AIR² Access to INFRASTRUCTURES for Radiation protection Research

Special Issue 7
March 2020

Editorial

Here comes the third part of our Special Issues dedicated to projects selected in CONCERT's calls for tenders. LDLensRad was selected during the first call for projects, with a duration of 3 years. This project tackles a priority topic for MELODI and EURAMED: non-cancer effects and in particular the effects of irradiation on the eye and cataract induction. This Issue is not organized around the Work Packages of the project but rather around the outstanding results that have been obtained. It should also be mentioned that LDLensRad has designed a very ambitious Data Management Plan. The partners have worked together with the STORE database, not only at the ultimate stage of data storage, but from the very beginning of the project to design the data architecture, ensure the best storage and potentially the best re-use of the data.

Dr Laure Sabatier, CEA

The floor to...

LDLensRad – Towards a full mechanistic understanding of low dose radiation cataracts

Human studies, for instance of the Atomic Bomb survivors, have led to the conclusion that the lens of the eye is more sensitive to ionising radiation (IR) exposure than previously thought. New, substantially reduced, dose limits came into force in Europe in early 2018. However, it is still very unclear how low dose IR might cause or be involved in development of cataracts. This is an important current public health issue, particularly for medical radiation workers, many of whom will need to amend their working practices despite a clear lack of understanding of the effects of low dose rate, low dose, IR exposure on the lens.

LDLensRad was a successful EJP CONCERT first call project, focused on the 'Improvement of health risk assessment associated with low dose/dose rate radiation.' The objective was to advance knowledge to solve the question of how radiation causes and/or promotes cataracts.

The aims were to answer the questions:

1) How does low dose radiation cause cataract?

2) Is there a dose rate effect?

3) How does genetic background influence cataract development after radiation exposure?

In addition, the shape of the dose response (in time), the nature of radiation induced cataract (deterministic or stochastic, or both), bioindicators of global response and training of early career scientists were important considerations for the project.

So, to the results. Firstly, we have clearly demonstrated that both dose and dose rate of IR are important in terms of how the lens of the eye responds to IR. Importantly, doses as low as < 500 mGy, were found to cause quantifiable changes in the lens. Further, the long-term studies clearly demonstrate that genetic background, age and sex are also involved in the response and that factors influence each other.

However, unanswered questions concerning mechanism, latency and threshold remain. Our data were obtained using animal and cellular models and human studies need to be carried out to better understand the mutual influence of these and other factors and, further, the implication that the current radiation protection legislation and guidance might need to be reviewed in due course.

Photo: Liz Ainsbury/PHE

LDLensRad Coordinator:
Elizabeth (Liz) Ainsbury, Senior Scientific Group Leader
PHE Cytogenetics and Pathology Group

Partners:
PHE, HMGU, ENEA, OBU, DU, and advisory board members: Nobby Hamada (CRIEPI, Japan), Joe Dynlacht (Indiana University, US), Larry Dauer (MSKCC, US), Rick Tanner (PHE, UK), Tamara Azizova (SUBI, Russian Federation);

Informal collaborators:
Gabriele Babini (UNIPV, Italy), Paul Schofield and Michael Gruenburger (UCam, UK)

This project has received funding from the Euratom research and training programme 2014-2018 under grant agreement No 662287

CONCERT CORNER

Future events:

28 Sept.-2 Oct. 2020
ERPW 2020, Estoril, Portugal
Deadline for abstract submission: 31 March

WP 6 News:

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Contents:

Long term studies

Mouse models: Scheimpflug imaging and associated data on cataract over the mouse lifetime

WP 1 focused on collection of long and short term data on a variety of endpoints associated with cataract. Here are described the results of long term studies focused on cataract development in the mouse models over an 18 month (mth) period.

Mice were exposed to doses of 0 to 2 Gy at an acute, high dose rate (0.3 Gy/min) or at a more protracted, low dose rate (0.063 Gy/min). The impact of genetic background was assessed using 6 strains of mice currently or previously used as models for radiation cataractogenesis were included in this study, as detailed in the figure below.

