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► To cite this version:

Thomas Jaylet, Roel Quintens, Mohamed Abderrafi Benotmane, Jukka Luukkonen, Ignacia Braga Tanaka, et al.. Development of an adverse outcome pathway for radiation-induced microcephaly via expert consultation and machine learning. *International Journal of Radiation Biology*, 2022, 98 (12), pp.1752-1762. 10.1080/09553002.2022.2110312 . hal-03966042

HAL Id: hal-03966042

<https://hal.science/hal-03966042>

Submitted on 21 Jul 2023

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1 **Development of an Adverse Outcome Pathway for radiation-induced**
2 **microcephaly via expert consultation and machine learning**

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74 mice and human brain organoids, and (2) the identification of novel transcriptional biomarkers
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98 systems toxicology methods. He is also involved in the development of the AOP-helpFinder
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115 **Development of an Adverse Outcome Pathway for radiation-induced** 116 **microcephaly via expert consultation and machine learning**

117 **Background:** Brain development during embryogenesis and in early postnatal
118 life is particularly complex and involves the interplay of many cellular processes
119 and molecular mechanisms, making it extremely vulnerable to exogenous insults,
120 including ionizing radiation (IR). Microcephaly is one of the most frequent
121 neurodevelopmental abnormalities that is characterized by small brain size, and is
122 often associated with intellectual deficiency. Decades of research span from
123 epidemiological data on *in utero* exposure of the A-bomb survivors, to studies on
124 animal and cellular models that allowed deciphering the most prominent
125 molecular mechanisms leading to microcephaly. The Adverse Outcome Pathway
126 (AOP) framework is used to organize, evaluate and portray the scientific
127 knowledge of toxicological effects spanning different biological levels of
128 organizations, from the initial interaction with molecular targets to the occurrence
129 of a disease or adversity. In the present study, the framework was used in an
130 attempt to organize the current scientific knowledge on microcephaly progression
131 in the context of ionizing radiation (IR) exposure. This work was performed by a
132 group of experts formed during a recent workshop organized jointly by the
133 Multidisciplinary European Low Dose Initiative (MELODI) and the European
134 Radioecology Alliance (ALLIANCE) associations to present the AOP approach
135 and tools. Here we report on the development of a putative AOP for congenital
136 microcephaly resulting from IR exposure based on discussions of the working
137 group and we emphasize the use of a novel machine-learning approach to assist
138 in the screening of the available literature to develop AOPs.

139 **Conclusion:** The expert consultation led to the identification of crucial biological
140 events for the progression of microcephaly upon exposure to IR, and highlighted
141 current knowledge gaps. The machine learning approach was successfully used to
142 screen the existing knowledge and helped to rapidly screen the body of evidence
143 and in particular the epidemiological data. This systematic review approach also
144 ensured that the analysis was sufficiently comprehensive to identify the most
145 relevant data and facilitate rapid and consistent AOP development. We anticipate
146 that as machine learning approaches become more user-friendly through easy-to-
147 use web interface, this would allow AOP development to become more efficient
148 and less time consuming.

149 Keywords: Adverse Outcome Pathway; AOP; microcephaly; brain; development;
150 ionizing radiation

151 **Abbreviation list**

152	AOP(s)	Adverse Outcome Pathway(s)
153	AO	Adverse outcome
154	BH	Bradford Hill criteria
155	DDR	DNA damage response
156	DSB(s)	DNA double-strand break(s)
157	KE(s)	Key event(s)
158	KER(s)	Key event relationship(s)
159	Gy	Gray
160	HR	Homologous recombination
161	IR	Ionizing radiation
162	LET	Linear energy transfer
163	MIE	Molecular initiating event
164	NHEJ	Nonhomologous end-joining
165	NPP	Nuclear power plant
166	qAOP(s)	Quantitative Adverse Outcome Pathway(s)
167	RGCs	Radial glia cell(s)
168	Sv	Sievert
169	TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labeling
170	WoE	Weight of evidence
171	WG	Working group
172	ZIKV	Zika virus

173

174 **Epidemiological studies on IR-induced microcephaly**

175 Microcephaly is a medical condition characterized by a small head size less than
176 the expected average for age, gender and considered population. Based on the literature,
177 its definition may vary qualitatively (e.g. exclusion of other syndromes) or
178 quantitatively (2 or 3 standard deviations below the mean, which may also reflect

179 severity, with dependence on the chosen reference population) (Passemaid et al. 2013;
180 Becerra-Solano et al. 2021). Microcephaly is classified either as primary when brain
181 development is altered during embryogenesis, or acquired when the brain is normal at
182 birth but suffer damages postnatally resulting in impaired growth (Passemaid et al.
183 2013). Both cases result in reduced brain size/volume and is frequently associated with
184 intellectual disability (Woods 2004). Several risk factors have been identified for
185 microcephaly, including infections during pregnancy (e.g. syphilis, herpes simplex,
186 HIV, Zika), maternal exposure to ionizing radiation or chemicals (e.g. mercury, arsenic,
187 alcohol), pre- and perinatal injuries to the developing brain (trauma, hypoxia-ischemia)
188 and genetic abnormalities. This wide range of risk factors can partly explain the
189 variations in incidence rates observed in normal populations (from 1 to 150 cases per
190 100,000 birth) (Passemaid et al. 2013; Becerra-Solano et al. 2021). In addition, the
191 consideration of additional criteria (e.g. mental disability) as indicators of microcephaly,
192 can create even more disparities in the number of cases accounted in the
193 epidemiological cohorts from different populations. Recent studies dealing with
194 microcephaly are related to Zika virus (ZIKV) infection during gestation, where the
195 estimated prevalence rate is of 2.3% (95% CI = 1.0-5.3%) among all pregnancies
196 (Coelho and Crovella 2017).

197 Associations between microcephaly and ionizing radiation (IR) have first been
198 suspected from a series of early clinical observations of newborns with small head size
199 and ophthalmic defects with or without mental disability, when pregnant women were
200 exposed to pelvic irradiation (more than 10 mSv of X-rays) (Goldstein and Murphy
201 1929; Goldstein 1930) . Data from Atomic-bomb survivors of Hiroshima and Nagasaki
202 exposed *in utero* between 8-25 weeks of gestation showed statistically significant
203 associations between IR exposure and small head sizes (defined here as head

204 circumference inferior to two standard deviations below the sex and age specific mean
205 of the studied population of 1473 children) (Plummer 1952; Miller and Blot 1972;
206 Otake and Schull 1993; Otake and Schull 1998). Some craniostenosis cases were
207 potentially included which raised some controversy (Otake and Schull 1998). The most
208 radiosensitive period of embryonic development of the human brain was established as
209 between 8-15 weeks of gestation (Miller and Blot 1972; Otake and Schull 1998). More
210 recently, three papers reported increased prevalence rates of microcephaly (defined as
211 occipito-frontal circumferences of at least three standard deviations below the standard
212 for age and sex proposed by the World Health Organization,
213 <https://www.who.int/tools/child-growth-standards>) in children of women residing in
214 areas impacted by the Chernobyl fall-out of the Rivne Province of Ukraine at the time
215 of pregnancy. However, as these papers considered geographically aggregated data
216 rather than individual data (Wertelecki et al. 2014; Wertelecki et al. 2017; Wertelecki et
217 al. 2018), they currently provide a lower weight of evidence compared to the atomic
218 bomb survivor studies in Hiroshima and Nagasaki.

219 **Aims of the AOP working group on IR induced microcephaly**

220 The Adverse Outcome Pathway (AOP) framework allows the description of
221 events from the molecular initiation of the toxic effects to the adverse effects observed
222 in the organism at levels relevant for human and biota populations. It describes the
223 fundamental biological events from a Molecular Initiating Event (MIE) and propagation
224 through essential Key Events (KEs) across the different biological scales of
225 organization to result in an Adverse Outcome (AO). The Key Event Relationships
226 (KERs) represent links between the KEs and are supported by experimental data
227 evaluated as empirical evidence using modified (tailored) Bradford Hill (BH) criteria
228 (Becker et al. 2015). Thus, the AOP describes in a simple way the scientific knowledge

229 of the most critical toxic effects at different biological levels of organization from
230 molecular mechanisms to the occurrence of the disease or adverse effects of regulatory
231 or research focus (Villeneuve et al. 2014; OECD 2018). This concept can also be used
232 to model the combined effects caused by single- or mixtures of different stressors to
233 identify and quantify the links between the biological events and the health outcomes
234 (Beyer et al. 2014; Salbu et al. 2019).

235 The Organization for Economic Cooperation and Development (OECD)
236 launched in 2012 a program on the development and assessment of AOPs to support
237 chemical risk assessment. Through this program, guidance and a dedicated web-based
238 platform (www.aopwiki.org) emerged to facilitate AOP development and uptake from
239 the scientific and regulatory communities. As the development of AOP are relatively
240 new to the field of low dose radiation research (Chauhan et al. 2022), a workshop
241 dedicated to AOP of IR effects was organized jointly by the Multidisciplinary European
242 Low Dose Initiative (MELODI) and the European Radioecology Alliance (ALLIANCE)
243 on April 12-16, 2021. The aim was to foster interactions between radiobiologists,
244 radioecotoxicologists and other communities, including chemical toxicologists, in order
245 to define the particularities of the AOP when applied to the radiation field and prioritize
246 the development of AOP relevant for health and environmental effects (Chauhan et al.
247 2021). Here we report on the discussion of a working group (WG) dedicated to the
248 development and proposing of an AOP for fetal neurotoxicity resulting from IR
249 exposure. The WG is composed of scientists knowledgeable in the field of brain
250 development, low dose radiation research (including epidemiology), chemical toxicity
251 research and computational science. Developmental toxicity encompasses many
252 pathologies, and it was rapidly recognized that brain development is particularly
253 vulnerable to IR. Microcephaly provided a favorable case study as : i) the outcome

254 (decrease in brain size) is clearly defined and measurable, ii) epidemiological data is
255 available to demonstrate an increased risk of microcephaly following *in utero* exposure
256 to IR, iii) mechanistic understanding of the effects is well documented, and iv)
257 microcephaly was not documented in AOP-Wiki (<https://aopwiki.org>).

