though the weight of evidence of this KER (1331) is high, the mechanisms of how MLL chromosomal translocation leads to infant leukaemia are not clear. This is already mentioned in the AOP as an important uncertainty. Several molecular events (e.g. altered gene expression, overexpression of BCL-2) have been described under the Key Event Relationship Description of KER 1331. Further development of this AOP could consider adding more key events between MLL chromosomal translocation and infant leukaemia even if the weight of evidence is not as high. 2. This might facilitate development of future AOPs related to other forms of (childhood) leukaemia. The AOP aims at a rare disease infant leukaemia, therefore overall biological plausibility as well as the empirical is only considered moderate. It might have been more appropriate to focus on other types of leukaemia first which also seems to be more relevant from a regulatory point of view. Moreover, animal reference data are not available.	We thank DE/BfR for the interest on this AOP and for the comments. 1. This AOP is indeed a prototype for possible regulatory uses and stimulate further research in the field. During the development of this AOP we investigate the possibility of including additional KE but eventually this was not possible and we therefore listed knowledges steps or events in the biological pathway as important source of uncertainties. At the moment, beyond the MLL translocation, it would be arguably correct to include additional KEs for which the translation into the regulatory applicability and the ability to measure them is still to uncertain/complex. 2. We agree and the WG spent a lot of time initially to come to a more comprehensive AOP for the childhood leukaemia. EFSA published an external report and a summary of this effort is also

[1] GC : General comments

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		3. Although the data are not as extensive as etoposide, potential inhibitors of DNA topo II, e.g. doxorubicin, bioflavonoids, chlorpyrifos, or benzene, could also be included as stressors for some of the events of the AOP. The additional information from other DNA topo II inhibitors could strengthen the scientific knowledge on some of the events in this AOP.	included in the EFSA Scientific Opinion of the PPR Panel. EFSA WG concluded that there is no sufficient information to do an AOP on childhood lekaemia which was indeed the initial scope of the work. Therefore, during the development of the AOP, with the support of experts in the field, we decided to move to a more specific disease (IFL) where some critical KEs are canonical and use it a starting point for possible further development of AOP of interest to be used for the inclusion of epidemiological data in the process of hazard characterization 3. The list of the stressors in the AOP includes the one for which some empirical support exists. We focus on etoposide and we sponsored experimental work to check the relevance of chlorpyrifos. It remains difficult to strength the empirical support and we hope to come to more experimental work for testing chemicals for the MIE and MLL translocation. A response-response analysis for this KER would represent a relevant experimental step to strength the KER and the AOP overall.

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DE/BfR	GC	Prior to publication, please check and correct the PDF document (and/or in the AOP-Wiki) for spelling errors. Misspelled words were frequently found in the PDF document of this AOP. We mentioned some in this table, but it is not feasible or practical to list them all in this table.	Noted
DE/BfR	1 (Cover)	Spelling correction: For the short name, it is infant leukaemia (instead of leukaemian), correct? Please check and amend as necessary.	Addressed
DE/BfR	4	IFL vs. AML, and AML – could be discussed in more detail here	Because this is the abstract, only the age of this population was added
DE/BfR	4 (Abstract)	It seems that there are several transcription errors in the pdf file. For example, the first sentence of the abstract states that 1 in 106 newborns will develop infant leukaemia, but according to the AOP wiki website, 10^6 is the correct number. In addition, some words are missing in later paragraphs, e.g., in the table "Stressors" on page 10, the evidence for chlorpyrifos.	Noted and addressed
DE/BfR	4 (Abstract)	Please review the following editing corrections and amend as appropriate. Second paragraph: "Following these distinct features a **M**olecular Initiating Event (MIE), two Key Event**s** (KE) and an Adverse Outcome (AO) were identified. The MIE was identified as "**(remove space)**DNA topoisomerase II poisons (interfer**e**s with) topo II enzyme" and epidemiological studies suggest that exposure to topoisomerase ~~s 2~~ **II poisons** may be involved in generation of the two KEs, DNA double strand break and MLL chromosomal rearrangement."	Addressed