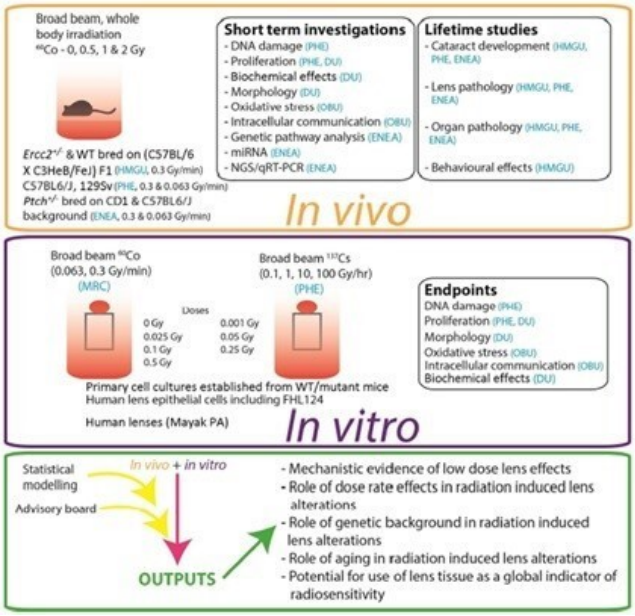


Photo: PHE

Summary of the work plan as initially proposed, role and interaction of the partners. WT= wildtype.

Mice were irradiated at 10 weeks (wk) old (mature lenses) and at neonatal age (postnatal day 2), in order to investigate the ageing effect in this particularly age-sensitive strain. The mice were then followed for up to 18 mth post exposure, with Scheimpflug imaging (Dalke et al., 2018) every 1 mth, together with Optical Coherance Tomography (OCT) and histology to track cataract appearance and development.

Irradiations and long term Scheimpflug imaging: at HMGU with wildtype (B6C3F1) and heterozygous mutant Ercc2$^{+/S737P}$ mice, 19 mth Scheimpflug data demonstrate no significant radiation-induced lens opacifications in 10 wk irradiated mice given doses of 0, 0.5, 1 and 2 Gy at 0.3 Gy/min. Strain, and the interaction of sex and strain, strain and dose were, however, statistically significant.

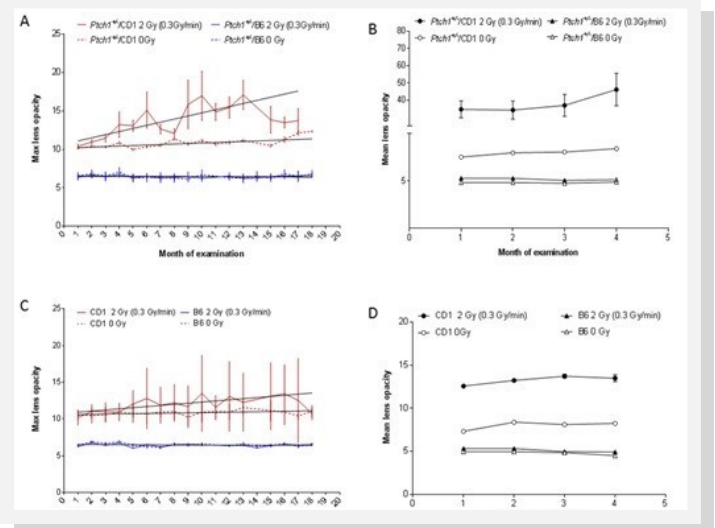
Data from the wildtype (WT) and Ptch1$^{+/-}$ mice on C57 (C57Bl6) and CD1 backgrounds irradiated at 10 wk and 2 days can be summarised as follows: Age related changes are clearly strain dependent. CD1 mice had more opacities than the C57 mice and, in contrast to the PHE C57 data, the C57 mice showed no increase in opacification with age over 18 mth of observation. There was a strain dependent dose rate effect, but in 10 wk mice no 'clinically significant' opacification was seen. In the P2 mice, there were significant strain dependent dose and dose rate effects resulting in opacification of the lens up to ~ 40% (likely vision impairing), which also markedly impacted survival.

OCT and histology data clearly show that OCT is the preferred modality for detection of PSC, compared with Scheimpflug imaging. New measurement techniques and new phenotypes have been defined and there is a clear effect of IR for posterior and anterior changes.

In the 10 wk mice, at 12 mth post irradiation, there appears to be a threshold of approximately 2 Gy for alterations in lens structure, however, by 20 mth the dose response is linear.