258 Discussions centered first on the exposure situation to IR. It was acknowledged
259 that most of the evidence, primarily epidemiological data, were obtained for acute
260 exposure at a relative low dose of IR (> 0.1 Gy) during embryonic development and that
261 most effects were studied using low LET IR, including both gamma and X-rays.
262 Virtually all data were obtained from human and rodent studies (mouse and rat),
263 although the existence of two studies on wild animals (monkey and birds in Fukushima
264 and Chernobyl, respectively (Møller et al. 2011; Hayama et al. 2017)) was
265 acknowledged, but not considered sufficiently supportive for expanding the taxonomic
266 application domains of the AOP. During the review of the available human data, the
267 group considered accidental *in utero* exposures to the dose > 0.1 Gy as the preferential
268 scenario, since occupational and environmental exposures are unlikely (Applegate et al.
269 2021). This dose is in the range of the threshold value for which therapeutic abortion
270 should be considered in human (0.1 Gy) (Nakagawa et al. 1997). The frequency and
271 severity of microcephaly is dose-dependent in epidemiological data and animal
272 experiments, but the dose-threshold of occurrence of microcephaly is different in human
273 and mouse (above 0.3 Gy in the mouse (Verreet, Verslegers, et al. 2016) and above 0.1
274 Gy for the human (Otake and Schull 1998)).

275 Data at a lower dose (< 0.1 Gy) are scarce, and microcephaly was proposed to be
276 an endpoint without dose threshold. To perform a comprehensive review, the group
277 decided to consider all available data irrespective of the dose, in order to detect potential
278 early molecular or cellular key events of microcephaly. It was also discussed that dose

279 and dose rates would be important for the development of quantitative AOPs (qAOP),
280 the quantitative characterization of stressor-response and response-response
281 relationships along an AOP (Conolly et al. 2017; Song et al. 2020; Moe et al. 2021),
282 although such effort was not undertaken in the present work. In this context, dose
283 dependencies were considered as potential strategy to assess the weight of evidence
284 (WoE) between adjacent KE events according to the modified Bradford-Hill criteria
285 (Becker et al. 2015).

286 The group considered different possible MIEs for the microcephaly AOP.
287 ‘Deposition of energy’ (found as MIE 1686 in AOP-Wiki) was proposed as a possible
288 consensus between the different WGs during the workshop (Chauhan et al. 2021). In the
289 context of microcephaly, such MIE can be linked directly to the first KE ‘increased
290 DNA damages’ for which compelling evidence of essentiality exist. A coherent parallel
291 can be made with an already existing MIE, ‘deposition of particles’, as ‘Deposition of
292 energy’ conveys the notion of interactions between photons (or high LET particles) and
293 living matter at the origin of the effects observed at the molecular level. However, this
294 MIE does not distinguish non-ionizing from ionizing radiation exposures, while the
295 body of evidence collected for microcephaly comprises only IR. Alternative proposed
296 MIE were ‘deposition of ionizing energy’ (MIE 1550) or ‘increase, DNA damages’
297 itself (KE 1194). However, for the sake of coherence and transposability with the
298 MELODI/Alliance WGs it was decided to keep ‘deposition of energy’ as the MIE for
299 microcephaly and highlighted that further discussion and harmonization of potential
300 MIEs could be relevant within the radiation community.

301 The integration of the effects observed at different levels of biological
302 organization, central for the AOP framework, was identified as closely linked to the
303 concept of systems biology. The existence of a high-throughput dataset describing the

304 effects of IR on gene expression leading to microcephaly in the mouse embryonic brain
305 (Mfossa et al. 2020) was discussed as an interesting approach to identify KEs and
306 characterize the KERs, but such analysis was beyond the reach of the group. Instead, the
307 screening of additional databases useful for AOP development, such as the Comparative
308 Toxicogenomics Database (CTD), GeneCards, AOP-Wiki and PubMed databases were
309 highlighted as useful approaches to capture and assess evidence systematically. The
310 integration of these data to the results of the scoping review, also performed by the WG,
311 was thus proposed as an innovative way to incorporate the existing knowledge to the
312 microcephaly AOP and ensure its comprehensive assembly.

313 **Identification of key events through weight of evidence considerations**

314 AOP development is based on the available knowledge from studies that support
315 each KE and KER. The analysis of the evidence collected in the scientific literature is a
316 tedious and challenging process made by experts that must understand, organize and
317 evaluate the WoE along the AOP. This process is at the basis of the mapping of the
318 knowledge made during the construction of AOPs, and is made through the effective
319 assessment of biological plausibility, causality and consistency of the empirical
320 evidence according to the modified Bradford-Hill criteria, as described by Becker *et al.*
321 (Becker et al. 2015). The WG performed the scoping review of the literature in several
322 steps: i) the constitution of a preliminary AOP (or AOP ‘skeleton’) based on experts’
323 knowledge, ii) consolidation of the AOP by evaluation of the scientific evidence, and
324 iii) proposition of a hypothetical AOP based on consensus among experts. The second
325 step is the heart of the compilation of evidence to develop AOPs. The WG decided to
326 prioritize the evaluation of scientific articles focusing on microcephaly induced by IR,
327 but, as these articles are limited in number, functional studies from genetic mutations
328 leading to microcephaly were also included. In addition, genetic studies on

329 microcephaly were considered important to provide evidence of essentiality and
330 causality between KEs. The group decided to focus pragmatically on the effects of IR
331 on neurogenesis, as a first step towards a more comprehensive and integrated evaluation
332 of the effects on other type of cells in the brain like oligodendrocyte, astrocyte or
333 endothelial cells implicated in the vascularization of the brain and transport across the
334 blood-brain barrier.

335 ***Increased DNA damage leads to TP53 activation and apoptosis of progenitors***
336 ***in the developing brain***

337 Controlling the number and differentiation of progenitor cells during brain
338 development is critical to generate the appropriate number of neurons (and other type of
339 cells) and to achieve normal expansion of the cerebral cortex (Noctor et al. 2004;
340 Kriegstein and Alvarez-Buylla 2009; Xing et al. 2021)(Pinson and Huttner 2021). The
341 best documented cause of primary microcephaly is the loss of radial glial cells (RGCs)
342 and intermediate progenitors by apoptosis (Baala et al. 2007; Sessa et al. 2008). Such
343 losses occur by the induction of genomic instability (Marthiens et al. 2013) and the
344 accumulation of unreparable DNA damages (through cell division) via mutation in
345 critical genes involved in the DNA damage response (DDR) (Buck et al. 2006;
346 O'Driscoll and Jeggo 2008; Zhou et al. 2013; Bianchi et al. 2017), or defective
347 chromosomal segregation during mitosis that leads to prolonged cell arrest and TP53-
348 mediated apoptosis (Chen et al. 2014; Insolera et al. 2014; Phan et al. 2021). In
349 addition, other mutations can lead to excessive and inappropriate TP53 activation in the
350 developing brain, ultimately leading to microcephaly (or microcephaly-associated
351 syndromes) because of increased apoptosis of neural progenitors (Bowen and Attardi
352 2019).

353 As a well-known mutagen, IR induces microcephaly. Compelling evidence from human

354 epidemiological studies and laboratory animals exposed *in utero* demonstrated the link
355 between radiation-induced DNA damage and apoptosis of progenitors in the mouse
356 cerebral cortex as the origin of microcephaly (Nowak et al. 2006; Saha et al. 2014;
357 Barazzuol and Jeggo 2016; Kashiwagi et al. 2018; Mfossa et al. 2020). Microcephaly is
358 observed after exposure throughout the neurogenesis period, from E11 to E17 in the
359 mice and between weeks 8 and 25 of gestation in humans (Verreet, Verslegers, et al.
360 2016), and is apparent in the domain of the low dose radiation (near 0.2 Gy for humans
361 and 0.3 Gy for the mouse). In both species, the decreased brain size was strongly
362 correlated to altered cognitive performance (Verreet, Rangarajan, et al. 2016; Verreet,
363 Verslegers, et al. 2016). Importantly, the central role of TP53-induced apoptosis was
364 demonstrated by transcriptome-wide expression studies of TP53 early transcriptional
365 response to radiation exposure in mouse (Quintens et al. 2015; Mfossa et al. 2020) and
366 zebrafish (Jaafar et al. 2013). Moreover, the partial rescue of the microcephaly
367 phenotype when conditional *Trp53* knock out mice are exposed to IR, provides a direct
368 causal link between radiation-induced apoptosis and microcephaly (Mfossa et al. 2020).