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		Third paragraph: "…agents promoting the ~~driver~~ **driving** genetic oncogenic event." Fourth paragraph, line 1: "…the anticancer drug etoposide can be considered as a model chemical for DNA topoisomerase **II** "poison"." Fourth paragraph, line 6: Instead of "tool compound" (term used more commonly in drug discovery; not really fit-for-purpose in this case), consider using rather "model compound" or "reference compound"? Fourth paragraph, line 11: "…additional elements are limiting the ~~strenght~~ **strength** of this AOP."	
DE/BfR	8	*pattern of genetic changes as observed in the IFL disease* abbreviation not introduced yet	Addressed
DE/BfR	8	*and eventually acute leukaemia by global (epi)genetic dysregulation* shouldn't epigenetic dysregulation become a key event as well even if there are knowledge gaps? Maybe it could be discussed why this hasn't been included in the AOP.	The epigenetic plasticity as a potential KE was discussed during the development of this AOP. It was considered more prudent and more in line with the current scientific knowledge to include the epigenetic plasticity in the uncertainties rather than a KE because of the lack of empirical data and by the fact that under the KE, MLL translocation, there is concomitant biological processes that would include the epigenetic modification but most of them are specific to each of the oncogenetic fusion proteins so far discovered as involved in the process (e.g.

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			AF4, AF6, AF9, AF10). The WG thought that epigenetic changes are not sufficiently substantiated to be included as a unique KE in this AOP at the moment should be considered as relevant part of the biological pathway but not necessarily of an AOP.
DE/BfR	9	In general, for the graphical representation of the AOP, the MIE is shown in green, KEs in orange, and the AO in red. Perhaps it might be good to change the colour of the "DNA double-strand break" box from green to orange for clarity purpose.	Noted
DE/BfR	10	In the stressors table the evidence for chlorpyrifos is missing	Addressed
DE/BfR	11	although the concentrations in **the corresponding** in vitro studies have been quite high	Addressed
DE/BfR	11	is based on small studies -> is based on a limited number of studies with few individuals only	Addressed
DE/BfR	11/ 12	Most of the substances mentioned in the three tables on pages 11 and 12 are not explained in the text. Furthermore, there is no link to the tables in the text. Therefore, the tables and their content seem a bit isolated. Perhaps it would be helpful to delete the substances that are not mentioned further in the text or to	Noted

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		somehow include them in the text with further explanations.	
DE/BfR	12	as detected by **the Fluorescence in situ hybridization** (FISH) assay	Addressed
DE/BfR	12	Etoposide was used a positive reference compound in these studies ~~**and it performed as expected**~~	Addressed
DE/BfR	12	For the sake of completeness, the results of Rodriguez-Cortez et al. 2020 (doi:10.2903/sp.efsa.2020.EN-1866) should be added in the section on chlorpyrifos.	Addressed
DE/BfR	12	Please insert the corresponding reference in the table "Environmental chemicals" in the last column after the aromatic compounds.	Addressed
DE/BfR	13	Please revise the typo in the following sentence: Topoisomerases are able to alter the topological state of the DNA and topoisomerases are important targets for many chemoterapeutic agent.	Addressed
DE/BfR	13	"*DNA topoisomerases II drugs, like doxorubicin and etoposide are therefore able to convert their target to DNA damaging chemicals."* It is not clear what is meant by this sentence. Perhaps the following revision	Addressed

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		could be used instead: *"For example, drugs that inhibit DNA topoisomerase II, such as doxorubicin and etoposide, can cause DNA damage."*	
DE/BfR	13	Tool chemical: shouldn't rather reference chemical be used here and throughout the document instead?	Addressed
DE/BfR	14	*MLL-AF4 fusion gene* AF4 has not been introduced	Addressed
DE/BfR	15	*the effect described by Lu et al. 2015 was not reproduced by Rodriguez* Which effect? Lu et al. is discussed on page 16, please rearrange	Addressed
DE/BfR	15	target cells **i**.e. the liver haematopoietic stem cell	Addressed
DE/BfR	17	it to the obligatory pathway to the adverse outcome of **infant** leukaemia.	Addressed
DE/BfR	20	AF9 and ENL have not been introduced, what is there biological function?	Addressed (translocated chromosome is now described in the text) They represent alternative fusion transcripts and are used