Visual acuity was impacted by radiation with sex and genotype influence. Only for female mutants was there a linear correlation between visual acuity and corneal clouding (opacification). This leads to further questions regarding the impact of radiation on vision in general – and, possibly, as the data can be interpreted to support a stochastic model for cataractogenesis, regarding the ICRP definition of detriment. Effects in female mice were greater than those in male mice throughout. C57 mice showed a similar dose response but no dose rate effect.

Photo: ENEA



Representative graphs of data obtained at ENEA showing the role of CD1 and C57 ('B6') genetic backgrounds with doses 0 or 2 Gy in the control of radiogenic lens opacification in Ptch1$^{+/-}$ mice. Scheimpflug data were presented in terms of temporal dynamics and statistically analyzed by linear regression curve fit (95% CI; best fit value using 2 parameters: y-intercept and slope). [A,C] Adult irradiation. [B,D] Neonatal irradiation.

ID Card:

Keywords:
Mouse models, cataract, ionising radiation, Scheimpflug imaging, OCT

Work Package leader:
Data presented here from HMGU and ENEA

Partners involved in WP1:
- PHE, United Kingdom
- HMGU, Germany
- ENEA, Italy
- DU, United Kingdom
- OBU, United Kingdom

Duration:
36 months

Total project budget:
~ 2.5M€

Infrastructures:
PHE, HMGU and ENEA Co-60 gamma irradiation facilities

Open Access of produced data:
Yes: https://www.storedb.org/store_v3//study.jsp?studyId=1111

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Related to:
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Lifetime study in mice after acute low-dose ionizing radiation: a multifactorial study with special focus on cataract risk, Dalke C., Neff F., Bains S. K., Bright S., Lord D., Reitmeir P., Rößler U., Samaga D., Unger K., Braselmann H., Wagner F., Greiter M. et al. (2018), Radiat Environ Biophys, 57 (2), 99-113

Spectral domain - Optical coherence tomography (SD-OCT) as a monitoring tool for alterations in mouse lenses, Pawliczek D., Dalke C., Fuchs H., Gailus-Durner V., Hrabě de Angelis M., Graw J., Amarie O. V. (2020), Exp Eye Res, 190, 107871

Short term endpoints

Mouse models: Mechanistic endpoints 4 hrs - 12 months post exposure

Further to the long term results described in the previous WP description, for each strain, dose and dose rate, lenses extracted from groups of mice were assessed for: initial DNA damage at 0, 4 and 24 hours (h) following exposure; intracellular communication, cell cycle effects, biochemical analyses and genetic pathway analyses at 0, 4 and 24 h, 4 and 12 mth; proliferative and morphological effects at 24 h, 4 and 12 mth; miRNA content using Next Generation Sequencing (NGS) at 4 h and qRT-PCR at 24 h, 4 and 12 mth.

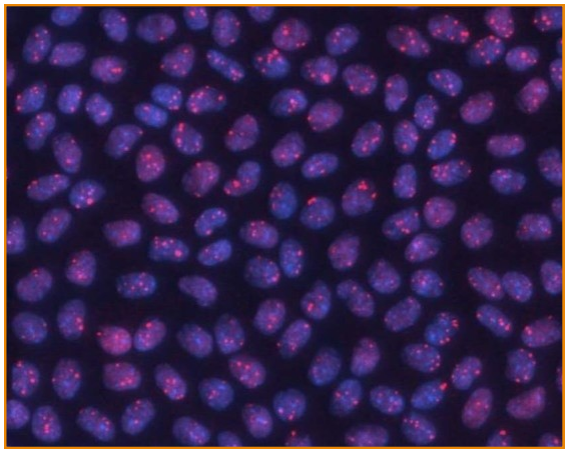

Photo: Adapted from Barnard et al., 2019

53BP1 foci in the nuclei of LEC located in the central region of the monolayer following 2 Gy irradiation (0.063 Gy/min) fixed and stained 4 h post-exposure.

In terms of initial DNA damage responses in the lens epithelial cells, a key output of the project is that an inverse dose rate effect for DNA damage (53BP1 foci) was observed.