369 The evidence describing the effects of IR on DNA is already present in two different
370 AOP (<https://aopwiki.org>, AOP#272, AOP#293) and it is out of the scope of this work
371 to present the detailed mechanisms of DNA damages and repair (see for instance (Lu et
372 al. 2019; Tang et al. 2022)). However, it remain important to consider that during
373 neurogenesis, the repair of DNA double-strand breaks (DSBs) via nonhomologous end-
374 joining (NHEJ) or homologous recombination (HR) depend on the developmental stage,
375 as DSB in early progenitors (E13 in the mouse) are mainly repaired by HR, while
376 progenitors present at later stage requires NHEJ (Orii et al. 2006; Li et al. 2020). The
377 causality between the KE increased DSB – TP53 activation – apoptosis, is well
378 described and the biological plausibility is considered strong. Increased DSB and

379 apoptosis are two KEs already present in AOP-Wiki (respectively KE 1194 and KE
380 1262 or KE 1365), but TP53 activation was not yet proposed as a KE (except KE 1923
381 ‘Altered gene expression, TP53 dependent apoptosis pathway). Many other functional
382 studies evidenced a role of DDR and TP53-dependent responses similar to those elicited
383 by IR, suggesting the activation of identical underlying mechanisms (Pilaz et al. 2016;
384 Mao et al. 2016; Baumgartner et al. 2018; Shi et al. 2019; Keil et al. 2020).

385 Beside apoptosis, mitotic spindle defects (Shimada et al. 2016) and endoplasmic
386 reticulum stress (Passemar et al. 2019) were also implicated in microcephaly in the
387 context of IR exposure. Finally, data showed that TP53 have also a role in cell-fate
388 determination during neurogenesis, independent of its classical function in the induction
389 of apoptosis. Indeed, *in vivo* study using *Trp53* mutant mice demonstrated that Trp53
390 hyperactivation led to a precocious differentiation of RGC into neurons during
391 embryonic development (Liu et al. 2013; Mfossa et al. 2020). In this case, the pool of
392 RGC is depleted too early (Gruber et al. 2011), limiting the production of neurons
393 throughout neurogenesis and thereby contributing to a decreased brain size. Given all
394 these data, the WG considered TP53 activation as a genuine KE essential for the
395 appearance of microcephaly via two independent KE, namely apoptosis and premature
396 neuronal differentiation.

397 ***Oxidative stress***

398 Deposition of ionizing energy may induce oxidative stress, an imbalance between
399 oxidants and antioxidants in favor of the former. In addition, IR can directly damage
400 DNA but also indirectly through the production of reactive oxygen species (Kim et al.
401 2019). Oxidative stress produces mainly single-strand DNA breaks, whereas more
402 detrimental effects of radiation are thought to be contributed by the radicals formed

403 along the radiation track where they induce clustered DNA-damage (Sharma et al.
404 2016). Although oxidative stress has been associated with a variety of diseases, such as
405 cancer and cardiac diseases, there is currently only limited evidence of its involvement
406 in radiation-induced microcephaly. This limited evidence includes the occurrence of
407 oxidative stress and cell cycle arrest in hippocampal neuronal progenitor cells (Oh et al.
408 2013) and increased neuroinflammation in the brain of adult Wistar albino rats (Kale et
409 al. 2018). Although it is challenging to directly link a measure of oxidative stress to
410 DNA damage, some studies have demonstrated quite clearly a key role played by
411 oxidative stress in radiosensitivity and IR-induced DDR, which remain to be
412 investigated in the case of microcephaly (Hong et al. 2013; Yazlovitskaya et al. 2015)
413 (Nikitaki et al. 2015). In addition to acute DNA damage by radiation-induced oxidants,
414 several studies have found radiation to cause persistent oxidative stress (Datta et al.
415 2012; Cosar et al. 2012), which in turn might result in a prolonged increase in DNA
416 damage. Taken together, we acknowledge that oxidative stress might be involved in
417 parallel or as a complementary pathway that can be described by an AOP network, but
418 we did not include it as a KE in the AOP due to the low weight of evidence of this
419 process during microcephaly progression.

420 *Neuroinflammation*

421 Neuroinflammation is considered as a key component of the stress response in the brain
422 after exposure to various stressors in adults and during embryogenesis. It has therefore
423 been considered as a possible KE during the construction of the preliminary AOP for
424 microcephaly. Recent studies associating neuroinflammation and microcephaly are
425 related to ZIKV infection during gestation. Indeed, many studies highlighted that
426 infections by ZIKV during pregnancy is characterized by polyfunctional immune
427 activation associated with increased levels of inflammatory cytokines in the amniotic

428 fluid of ZIKV-positive pregnant women, including the presence of interferon-gamma-
429 inducible protein 10, interleukin-6 (IL-6) and IL-8, vascular endothelial growth factor
430 (VEGF), monocyte chemoattractive protein 1 (MCP-1) and granulocyte colony-
431 stimulating factor (G-CSF) (Ghouzzi et al. 2017). This inflammatory response is
432 associated with neuronal damages during neurogenesis, leading to microcephaly (Tappe
433 et al. 2016; Ornelas et al. 2017; Lima et al. 2019). In the context of IR, several studies
434 reported a microglial activation after exposure of embryonic, young or adult rodents to
435 IR, which elicited neuroinflammation associated with neuronal loss and cognitive
436 deficit (Quintens et al. 2015; Verreet, Verslegers, et al. 2016; Osman et al. 2020;
437 Constanzo et al. 2020). Gene expression changes in irradiated microglia of juvenile
438 mice highly overlap with those seen in the developing mouse brain and converge on the
439 TP53 pathway and inflammation (Osman et al. 2020; Mfossa et al. 2020). Importantly,
440 pharmacological inhibition of several cytokines can confer neuroprotection depending
441 on the stage of development and type of injury, highlighting the contribution of
442 neuroinflammation in the appearance of neurological disorders (Hagberg et al. 2015). In
443 addition there is a strong relationship between chronic inflammation and oxidative
444 damage after exposure to IR (Najafi et al. 2017; Yahyapour et al. 2018). Indeed, after
445 exposure to very high dose of IR (> 1Gy), necrosis is more common than apoptosis,
446 which leads to the release of damage-associated molecular patterns by necrotic cells
447 which stimulate more T-cells and dendritic cells, amplifying the production of reactive
448 oxygen species (ROS) and inflammatory mediators (Multhoff et al. 2011; Yahyapour et
449 al. 2018). In contrast, at lower doses of IR, macrophages detect apoptotic cells and can
450 release anti-inflammatory cytokines such as transforming growth factor beta (TGF- β)
451 that keep inflammation under control. Disruption of non-cell autonomous signaling like
452 TGF- β can have a role in the progression of microcephaly as they are also essential for

453 cortical migration during development (Zhang et al. 2016). The role of these factors in
454 the sense of bystander effects of radiation has so far not been demonstrated. The
455 interaction between inflammation and oxidative stress is thus complex. It is noteworthy
456 that infection of human neural progenitor cells by Zika virus (Ghouzzi et al. 2017),
457 irradiation of the mouse embryonic brain (Quintens et al. 2015) and mutation in the
458 DDR gene citron kinase (Di Cunto et al. 2000), all results in TP53-mediated apoptosis
459 of the developing neuronal progenitors (Quintens 2017). As radiation-induced apoptosis
460 triggers inflammation (Boyd et al. 2021), it is possible that neuroinflammation
461 contributes to microcephaly, but this process remains poorly documented and could also
462 be considered as secondary compared to apoptosis. The WG decided thus to exclude
463 neuroinflammation from the list of possible KE until a causative role has been
464 established.

465 **Radiation-induced microcephaly, test-case application of a machine learning** 466 **method**

467 Given the enormous amount of existing knowledge in the scientific literature and other
468 data sources, the identification of relevant biological information to build AOP is a
469 complex and time-consuming task, especially in deciphering sparse data. Thus, to assist
470 in the AOP development process, the WG discussed that the use of artificial intelligence
471 (AI) and automated data mining methods can represent a complementary approach to
472 the expert manual review of the available data. From this perspective, an innovative AI
473 tool, called AOP-helpFinder, that combines graph theory and text mining, was
474 developed to automatically explore the scientific abstracts from the PubMed database
475 (Carvaillo et al. 2019; Jornod et al. 2021). This tool was previously successfully applied
476 in several studies to identify and extract associations between stressors (bisphenol S,
477 bisphenol F and pesticides) and KEs of interest (Carvaillo et al. 2019; Rugard et al.

478 2020; Jornod et al. 2021). Epidemiological studies linking microcephaly and IR
479 exposures being sparse in the literature, and then difficult to identify, we used the AOP-
480 helpFinder tool to quickly identify and extract, in a systematic manner, all published
481 existing information that could help AOP development. To do so, the user-friendly
482 AOP-helpFinder webserver was applied using the key words ‘ionizing radiation’ and
483 biological events ‘Microcephaly’ and ‘Small head size’ (<http://aop-helpfinder.u-paris->
484 [sciences.fr/](http://aop-helpfinder.u-paris-sciences.fr/), accessed on October, 19), with the options “Reduced search: 20%” and
485 “Refinement filter: yes”, in order to extract as much information as possible. Doing so
486 the co-occurrence of ‘ionizing radiation’ with ‘Microcephaly’ or ‘Small head size’ in
487 the abstract of PubMed database was scanned. Other phenotypical terminology such as
488 brain atrophy, gliosis, or reduced neurogenesis were considered less precise. After
489 automatic computational exploration of 125,226 abstracts related to IR from PubMed
490 database, the AOP-helpFinder tool allowed to gather 47 articles associating IR and
491 microcephaly (or small head size). Among these 47 links, a manual curation of the
492 abstracts allowed to keep only the 9 epidemiological articles dealing with exposure to
493 IR in humans. All these 9 publications were fully evaluated and discussed in the WG.
494 Two types of epidemiological studies were discriminated, either related to A-bomb
495 survivors in Hiroshima and Nagasaki (Plummer 1952; Miller and Blot 1972; Otake and
496 Schull 1998) or exposures after the Chernobyl NPP accident (Wertelecki et al. 2014;
497 Wertelecki et al. 2017; Wertelecki et al. 2018). After removing potential redundancy
498 within these studies (including metanalysis and confirmational studies), the WG
499 proposed to incorporate this knowledge via non-adjacent KER between the MIE ‘energy
500 deposition’ and microcephaly, as these studies demonstrated a clear link between the
501 two events, but without intermediate KEs.