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			as part of the characterization of the translocation and of the disease
DE/BfR	20	*However, there is a specific need to execute these studies in an appropriate experimental system with a proper target cell within a proper molecular and physiological environment.* It is suggested to delete this rather broad sentence which applies for all types of studies	Addressed
DE/BfR	21	factor for the development of the A**O**	Addressed
DE/BfR	22	Please specify the standard genotoxicity test battery	Addressed
DE/BfR	22	representing an important uncertaintie~~s~~ for this AOP	Addressed
DE/BfR	23	*is an adequate and robust experimental model system* how would this look like?	Addressed

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DE/BfR	24	*This AOP is however indicating that the MIE and the KE1 can be measured in scientific and/or regulatory validated test assays*. Which are the validated assays or are promising ones to be validated?	addressed
DE/BfR	28	The text under heading 'Overview for Molecular Initiating Event' is partly redundant to the following sections and could be streamlined	Noted
DE/BfR	28	Etoposide quinone**, a metabolite of etoposide,** induces DNA cleavage	Addressed
DE/BfR	28	The catechol **metabolite** displayed properties	Addressed
DE/BfR	30	topoisomerase IIÞ and IIß On page 28 only IIß is mentioned, is this the only relevant isoform since it is active during development? Maybe the roles for the two isoforms in this AOP could be explained in more detail.	Noted: already described in the AOP. Mammalian cells are known to possess two isoforms of topo II, α and β; they are similar in primary structure and have almost identical catalytic properties in vitro (Austin and Marsh, 1998; Drake et al., 1987; Jenkins et al., 1992). Several lines of evidence suggest that topo IIα is the main isoform involved in mitotic processes. First, there is a positive correlation between the cellular concentration of topo IIα and the rate of cell proliferation (Drake et al., 1989). Second, the expression of topo IIα mRNA is higher

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			in tissues containing proliferating cells (Capranico et al., 1992). Third, the level of topo IIα protein peaks at G2/M phase during the cell cycle (Woessner et al., 1991) and, finally, topo IIα localizes to the centromeres and axes of metaphase chromosomes (De, 2002). By contrast, the function of topo IIβ at the cellular level remains obscure (Sakaguchi et al., 2001). Topo II inhibitors, such as 2,6-dioxopiperazines (ICRF-159 and ICRF-187) and epipodophyllotoxins (VP-16 and VM-26; Schneider et al., 1990), are commonly used to investigate the roles of topo II (Gorbsky, 1994); however, these drugs inhibit the enzymatic activity of both topo IIα and topo IIβ.
DE/BfR	31	are interfacial inhibitors **which bind selectively to interfaces as macromolecular machines assemble.**	Addressed
DE/BfR	31	Alternate Protocol -> alternate protocol covalent comple -> covalent complexes mewasuring -> measuring	Addressed
DE/BfR	31	In vivo complex enzyme assay An experimental description is only included here but not for the in vitro assays. It is suggested to shorten this paragraph.	Noted

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DE/BfR	41	The method uses ~~**long-distance inverse PCR (**~~LDI-PCR~~)~~	Addressed
DE/BfR	42	There are hyperlinks in one of the references which should be removed	Noted
DE/BfR	45	the extended one generation test (OECD **TG** 443)	Addressed
DE/BfR	45	*in the extended one generation test, no treatment is occurring during the early in-utero development phase in the carcinogenicity assay* unclear what is meant here, TG 443 is not a carcinogenicity assay. Moreover, the dosing also spans early development. Dosing for the parent generation in TG 443 is daily and begins at least 2 weeks before mating and continues for females until the end of weaning. Dosing for the F1 generation begins at weaning and continues until adulthood.	Addressed
DE/BfR	45/46	Since infant leukaemia is a rare disease, the regulatory relevance of the AO seems questionable.	Noted The author understand this comment and agrees that the AO is a rare disease. However, animal models for IFL are not existing and therefore the outcome of a chemically induced MLL translocation can likely only be tested at KE levels.In addition, MLL translocation is clearly a common

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			node to alternative AOs not described in this AOP (chemotherapy induced leukaemia) and genotoxicity per se should be considered as adverse.
DE/BfR	52	In vitro, a single-pulse of ETO induced DSBs measured ETO as abbreviation for etoposide is not consistently used throughout the document, suggested to remove	Addressed
DE/BfR	56	AF6 and AF10 are only introduced here (earlier on only AF4, AF9 and ENL were mentioned), AF10 is only mentioned here, shouldn't these be mentioned earlier on as well? Also their function should be briefly introduced.	Addressed