The different mouse strains showed differential repair in terms of detection of 53BP1 foci at 4 and 24 h post-irradiation, indicating Ercc2$^{+/-}$ mice are either more efficient in DNA damage repair or there is less damage induced in the lens. The influence of single strand breaks was discussed together with a hypothesis around the potential role of growth factors as discussed in Barnard et al., 2019 and transcription factors for the XPD DNA damage repair protein, in which the Ercc2$^{+/-}$ mice are deficient. In lymphocytes, *in vivo*, repair was slower, perhaps suggesting differential repair mechanisms in the lens as compared to other tissues. Further investigation into this interesting phenomenon is still to be carried out.

To date inverse dose rate effects, whereby lower dose rates lead to larger effects, have only been reported at low doses in very few studies, these references should be reported with these data to prompt further investigation into the potential implications.

The proliferation analyses completed to date in the PHE and HMGU mice indicate that IR reduces proliferation and also cell density, with region of the lens epithelium (i.e., the central or peripheral areas), dose and dose rate all significantly contributing to the response.

The morphology data indicate slow, but dynamic, development of opacities associated with radiation exposures of 0.5 - 2 Gy, 12 mth post exposure, with clear evidence of lens repair too, and gender and strain effects, similar to those seen in other tissues.

Regarding the NGS carried out at ENEA, initial analysis of miRNome identified miRNA indicative of a variety of well known radiation responses.

The lipidomic and proteomic work addressed the hypothesis that IR causes oxidative stress, leading to protein carbonylation and oxysterol formation from cholesterol respectively, ultimately causing cataracts. Oxysterol levels were impacted with a trend of dose dependent increase, with differential responses in the lens nucleus and cortex.

Further analysis, including the *in vitro* work on the additional endpoints, is ongoing.

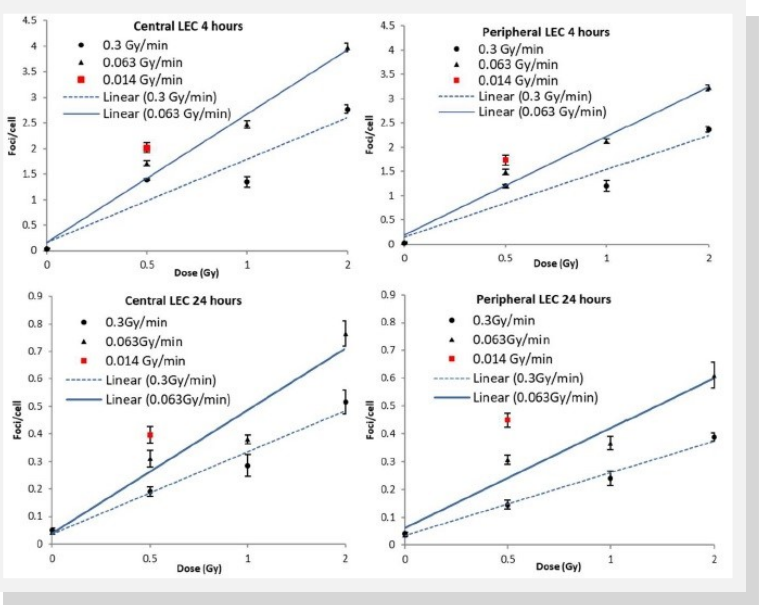

Photo: Adapted from Barnard et al., 2019

Mean 53BP1 foci/cell within central and peripheral region LEC (LEC) both 4 and 24 h post-irradiation to 0.5, 1 and 2 Gy (plus control) Co-60 γ-radiation at 0.3, 0.063 and 0.014 (0.5 Gy dose only as discussed) Gy/min dose rates. Note mean foci/cell axis is to a different scale at 24 h post-exposure compared to 4 h for ease of reading.

Inverse dose-rate effect of ionising radiation on residual 53BP1 foci in the eye lens, Barnard S. G. R., McCarron R., Moquet J., Quinlan R., Ainsbury E. (2019), Sci Rep, 9 (1), 10418

ID Card:

Keywords:
Mouse models, cataract, ionising radiation, DNA damage, 53BP1

Work Package leader:
Data presented here from Barnard et al., 2019

Partners involved in WP1:
- PHE, United Kingdom
- HMGU, Germany
- ENEA, Italy
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Open Access of produced data:
Yes: 10.20348/STOREDB/1112/1221

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Lens as an indicator of global radiosensitivity: behaviour and pathological effects

The wider effects in the mouse brain together with behavioural testing were carried out concurrently and, at the end of the long term study, with the aim of comparing the data with the lens results, to test the hypothesis that lens effects can be used as an indicator of global radiation effects.