502 **Preliminary AOP based on expert consultation**

503 Based on the discussion in the WG, it was possible to propose a preliminary AOP
504 related to radiation-induced microcephaly (Figure1).

505 The MIE (MIE1686, Deposition of Energy) leads to the increased DNA damage at the
506 molecular scale (KE 1194, Increase, DNA damage), which is considered in this AOP as
507 the main route by which IR exerts their effects at higher levels of biological
508 organization. The large amount of data describing the link between IR and DNA
509 damages already present in AOP-Wiki, and the additional evidences gathered in the
510 context of neurogenesis (Table1), led to WG to propose a ‘High’ WoE for this KE.
511 ‘Activation of TP53’ (KE 1974) is proposed as a novel essential KE. The existing KE
512 1923 ‘altered gene expression, P53 dependent apoptosis pathway’ was not used as it is
513 restricted to apoptosis. This KE is first supported by a time course analysis by
514 immunoblot of phosphorylated TP53 on (E14) primary mouse striatal stem irradiated
515 acutely to 1 Gy and 2 Gy of X-rays (Kashiwagi et al. 2018). The authors also quantified
516 TP53BP1 by immunofluorescence and describe that the peak of DNA damages (10 min
517 after irradiation) precedes, as expected, the peak of TP53 activation (3h to 5h after
518 irradiation). Similar data were obtained by Limoli *et al.* (Limoli et al. 2004), but with
519 higher dose (5 Gy). In the context of microcephaly, the link between increased DNA-
520 damages and TP53 activation was demonstrated by immunofluorescence using γ H2Ax
521 and TP53BP1 on brain cryosections and immunoblotting against phosphorylated TP53
522 in *Citk*^{-/-} E14.5 mouse embryos (Bianchi et al. 2017; Mfossa et al. 2020). Together, this
523 WoE for ‘Activation of TP53’ (KE 1974) was considered high. TP53 activation is
524 necessary for the induction of two possible cellular outcomes in the developing brain,
525 increased apoptosis (KE1365, ‘Increase apoptosis’) or ‘Premature cell differentiation’
526 (KE 1979), which could both contribute to ‘microcephaly’ (AO 1978). The WG

527 identified the KE1365, 'Increase apoptosis' (already present in AOP-Wiki), with high
528 level of confidence in the context of brain development, as described by analysis using
529 cleaved Caspase-3 (CC3) (Shimada et al. 2016; Mfossa et al. 2020), TUNEL assay
530 (Saha et al. 2014; Barazzuol and Jeggo 2016; Bianchi et al. 2017; Mfossa et al. 2020) on
531 mouse brain cryosections, western blotting (Kashiwagi et al. 2018) and microscopic
532 analysis of pyknotic nuclei (Nowak et al. 2006; Etienne et al. 2012). The KE 'Premature
533 cell differentiation' (KE 1979) was supported by immunostaining of the neuronal
534 markers on mouse brain cryosections (Liu et al. 2013; Mfossa et al. 2020). We noted the
535 existence of the KE 1560 'altered differentiation', but considered that this KE was not
536 adequate as altered differentiation can also lead to defective cell function and this term
537 does not convey the notion of temporality during development. In addition, both IR-
538 induced apoptosis and premature differentiation were ablated in conditional *Trp53*
539 knock out mouse, which adds to the WoE (Mfossa et al. 2020). But, together, as few
540 studies were identified, this WoE was considered moderate. Finally, the abundant
541 epidemiological data in the field of radiation is incorporated as a non-adjacent KER
542 between energy deposition and the adverse outcome (AOP 441, <https://aopwiki.org>).

543 The proposed AOP represents the foundation on which more dedicated work will be
544 needed. Indeed, despite the willingness of the WG to follow the modified Bradford-Hill
545 criteria to assess the WoE for the KER, this process was only initiated, and it was
546 evident that additional effort will be needed to compile the evidence in a systematic and
547 transparent manner. Additional potential KE were considered in the initial phase of the
548 AOP construction and might be considered for future development of the AOP towards
549 an AOP network. For transparency the complete list of the unused events is provided in
550 Supplementary Table 1.

551 **Conclusion**

552 In this work, we proposed an AOP for radiation-induced microcephaly and highlighted
553 the important molecular and cellular processes leading to this adverse outcome. The
554 WG considered accidental exposure to IR (> 0.1 Gy) of low LET energy (gamma and
555 X-rays) during pregnancy (between 8 and 25 weeks post-fertilization in human), as a
556 well-known risk factor for microcephaly. The effects of IR on RGs and intermediate
557 progenitors in the developing brain were considered as the main KEs leading to
558 microcephaly. The AOP proposed here is a simplistic representation of the known
559 mechanisms leading to microcephaly after exposure to IR. It relies on epidemiological
560 data and is completed by mechanistic evidences that support the relationship between
561 IR and microcephaly. As such, this AOP can facilitate the predictive extrapolation to
562 other animal species, given that the processes of neurogenesis are to a certain extent
563 similar and the KERs conserved, and can thus help understand the effects on brain size
564 observed on wild animals after the Fukushima and Chernobyl nuclear accidents (Møller
565 et al. 2011; Hayama et al. 2017).

566 Besides a classical knowledge gap in the field of low-dose (< 0.1 Gy) and low-dose rate
567 (< 5 mGy/h), the development of the AOP also emphasized the need for more studies on
568 cell fate after irradiation. Indeed, if apoptosis is well documented, the effects of IR on
569 premature neuronal differentiation, cell migration, cytokinesis defects and their link
570 with cellular senescence, oxidative stress and neuroinflammation remain scarce, as well
571 as their contribution to microcephaly. The WG also identified the effect on non-
572 neuronal tissues as a major gap, especially for the vascular system and microglia. More
573 functional analysis (i.e. mutants, transgenesis and other mechanistic data) are thus
574 required to delineate causes and consequences in the context of DNA damage induced
575 by IR. It is also noteworthy that most AOP related to DNA damage in the AOP-Wiki

576 database relate to cancer effects. If cancer is indeed a major outcome following
577 exposure to a relative low to moderate dose of IR, non-cancer effects (including
578 developmental effects but also cardiovascular or cognitive effects in adults) remain less
579 studied and should not be neglected. The final proposed AOP is relatively simple and
580 more dedicated work is required to assess WoE in a systematic and transparent manner
581 as well as identifying additional MIEs and KEs relevant for the same AO. This effort
582 would entail developing additional AOPs that collectively can form an AOP network
583 where the relevance of each AOP can be assessed in terms of developing quantitative
584 stressor-response and response-response relationships. In this regard, automated
585 analysis by machine learning approaches will help to screen the huge literature rapidly,
586 and permits consolidation of KEs, but limits still exists for this automated process as
587 different AO and KE terminology can affect the search. More generally, system biology
588 approaches including omics data will be useful to consolidate the molecular events of
589 the AOP, and will be of use by bringing a quantitative aspect to the AOP.

590

591 Acknowledgements:

592 This project was supported by funding from the Euratom research and training program 2019-
593 2020 under grant agreement No. 900009 (RadoNorm) ; the Research Council of Norway (RCN)
594 through its Centre for Excellence funding scheme (Project No. 223268 and project No. 268294
595 ‘MixRisk’) and NIVAS Computational Toxicology Program, NCTP (www.niva.no/nctp, RCN
596 project No. 160016).

597

598 Disclosure statement

599 All authors declare that they have no competing interests. The opinions expressed and
600 arguments employed herein are those of the authors and do not necessarily reflect the
601 official views of the OECD or of the governments of the OECD Member Countries.

602

603 References:

- 604 Applegate KE, Findlay Ú, Fraser L, Kinsella Y, Ainsbury L, Bouffler S. 2021.
605 Radiation exposures in pregnancy, health effects and risks to the embryo/foetus-
606 information to inform the medical management of the pregnant patient. *J Radiol Prot.*
607 41(4). <https://doi.org/10.1088/1361-6498/ac1c95>
- 608 Baala L, Briault S, Etchevers HC, Laumonnier F, Natiq A, Amiel J, Boddaert N, Picard
609 C, Sbiti A, Asermouh A, et al. 2007. Homozygous silencing of T-box transcription
610 factor EOMES leads to microcephaly with polymicrogyria and corpus callosum
611 agenesis. *Nat Genet.* 39(4):454–456. <https://doi.org/10.1038/ng1993>
- 612 Barazzuol L, Jeggo PA. 2016. In vivo sensitivity of the embryonic and adult neural stem
613 cell compartments to low-dose radiation. *J Radiat Res.* 57(Suppl 1):i2–i10.
614 <https://doi.org/10.1093/jrr/rrw013>
- 615 Baumgartner M, Olthof AM, Aquino GS, Hyatt KC, Lemoine C, Drake K, Sturrock N,
616 Nguyen N, al Seesi S, Kanadia RN. 2018. Minor spliceosome inactivation causes
617 microcephaly, owing to cell cycle defects and death of self-amplifying radial glial cells.
618 *Development.* 145(17):dev166322. <https://doi.org/10.1242/dev.166322>
- 619 Becerra-Solano LE, Mateos-Sánchez L, López-Muñoz E. 2021. Microcephaly, an
620 etiopathogenic vision. *Pediatr Neonatol.* 62(4):354–360.
621 <https://doi.org/10.1016/j.pedneo.2021.05.008>
- 622 Becker RA, Ankley GT, Edwards SW, Kennedy SW, Linkov I, Meek B, Sachana M,
623 Segner H, Van Der Burg B, Villeneuve DL, et al. 2015. Increasing Scientific
624 Confidence in Adverse Outcome Pathways: Application of Tailored Bradford-Hill
625 Considerations for Evaluating Weight of Evidence. *Regul Toxicol Pharmacol.*
626 72(3):514–537. <https://doi.org/10.1016/j.yrtph.2015.04.004>
- 627 Beyer J, Petersen K, Song Y, Ruus A, Grung M, Bakke T, Tollefsen KE. 2014.
628 Environmental risk assessment of combined effects in aquatic ecotoxicology: A
629 discussion paper. *Marine Environmental Research.* 96:81–91.
630 <https://doi.org/10.1016/j.marenvres.2013.10.008>
- 631 Bianchi FT, Tocco C, Pallavicini G, Liu Y, Vernì F, Merigliano C, Bonaccorsi S, El-
632 Assawy N, Priano L, Gai M, et al. 2017. Citron Kinase Deficiency Leads to
633 Chromosomal Instability and TP53-Sensitive Microcephaly. *Cell Rep.* 18(7):1674–
634 1686. <https://doi.org/10.1016/j.celrep.2017.01.054>
- 635 Bowen ME, Attardi LD. 2019. The role of p53 in developmental syndromes. *J Mol Cell*
636 *Biol.* 11(3):200–211. <https://doi.org/10.1093/jmcb/mjy087>
- 637 Boyd A, Byrne S, Middleton RJ, Banati RB, Liu G-J. 2021. Control of
638 Neuroinflammation through Radiation-Induced Microglial Changes. *Cells.* 10(9):2381.
639 <https://doi.org/10.3390/cells10092381>
- 640 Buck D, Malivert L, de Chasseval R, Barraud A, Fondanèche M-C, Sanal O, Plebani A,
641 Stéphan J-L, Hufnagel M, le Deist F, et al. 2006. Cernunnos, a novel nonhomologous
642 end-joining factor, is mutated in human immunodeficiency with microcephaly. *Cell.*
643 124(2):287–299. <https://doi.org/10.1016/j.cell.2005.12.030>

- 644 Carvaillo J-C, Barouki R, Coumoul X, Audouze K. 2019. Linking Bisphenol S to
645 Adverse Outcome Pathways Using a Combined Text Mining and Systems Biology
646 Approach. *Environ Health Perspect.* 127(4):47005. <https://doi.org/10.1289/EHP4200>
- 647 Chauhan V, Beaton D, Hamada N, Wilkins R, Burt J, Leblanc J, Cool D, Garnier-
648 Laplace J, Laurier D, Le Y, et al. 2022. Adverse outcome pathway: a path toward better
649 data consolidation and global co-ordination of radiation research. *Int J Radiat Biol.*:1–
650 10. <https://doi.org/10.1080/09553002.2021.2020363>
- 651 Chauhan V, Hamada N, Monceau V, Ebrahimian T, Adam N, Wilkins RC, Sebastian S,
652 Patel ZS, Huff JL, Simonetto C, et al. 2021. Expert consultation is vital for adverse
653 outcome pathway development: a case example of cardiovascular effects of ionizing
654 radiation. *Int J Radiat Biol.* 97(11):1516–1525.
655 <https://doi.org/10.1080/09553002.2021.1969466>
- 656 Chen J-F, Zhang Y, Wilde J, Hansen KC, Lai F, Niswander L. 2014. Microcephaly
657 disease gene *Wdr62* regulates mitotic progression of embryonic neural stem cells and
658 brain size. *Nat Commun.* 5:3885. <https://doi.org/10.1038/ncomms4885>
- 659 Coelho AVC, Crovella S. 2017. Microcephaly Prevalence in Infants Born to Zika Virus-
660 Infected Women: A Systematic Review and Meta-Analysis. *International Journal of*
661 *Molecular Sciences.* 18(8):1714. <https://doi.org/10.3390/ijms18081714>
- 662 Conolly RB, Ankley GT, Cheng W, Mayo ML, Miller DH, Perkins EJ, Villeneuve DL,
663 Watanabe KH. 2017. Quantitative Adverse Outcome Pathways and Their Application to
664 Predictive Toxicology. *Environ Sci Technol.* 51(8):4661–4672.
665 <https://doi.org/10.1021/acs.est.6b06230>
- 666 Constanzo J, Midavaine É, Fouquet J, Lepage M, Descoteaux M, Kirby K, Tremblay L,
667 Masson-Côté L, Geha S, Longpré J-M, et al. 2020. Brain irradiation leads to persistent
668 neuroinflammation and long-term neurocognitive dysfunction in a region-specific
669 manner. *Prog Neuropsychopharmacol Biol Psychiatry.* 102:109954.
670 <https://doi.org/10.1016/j.pnpbp.2020.109954>
- 671 Cosar R, Yurut-Caloglu V, Eskiocak S, Ozen A, Altaner S, Ibis K, Turan N, Denizli B,
672 Uzal C, Saynak M, et al. 2012. Radiation-induced chronic oxidative renal damage can
673 be reduced by amifostine. *Med Oncol.* 29(2):768–775. [https://doi.org/10.1007/s12032-](https://doi.org/10.1007/s12032-011-9870-7)
674 [011-9870-7](https://doi.org/10.1007/s12032-011-9870-7)
- 675 Datta K, Suman S, Kallakury BVS, Fornace AJ. 2012. Exposure to heavy ion radiation
676 induces persistent oxidative stress in mouse intestine. *PLoS One.* 7(8):e42224.
677 <https://doi.org/10.1371/journal.pone.0042224>
- 678 Di Cunto F, Imarisio S, Hirsch E, Broccoli V, Bulfone A, Migheli A, Atzori C, Turco E,
679 Triolo R, Dotto GP, et al. 2000. Defective neurogenesis in citron kinase knockout mice
680 by altered cytokinesis and massive apoptosis. *Neuron.* 28(1):115–127.
681 [https://doi.org/10.1016/s0896-6273\(00\)00090-8](https://doi.org/10.1016/s0896-6273(00)00090-8)
- 682 Etienne O, Roque T, Haton C, Boussin FD. 2012. Variation of radiation-sensitivity of
683 neural stem and progenitor cell populations within the developing mouse brain. *Int J*
684 *Radiat Biol.* 88(10):694–702. <https://doi.org/10.3109/09553002.2012.710927>

- 685 Ghouzzi VE, Bianchi FT, Molineris I, Mounce BC, Berto GE, Rak M, Lebon S, Aubry
686 L, Tocco C, Gai M, et al. 2017. ZIKA virus elicits P53 activation and genotoxic stress
687 in human neural progenitors similar to mutations involved in severe forms of genetic
688 microcephaly and p53. *Cell Death Dis.* 8(1):e2567.
689 <https://doi.org/10.1038/cddis.2016.446>
- 690 Goldstein L. 1930. RADIOGENIC MICROCEPHALY: A SURVEY OF NINETEEN
691 RECORDED CASES, WITH SPECIAL REFERENCE TO OPHTHALMIC DEFECTS.
692 *Archives of Neurology & Psychiatry.* 24(1):102–115.
693 <https://doi.org/10.1001/archneurpsyc.1930.02220130105010>
- 694 Goldstein L, Murphy DP. 1929. Microcephalic idiocy following radium therapy for
695 uterine cancer during pregnancy. *American Journal of Obstetrics and Gynecology.*
696 18(2):189–195. [https://doi.org/10.1016/S0002-9378\(29\)91057-7](https://doi.org/10.1016/S0002-9378(29)91057-7)
- 697 Gruber R, Zhou Z, Sukchev M, Joerss T, Frappart P-O, Wang Z-Q. 2011. MCPH1
698 regulates the neuroprogenitor division mode by coupling the centrosomal cycle with
699 mitotic entry through the Chk1-Cdc25 pathway. *Nat Cell Biol.* 13(11):1325–1334.
700 <https://doi.org/10.1038/ncb2342>
- 701 Hagberg H, Mallard C, Ferriero DM, Vannucci SJ, Levison SW, Vexler ZS, Gressens P.
702 2015. The role of inflammation in perinatal brain injury. *Nat Rev Neurol.* 11(4):192–
703 208. <https://doi.org/10.1038/nrneurol.2015.13>
- 704 Hayama S-I, Tsuchiya M, Ochiai K, Nakiri S, Nakanishi S, Ishii N, Kato T, Tanaka A,
705 Konno F, Kawamoto Y, Omi T. 2017. Small head size and delayed body weight growth
706 in wild Japanese monkey fetuses after the Fukushima Daiichi nuclear disaster. *Sci Rep.*
707 7(1):3528. <https://doi.org/10.1038/s41598-017-03866-8>
- 708 Hong Z, Kase Y, Moritake T, Gerelchuluun A, Sun L, Suzuki K, Terunuma T, Yasuoka
709 K, Kumada H, Anzai K, et al. 2013. Lineal energy-based evaluation of oxidative DNA
710 damage induced by proton beams and X-rays. *Int J Radiat Biol.* 89(1):36–43.
711 <https://doi.org/10.3109/09553002.2012.715791>
- 712 Insolera R, Bazzi H, Shao W, Anderson KV, Shi S-H. 2014. Cortical neurogenesis in
713 the absence of centrioles. *Nat Neurosci.* 17(11):1528–1535.
714 <https://doi.org/10.1038/nn.3831>
- 715 Jaafar L, Podolsky RH, Dynan WS. 2013. Long-term effects of ionizing radiation on
716 gene expression in a zebrafish model. *PLoS One.* 8(7):e69445.
717 <https://doi.org/10.1371/journal.pone.0069445>
- 718 Jornod F, Jaylet T, Blaha L, Sarigiannis D, Tamisier L, Audouze K. 2021. AOP-
719 helpFinder webserver: a tool for comprehensive analysis of the literature to support
720 adverse outcome pathways development. *Bioinformatics.*:btab750.
721 <https://doi.org/10.1093/bioinformatics/btab750>
- 722 Kale A, Piskin Ö, Bas Y, Aydin BG, Can M, Elmas Ö, Büyükuysal Ç. 2018.
723 Neuroprotective effects of Quercetin on radiation-induced brain injury in rats. *J Radiat*
724 *Res.* 59(4):404–410. <https://doi.org/10.1093/jrr/rry032>