Studies on behaviour of the HMGU mice was focused on spontaneous and cognitive behaviour assessed by open field, prepulse inhibition, social discrimination and y-maze following IR of the HMGU (ERCC2$^{+/-}$ and wt) mice at 10 wk to 0 – 2 Gy (0.3 Gy/min) at 4, 12 and 18 mth post irradiation (PI). The strongest dose-dependent IR effects were observed at 4 mth PI, but overall the responses were clearly also dependent on age, sex, genotype, with various factor interactions identified for the different endpoints. At 4 mth PI, visual acuity was not yet affected, indicating that it is not the lens which is driving behavioural alterations at this timepoint. The relationship with neurogenesis is likely to be important.

Irradiation effects on the mouse brain at ENEA are still under investigation, but the role of the sonic hedgehog (shh) pathway for wide regulation of cell growth and differentiation (and which is deficient in Ptch1$^{+/-}$ mice) is now much better understood. Key outputs include that doses of IR > 0.5 Gy significantly reduced survival of the CD1 background mice due to development of medulloblastoma (MB); this was not observed in any unirradiated mice. There were no significant effects of dose rate on survival or MB induction in the CD1 mice. In the C57 mice, IR reduced survival less significantly, and there was no MB induction above background in the P2 irradiated C57 mice. Genetic background was thus the dominating factor in MB development in this study.

In terms of neurogenesis, the results show a significantly slower rate of basal neurogenesis in C57 (-50%) compared to CD1 mice pointing to important genetic background related differences between the two mouse strains. No difference in the long-term response (4 mth PI) of the neuronal population of the dentate gyrus to adult irradiation with 2 Gy were observed at any of the two dose-rates in WT CD1 and C57 mice, although the genetic background-dependent differences were maintained (Figure below).

At 6 wk post exposure, impairment of Sox2 and Dcx populations was observed in the C57-Ptch1$^{+/-}$ mice only. It is thus concluded that sensitivity of Ptch1$^{+/-}$ mice to irradiation was strongly exacerbated on a C57 background both after neonatal or adult irradiation. The link between these observations and the NGS data are still being considered. Efforts will continue to attempt to understand the molecular mechanisms accounting for the different response to IR in Ptch1$^{+/-}$/CD1 and Ptch1$^{+/-}$/C57 mice.

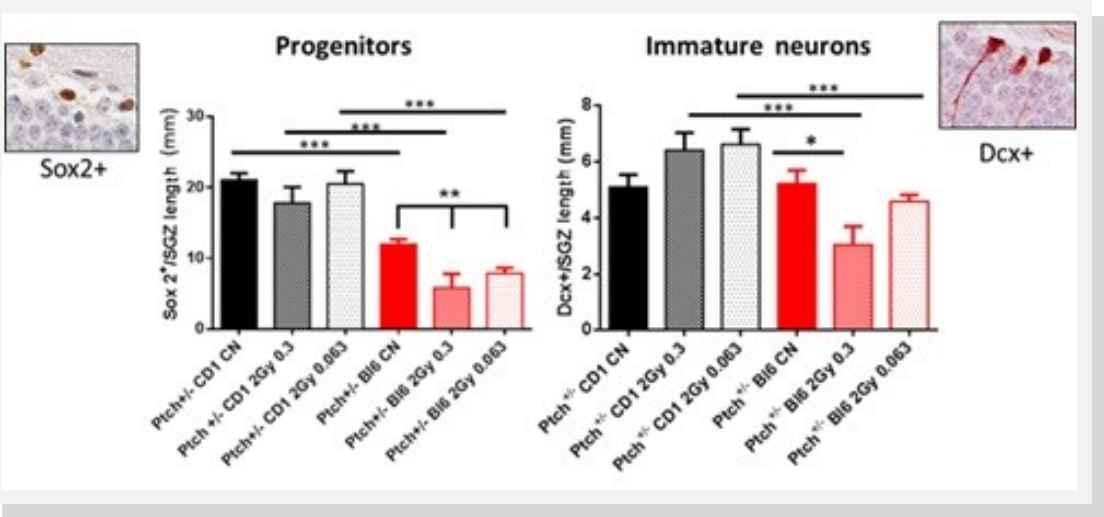

Photo: ENEA

Genetic background-related changes in neurogenesis in CD1-Ptch1$^{+/-}$ and C57 ('Bl6')-Ptch1$^{+/-}$ mice exposed to 2 Gy (0.3 Gy/min and 0.063 Gy/min) at 10 wk of age and examined 4 mth post-IR.