- 725 Kashiwagi H, Shiraishi K, Sakaguchi K, Nakahama T, Kodama S. 2018. Repair kinetics
726 of DNA double-strand breaks and incidence of apoptosis in mouse neural
727 stem/progenitor cells and their differentiated neurons exposed to ionizing radiation. *J*
728 *Radiat Res.* 59(3):261–271. <https://doi.org/10.1093/jrr/rrx089>
- 729 Keil JM, Doyle DZ, Qalieh A, Lam MM, Funk OH, Qalieh Y, Shi L, Mohan N, Sorel
730 A, Kwan KY. 2020. Symmetric neural progenitor divisions require chromatin-mediated
731 homologous recombination DNA repair by Ino80. *Nat Commun.* 11(1):3839.
732 <https://doi.org/10.1038/s41467-020-17551-4>
- 733 Kim W, Lee S, Seo D, Kim D, Kim K, Kim E, Kang J, Seong KM, Youn H, Youn B.
734 2019. Cellular Stress Responses in Radiotherapy. *Cells.* 8(9):1105.
735 <https://doi.org/10.3390/cells8091105>
- 736 Kriegstein A, Alvarez-Buylla A. 2009. The glial nature of embryonic and adult neural
737 stem cells. *Annu Rev Neurosci.* 32:149–184.
738 <https://doi.org/10.1146/annurev.neuro.051508.135600>
- 739 Li Jingzheng, Shang Y, Wang L, Zhao B, Sun C, Li Jiali, Liu S, Li C, Tang M, Meng F-
740 L, Zheng P. 2020. Genome integrity and neurogenesis of postnatal hippocampal neural
741 stem/progenitor cells require a unique regulator Filia. *Science Advances.*
742 6(44):eaba0682. <https://doi.org/10.1126/sciadv.aba0682>
- 743 Lima MC, de Mendonça LR, Rezende AM, Carrera RM, Aníbal-Silva CE, Demers M,
744 D’Aiuto L, Wood J, Chowdari KV, Griffiths M, et al. 2019. The Transcriptional and
745 Protein Profile From Human Infected Neuroprogenitor Cells Is Strongly Correlated to
746 Zika Virus Microcephaly Cytokines Phenotype Evidencing a Persistent Inflammation in
747 the CNS. *Front Immunol.* 10:1928. <https://doi.org/10.3389/fimmu.2019.01928>
- 748 Limoli CL, Giedzinski E, Rola R, Otsuka S, Palmer TD, Fike JR. 2004. Radiation
749 response of neural precursor cells: linking cellular sensitivity to cell cycle checkpoints,
750 apoptosis and oxidative stress. *Radiat Res.* 161(1):17–27. <https://doi.org/10.1667/rr3112>
- 751 Liu H, Jia D, Li A, Chau J, He D, Ruan X, Liu F, Li J, He L, Li B. 2013. p53 Regulates
752 Neural Stem Cell Proliferation and Differentiation via BMP-Smad1 Signaling and Id1.
753 *Stem Cells Dev.* 22(6):913–927. <https://doi.org/10.1089/scd.2012.0370>
- 754 Lu H, Saha J, Beckmann PJ, Hendrickson EA, Davis AJ. 2019. DNA-PKcs promotes
755 chromatin decondensation to facilitate initiation of the DNA damage response. *Nucleic*
756 *Acids Research.* 47(18):9467–9479. <https://doi.org/10.1093/nar/gkz694>
- 757 Mao H, McMahon JJ, Tsai Y-H, Wang Z, Silver DL. 2016. Haploinsufficiency for Core
758 Exon Junction Complex Components Disrupts Embryonic Neurogenesis and Causes
759 p53-Mediated Microcephaly. *PLoS Genet.* 12(9):e1006282.
760 <https://doi.org/10.1371/journal.pgen.1006282>
- 761 Marthiens V, Rujano MA, Pennetier C, Tessier S, Paul-Gilloteaux P, Basto R. 2013.
762 Centrosome amplification causes microcephaly. *Nat Cell Biol.* 15(7):731–740.
763 <https://doi.org/10.1038/ncb2746>
- 764 Mfossa A-CM, Verslegers M, Verreet T, Fida H bin, Mysara M, IJcken WFJV, Vos
765 WHD, Moons L, Baatout S, Benotmane MA, et al. 2020. p53 drives premature neuronal

766 differentiation in response to radiation-induced DNA damage during early neurogenesis
767 [Internet]. [accessed 2022 Feb 22]:2020.06.26.171132.
768 <https://doi.org/10.1101/2020.06.26.171132>

769 Miller RW, Blot WJ. 1972. Small head size after in-utero exposure to atomic radiation.
770 *Lancet*. 2(7781):784–787. [https://doi.org/10.1016/s0140-6736\(72\)92145-9](https://doi.org/10.1016/s0140-6736(72)92145-9)

771 Moe SJ, Wolf R, Xie L, Landis WG, Kotamäki N, Tollefsen KE. 2021. Quantification
772 of an Adverse Outcome Pathway Network by Bayesian Regression and Bayesian
773 Network Modeling. *Integr Environ Assess Manag*. 17(1):147–164.
774 <https://doi.org/10.1002/ieam.4348>

775 Møller AP, Bonisoli-Alquati A, Rudolfson G, Mousseau TA. 2011. Chernobyl birds
776 have smaller brains. *PLoS One*. 6(2):e16862.
777 <https://doi.org/10.1371/journal.pone.0016862>

778 Multhoff G, Molls M, Radons J. 2011. Chronic inflammation in cancer development.
779 *Front Immunol*. 2:98. <https://doi.org/10.3389/fimmu.2011.00098>

780 Najafi M, Shirazi A, Motevaseli E, Geraily G, Norouzi F, Heidari M, Rezapoor S. 2017.
781 The melatonin immunomodulatory actions in radiotherapy. *Biophys Rev*. 9(2):139–148.
782 <https://doi.org/10.1007/s12551-017-0256-8>

783 Nakagawa K, Aoki Y, Kusama T, Ban N, Nakagawa S, Sasaki Y. 1997. Radiotherapy
784 during pregnancy: effects on fetuses and neonates. *Clin Ther*. 19(4):770–777.
785 [https://doi.org/10.1016/s0149-2918\(97\)80101-4](https://doi.org/10.1016/s0149-2918(97)80101-4)

786 Nikitaki Z, Hellweg CE, Georgakilas AG, Ravanat J-L. 2015. Stress-induced DNA
787 damage biomarkers: applications and limitations. *Front Chem*. 3:35.
788 <https://doi.org/10.3389/fchem.2015.00035>

789 Noctor SC, Martínez-Cerdeño V, Ivic L, Kriegstein AR. 2004. Cortical neurons arise in
790 symmetric and asymmetric division zones and migrate through specific phases. *Nat*
791 *Neurosci*. 7(2):136–144. <https://doi.org/10.1038/nn1172>

792 Nowak E, Etienne O, Millet P, Lages CS, Mathieu C, Mouthon M-A, Boussin FD.
793 2006. Radiation-induced H2AX phosphorylation and neural precursor apoptosis in the
794 developing brain of mice. *Radiat Res*. 165(2):155–164. <https://doi.org/10.1667/rr3496.1>

795 O’Driscoll M, Jeggo PA. 2008. The role of the DNA damage response pathways in
796 brain development and microcephaly: insight from human disorders. *DNA Repair*
797 (Amst). 7(7):1039–1050. <https://doi.org/10.1016/j.dnarep.2008.03.018>

798 OECD. 2018. Handbook supplement to the Guidance Document for developing and
799 assessing Adverse Outcome Pathways [Internet]. [https://www.oecd-](https://www.oecd-ilibrary.org/content/paper/5jlv1m9d1g32-en)
800 [ilibrary.org/content/paper/5jlv1m9d1g32-en](https://www.oecd-ilibrary.org/content/paper/5jlv1m9d1g32-en)

801 Oh SB, Park HR, Jang YJ, Choi SY, Son TG, Lee J. 2013. Baicalein attenuates
802 impaired hippocampal neurogenesis and the neurocognitive deficits induced by γ -ray
803 radiation. *Br J Pharmacol*. 168(2):421–431. [https://doi.org/10.1111/j.1476-](https://doi.org/10.1111/j.1476-5381.2012.02142.x)
804 [5381.2012.02142.x](https://doi.org/10.1111/j.1476-5381.2012.02142.x)

- 805 Orii KE, Lee Y, Kondo N, McKinnon PJ. 2006. Selective utilization of nonhomologous
806 end-joining and homologous recombination DNA repair pathways during nervous
807 system development. *Proc Natl Acad Sci U S A*. 103(26):10017–10022.
808 <https://doi.org/10.1073/pnas.0602436103>
- 809 Ornelas AMM, Pezzuto P, Silveira PP, Melo FO, Ferreira TA, Oliveira-Szejnfeld PS,
810 Leal JI, Amorim MMR, Hamilton S, Rawlinson WD, et al. 2017. Immune activation in
811 amniotic fluid from Zika virus-associated microcephaly. *Ann Neurol*. 81(1):152–156.
812 <https://doi.org/10.1002/ana.24839>
- 813 Osman AM, Sun Y, Burns TC, He L, Kee N, Oliva-Vilarnau N, Alevyzaki A, Zhou K,
814 Louhivuori L, Uhlén P, et al. 2020. Radiation Triggers a Dynamic Sequence of
815 Transient Microglial Alterations in Juvenile Brain. *Cell Rep*. 31(9):107699.
816 <https://doi.org/10.1016/j.celrep.2020.107699>
- 817 Otake M, Schull WJ. 1993. Radiation-related small head sizes among prenatally
818 exposed A-bomb survivors. *Int J Radiat Biol*. 63(2):255–270.
819 <https://doi.org/10.1080/09553009314550341>
- 820 Otake M, Schull WJ. 1998. Radiation-related brain damage and growth retardation
821 among the prenatally exposed atomic bomb survivors. *Int J Radiat Biol*. 74(2):159–171.
822 <https://doi.org/10.1080/095530098141555>
- 823 Passemard S, Kaindl AM, Verloes A. 2013. Chapter 13 - Microcephaly. In: Dulac O,
824 Lasseigne M, Sarnat HB, editors. *Handbook of Clinical Neurology* [Internet]. Vol. 111.
825 [place unknown]: Elsevier; [accessed 2022 Mar 3]; p. 129–141.
826 <https://doi.org/10.1016/B978-0-444-52891-9.00013-0>
- 827 Passemard S, Perez F, Gressens P, El Ghouzi V. 2019. Endoplasmic reticulum and
828 Golgi stress in microcephaly. *Cell Stress*. 3(12):369–384.
829 <https://doi.org/10.15698/cst2019.12.206>
- 830 Phan TP, Maryniak AL, Boatwright CA, Lee J, Atkins A, Tjihuis A, Spierings DC,
831 Bazzi H, Fojer F, Jordan PW, et al. 2021. Centrosome defects cause microcephaly by
832 activating the 53BP1-USP28-TP53 mitotic surveillance pathway. *EMBO J*.
833 40(1):e106118. <https://doi.org/10.15252/emboj.2020106118>
- 834 Pilaz L-J, McMahon JJ, Miller EE, Lennox AL, Suzuki A, Salmon E, Silver DL. 2016.
835 Prolonged mitosis of neural progenitors alters cell fate in the developing brain. *Neuron*.
836 89(1):83–99. <https://doi.org/10.1016/j.neuron.2015.12.007>
- 837 Pinson A, Huttner WB. 2021. Neocortex expansion in development and evolution—from
838 genes to progenitor cell biology. *Curr Opin Cell Biol*. 73:9–18.
839 <https://doi.org/10.1016/j.ceb.2021.04.008>
- 840 Plummer G. 1952. Anomalies occurring in children exposed in utero to the atomic
841 bomb in Hiroshima. *Pediatrics*. 10(6):687–693.
- 842 Quintens R. 2017. Convergence and divergence between the transcriptional responses to
843 Zika virus infection and prenatal irradiation. *Cell Death Dis*. 8(3):e2672.
844 <https://doi.org/10.1038/cddis.2017.109>

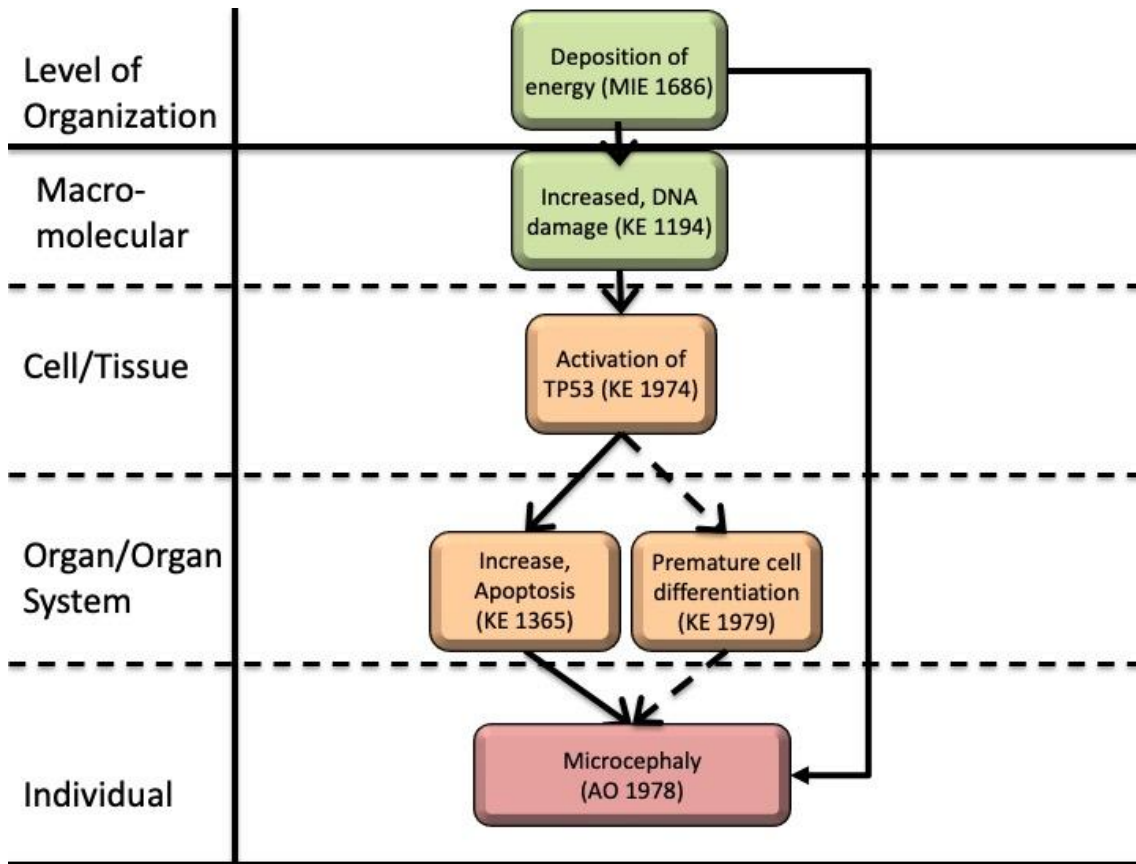
- 845 Quintens R, Verreet T, Janssen A, Neefs M, Leysen L, Michaux A, Verslegers M,
846 Samari N, Pani G, Verheyde J, et al. 2015. Identification of novel radiation-induced
847 p53-dependent transcripts extensively regulated during mouse brain development. *Biol*
848 *Open*. 4(3):331–344. <https://doi.org/10.1242/bio.20149969>
- 849 Rugard M, Coumoul X, Carvaillo J-C, Barouki R, Audouze K. 2020. Deciphering
850 Adverse Outcome Pathway Network Linked to Bisphenol F Using Text Mining and
851 Systems Toxicology Approaches. *Toxicol Sci*. 173(1):32–40.
852 <https://doi.org/10.1093/toxsci/kfz214>
- 853 Saha S, Woodbine L, Haines J, Coster M, Ricket N, Barazzuol L, Ainsbury E,
854 Sienkiewicz Z, Jeggo P. 2014. Increased apoptosis and DNA double-strand breaks in
855 the embryonic mouse brain in response to very low-dose X-rays but not 50 Hz magnetic
856 fields. *J R Soc Interface*. 11(100):20140783. <https://doi.org/10.1098/rsif.2014.0783>
- 857 Salbu B, Teien HC, Lind OC, Tollefsen KE. 2019. Why is the multiple stressor concept
858 of relevance to radioecology? *Int J Radiat Biol*. 95(7):1015–1024.
859 <https://doi.org/10.1080/09553002.2019.1605463>
- 860 Sessa A, Mao C, Hadjantonakis A-K, Klein WH, Broccoli V. 2008. Tbr2 Directs
861 Conversion of Radial Glia into Basal Precursors and Guides Neuronal Amplification by
862 Indirect Neurogenesis in the Developing Neocortex. *Neuron*. 60(1):56–69.
863 <https://doi.org/10.1016/j.neuron.2008.09.028>
- 864 Sharma V, Collins LB, Chen T, Herr N, Takeda S, Sun W, Swenberg JA, Nakamura J.
865 2016. Oxidative stress at low levels can induce clustered DNA lesions leading to NHEJ
866 mediated mutations. *Oncotarget*. 7(18):25377–25390.
867 <https://doi.org/10.18632/oncotarget.8298>
- 868 Shi L, Qalieh A, Lam MM, Keil JM, Kwan KY. 2019. Robust elimination of genome-
869 damaged cells safeguards against brain somatic aneuploidy following Knl1 deletion.
870 *Nat Commun*. 10(1):2588. <https://doi.org/10.1038/s41467-019-10411-w>
- 871 Shimada M, Matsuzaki F, Kato A, Kobayashi J, Matsumoto T, Komatsu K. 2016.
872 Induction of Excess Centrosomes in Neural Progenitor Cells during the Development of
873 Radiation-Induced Microcephaly. *PLoS One*. 11(7):e0158236.
874 <https://doi.org/10.1371/journal.pone.0158236>
- 875 Song Y, Xie L, Lee Y, Tollefsen KE. 2020. De Novo Development of a Quantitative
876 Adverse Outcome Pathway (qAOP) Network for Ultraviolet B (UVB) Radiation Using
877 Targeted Laboratory Tests and Automated Data Mining. *Environ Sci Technol*.
878 54(20):13147–13156. <https://doi.org/10.1021/acs.est.0c03794>
- 879 Tang J, Li Z, Wu Q, Irfan M, Li W, Liu X. 2022. Role of Paralogue of XRCC4 and
880 XLF in DNA Damage Repair and Cancer Development. *Front Immunol*. 13:852453.
881 <https://doi.org/10.3389/fimmu.2022.852453>
- 882 Tappe D, Pérez-Girón JV, Zammarchi L, Rissland J, Ferreira DF, Jaenisch T, Gómez-
883 Medina S, Günther S, Bartoloni A, Muñoz-Fontela C, Schmidt-Chanasit J. 2016.
884 Cytokine kinetics of Zika virus-infected patients from acute to convalescent phase.
885 *Med Microbiol Immunol*. 205(3):269–273. <https://doi.org/10.1007/s00430-015-0445-7>