Dose-dependent long-term effects of a single radiation event on behaviour and glial cells, Ung et al., submitted

Long-term effects of radiation on neuroinflammation and behaviour in mice, Hölter et al. Presentation at Federation of European Neuroscience Societies Meeting, Glasgow, UK; 11.-15.7.2020

ID Card:

Keywords:
Mouse models, cataract, ionising radiation, behavioural studies, brain analysis

Work Package leader:
Dr Sabine M. Hölter, HMGU and Dr Mariateresa Mancuso, ENEA

Partners involved in WP1:
- PHE, United Kingdom
- HMGU, Germany
- ENEA, Italy
- DU, United Kingdom
- OBU, United Kingdom

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Open Access of produced data:
Yes: https://www.storedb.org/store_v3//study.jsp?studyId=1111

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Access to INFRASTRUCTURES

Issue	Exposure platforms	Databases, Sample banks, Cohorts	Analytical platforms, Models & Tools
Published to date:			
Oct 2015, #1	FIGARO	FREDERICA	RENEB
Nov 2015, #2	B3, Animal Contamination Facility	The Wismut Cohort and Biobank	The Hungarian Genomics Research Network
Dec 2015, #3	Pulex Cosmic Silence	STORE	METABOHUB
Feb 2016, #4	SNAKE	French Haemangioma Cohort and Biobank	Dose Estimate, CABAS, NETA
Mar 2016, #5	Radon exposure chamber	3-Generations exposure study	PROFI
Apr 2016, #6	Biological Irradiation Facility	Wildlife TransferDatabase	Radiobiology and immunology platform (CTU-FBME)
May2016, #7	CIRIL	Portuguese Tinea Capitis Cohort	LDRadStatsNet
Jun 2016, #8	Mixed alpha and X-ray exposure facility	Elfe Cohort	ERICA Tool
Jul 2016, #9	SCRS-GIG	RES^{3}T	CROM-8
Sep 2016, #10	Facility radionuclides availability, transfer and migration	INWORKS cohort	France Génomique
Oct 2016 #11	LIBIS gamma low dose rate facility ISS	JANUS	Transcriptomics platform SCKCEN
Nov 2016, #12	Microtron laboratory	EPI-CT Scan cohort	CATI
Dec 2016, #13	Nanoparticle Inhalation Facility	UEF Biobanking	The Analytical Platform of the PREPARE project
Feb 2017, #14	Infrastructure for retrospective radon & thoron dosimetry	Chernobyl Tissue Bank	HZDR Radioanalytical Laboratories
Special Issue 1	1st CONCERT Call: CONFIDENCE, LDLensRad, TERRITORIES	1st CONCERT Call: CONFIDENCE, LDLensRad, TERRITORIES	1st CONCERT Call: CONFIDENCE, LDLensRad, TERRITORIES
Mar 2017, #15	Alpha Particles Irradiator Calibration Laboratory at KIT		SYMBIOSE
Apr 2017, #16	Changing Dose rate (SU) Low dose rate (SU)		Advanced Technologies Network Center
May 2017, #17	Chernobyl Exclusion Zone	Chernobyl clean-up workers from Latvia	BfS whole and partial body Counting
Jun 2017, #18	MELAF	Belgian Soil Collection	INFRAFONTIER
Jul 2017, #19	MICADO'LAB	Estchern Cohort	ECORITME
Sep 2017, #20	DOS NDS		CERES
Oct 2017, #21	CALLAB Radon Calibration Laboratory		CORIF
Nov 2017, #22	Calibration and Dosimetry Laboratory (INTE-UPC)	German airline crew cohort	Centre for Omic Sciences (COS)

Future events:

CONCERT Short Courses

15-29 March 2020

Monitoring strategies applied in NORM involving industries – evaluation of occupational exposure and environmental impact,
Central Mining Institute, Katowice, Poland
Contact:
Boguslav Michalik