- 886 Verreet T, Rangarajan JR, Quintens R, Verslegers M, Lo AC, Govaerts K, Neefs M,
887 Leysen L, Baatout S, Maes F, et al. 2016. Persistent Impact of In utero Irradiation on
888 Mouse Brain Structure and Function Characterized by MR Imaging and Behavioral
889 Analysis. *Front Behav Neurosci.* 10:83. <https://doi.org/10.3389/fnbeh.2016.00083>
- 890 Verreet T, Verslegers M, Quintens R, Baatout S, Benotmane MA. 2016. Current
891 Evidence for Developmental, Structural, and Functional Brain Defects following
892 Prenatal Radiation Exposure. *Neural Plast.* 2016:1243527.
893 <https://doi.org/10.1155/2016/1243527>
- 894 Villeneuve DL, Crump D, Garcia-Reyero N, Hecker M, Hutchinson TH, LaLone CA,
895 Landesmann B, Lettieri T, Munn S, Nepelska M, et al. 2014. Adverse outcome pathway
896 (AOP) development I: strategies and principles. *Toxicol Sci.* 142(2):312–320.
897 <https://doi.org/10.1093/toxsci/kfu199>
- 898 Wertelecki W, Chambers CD, Yevtushok L, Zymak-Zakutnya N, Sosyniuk Z,
899 Lapchenko S, Ievtushok B, Akhmedzhanova D, Komov O. 2017. Chornobyl 30 years
900 later: Radiation, pregnancies, and developmental anomalies in Rivne, Ukraine. *Eur J*
901 *Med Genet.* 60(1):2–11. <https://doi.org/10.1016/j.ejmg.2016.09.019>
- 902 Wertelecki W, Yevtushok L, Kuznietsov I, Komov O, Lapchenko S, Akhmedzhanova D,
903 Ostapchuk L. 2018. Chornobyl, radiation, neural tube defects, and microcephaly. *Eur J*
904 *Med Genet.* 61(9):556–563. <https://doi.org/10.1016/j.ejmg.2018.06.005>
- 905 Wertelecki W, Yevtushok L, Zymak-Zakutnia N, Wang B, Sosyniuk Z, Lapchenko S,
906 Hobart HH. 2014. Blastopathies and microcephaly in a Chornobyl impacted region of
907 Ukraine. *Congenit Anom (Kyoto).* 54(3):125–149. <https://doi.org/10.1111/cga.12051>
- 908 Woods CG. 2004. Human microcephaly. *Current Opinion in Neurobiology.* 14(1):112–
909 117. <https://doi.org/10.1016/j.conb.2004.01.003>
- 910 Xing L, Wilsch-Bräuninger M, Huttner WB. 2021. How neural stem cells contribute to
911 neocortex development. *Biochem Soc Trans.* 49(5):1997–2006.
912 <https://doi.org/10.1042/BST20200923>
- 913 Yahyapour R, Amini P, Rezapour S, Cheki M, Rezaeyan A, Farhood B, Shabeeb D,
914 Musa AE, Fallah H, Najafi M. 2018. Radiation-induced inflammation and autoimmune
915 diseases. *Military Medical Research.* 5(1):9. <https://doi.org/10.1186/s40779-018-0156-7>
- 916 Yazlovitskaya EM, Voziyan PA, Manavalan T, Yarbrough WG, Ivanova AV. 2015.
917 Cellular oxidative stress response mediates radiosensitivity in Fus1-deficient mice. *Cell*
918 *Death Dis.* 6:e1652. <https://doi.org/10.1038/cddis.2014.593>
- 919 Zhang F, Yu J, Yang T, Xu D, Chi Z, Xia Y, Xu Z. 2016. A Novel c-Jun N-terminal
920 Kinase (JNK) Signaling Complex Involved in Neuronal Migration during Brain
921 Development. *J Biol Chem.* 291(22):11466–11475.
922 <https://doi.org/10.1074/jbc.M116.716811>
- 923 Zhou Z-W, Tapias A, Bruhn C, Gruber R, Sukchev M, Wang Z-Q. 2013. DNA damage
924 response in microcephaly development of MCPH1 mouse model. *DNA Repair (Amst).*
925 12(8):645–655. <https://doi.org/10.1016/j.dnarep.2013.04.017>

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928 Figure 1. Description of the preliminary AOP: Ionizing radiation-induced DNA damage
929 leads to microcephaly via apoptosis and premature cell differentiation (AOP 441,
930 <https://aopwiki.org/aops/441>). MIE (Molecular Initiating Event), Key Events (KE),
931 Adverse Outcome (AO). Key event relationship with high and moderate confidence are
932 depicted respectively by solid and dashed lines.



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938 Table 1. Description of the Weight of Evidence (WoE) used for the preliminary AOP .
 939 MIE (Molecular Initiating Event), Key Events (KE), Adverse Outcome (AO). The
 940 event-identities (Event ID) in AOP-Wiki are indicated, as well as the bibliographical
 941 references used.

Type	Event ID	Title	Detection method	References	WoE
MIE	1686	Direct Deposition of Energy	Dosimetry	Chauhan V et al. 2021	High
KE	1194	Increase, DNA damage	Immunostaining Western Blot	Barazzuol and Jeggo 2016 Saha et al. 2014 Kashiwagi et al. 2018 Nowak et al. 2006 Mfossa et al. 2020 Bianchi et al. 2017	High
KE	1974	Activation of TP53	Immunostaining Western Blot	Kashiwagi et al. 2018 Limoli et al. 2004 Bianchi et al. 2017 Mfossa et al. 2020	High
KE	1365	Increase, Apoptosis	Immunostaining Western Blot TUNNEL assay Pycnotic nucleus	Shimada et al. 2016 Saha et al. 2014 Barazzuol and Jeggo 2016 Kashiwagi et al. 2018 Nowak et al. 2006 Mfossa et al. 2020 Bianchi et al. 2017 Etienne et al. 2012	High
KE	1979	Premature cell differentiation	Immunostaining	Mfossa et al. 2020 Liu et al 2013	Moderate
AO	1978	Microcephaly	Head size	Plummer 1952 Miller and Blot 1972 Otake and Schull 1998 Otake and Schull 1993 Shimada et al. 2016 Bianchi et al. 2017 Mfossa et al. 2020	High

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944 Supplementary Table 1. Description of the potential KE explored in the initial phase of
 945 AOP construction. The comment indicates how the potential KE was considered in the
 946 preliminary AOP after expert consultation.

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Term	KE in AOP wiki	Comment
Increased, oxydative stress	1088	Insufficient direct evidence
Oxidative Stress	1392	Insufficient direct evidence
Increase in reactive oxygen and nitrogen species (RONS)	1632	Insufficient direct evidence
Neuroinflammation	188	Insufficient direct evidence
Increase in inflammation	1633	Insufficient direct evidence
Increased DNA double breaks	1635	Related to DNA damage
Cell cycle arrest	None	Insufficient direct evidence
Cell cycle, disrupted	1505	Insufficient direct evidence
Defective cell division	None	Insufficient direct evidence
Defective spindle assembly	None	Insufficient direct evidence
Senescence	None	Insufficient direct evidence
Lipid peroxidation	1511	Insufficient direct evidence
Increase, Lipid peroxidation	1445	Insufficient direct evidence
Protein carbonylation	None	Insufficient direct evidence
Peptide Oxidation	209	Insufficient direct evidence
Mitochondria dysfunction	1816	Insufficient direct evidence
Altered gene expression, P53 dependent apoptosis pathway	1923	Difficulty to assess essentiality
Change in gene expression levels (omics data)	None	Difficulty to assess essentiality
Increased gliosis	None	Insufficient direct evidence
Brain atrophy	None	Related to microcephaly
Reduced brain size	None	Related to microcephaly
Reduced neurogenesis	None	Related to premature neuronal differentiation
General Apoptosis	1513	Related to Increase apoptosis
Neuronal death by apoptosis	None	Related to Increase apoptosis
Premature Neuronal differentiation	None	Related to Premature cell differentiation
Altered vascularization	None	Insufficient direct evidence
948 Impaired blood-brain transport	None	Insufficient direct evidence

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