16-27 March 2020

Health effects induced by radiation and space conditions,
SCK•CEN Mol, Belgium
Contact:
Sarah Baatout

30 March 2020

EU CONCERT Radiation Protection Research Projects and UK NIHR HPRU in Chemical and Radiation Threats and Hazards Medical Radiation Theme - Final Stakeholder Dissemination Meeting,
Newcastle, United Kingdom
Contact:
Liz Ainsbury

20 April-1 May 2020

Assessment of long-term radiological risks from environmental releases,
Technical University of Denmark, Risø Campus, Denmark
Contact:
Kasper Andersson

18-29 May 2020

Modelling radiation effects from initial physical events,
University of Pavia, Italy
Contact:
Andrea Ottolenghi

Due to the COVID-19 outbreak you will need to verify the current status of each event!

See also on CONCERT website

Editorial Committee: Maria Panagiotopoulou, Jean-Michel Dolo, Laure Sabatier

Special Issue 7
March 2020

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Access to INFRASTRUCTURES

Issue	Exposure platforms	Databases, Sample banks, Cohorts	Analytical platforms, Models & Tools
Published to date:			
Dec 2017, #23	NMG	Techa River Cohort (TRC)	iGE3
Special Issue 2	MEDIRAD	MEDIRAD	MEDIRAD
Feb 2018, #24	UNIPI-AmBe	Greek interventional cardiologists cohort	SNAP
Special Issue 3	2nd CONCERT Call: LEU-TRACK, PODIUM, SEPARATE, VERIDIC, ENGAGE, SHAMISEN-SINGS	2nd CONCERT Call: LEU-TRACK, PODIUM, SEPARATE, VERIDIC, ENGAGE, SHAMISEN-SINGS	2nd CONCERT Call: LEU-TRACK, PODIUM, SEPARATE, VERIDIC, ENGAGE, SHAMISEN-SINGS
Mar 2018, #25	IRRAD	MARiS	BIANCA
Apr 2018, #26	Forest observatory site in Yamakiya	BBM	OEDIPE
May 2018, #27	Belgian NORM Observatory Site	The German Thorotrast Cohort Study	VIB Proteomics Core
Jun 2018, #28	CERF	Mayak PA worker cohort	Geant4-DNA
Jul 2018, #29	TIFPA	RHRTR	D-DAT
Sep 2018, #30	HIT	The TRACY cohort	COOLER
Oct 2018, #31	PTB Microbeam	The BRIDE platform	BRENDA
Nov 2018, #32	AGOR Facility at KVI-CART LNK		MARS beamline at SOLEIL
Dec 2018, #33	PARISII	The ISIBELa cohort	CIEMAT WBC
Feb 2019, #34	The MIRCOM microbeam	The ISE cohort	EFFTRAN
Special Issue 4	NSRL	LSAH & LSDA	GeneLab
Mar 2019, #35	IRSE Experimental Farm	The MWF database	DSA Environmental Laboratory
Apr 2019, #36	PG stack at Barreiro, Portugal	CONSTANCES	The MCDA Tool
May 2019, #37	LERF	IMMO-LDRT01 cohort	Radiochemical and Radioactive Analysis Laboratory (INTE-UPC)
Jun 2019, #38	FAIR	The BACCARAT study	CIEMAT *In Vitro* Internal Dosimetry Laboratories
Jul 2019, #39	AMBIC	LSS	LRM
Sep 2019, #40	FRM II	REQUITE	TU Dublin Analytical Platform
Special Issue 5	CONFIDENCE	CONFIDENCE	CONFIDENCE
Special Issue 6	PODIUM	PODIUM	PODIUM
Special Issue 7	LDLensRad	LDLensRad	LDLensRad

Future events:

Other Events

19-24 April 2020
ICRER: 5th International Conference on Radioecology & Environmental Radioactivity, Amsterdam, The Netherlands

19-24 April 2020
IM2020: International Conference on Individual Monitoring, Budapest, Hungary

5-8 May 2020
1st ISORED scientific and organisation meeting, Sitges, Spain

27-29 May 2020
6th NERIS workshop: Operational and research achievements and needs to further strengthen preparedness in emergency management, recovery and response, Barcelona, Spain

28 September-2 October 2020
ERPW2020: European Radiation Protection Week 2020, Estoril, Portugal
Deadline for abstract submission: 31st March 2020

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