In utero exposure and cancer in children

Report by Ian Jones of a workshop sponsored by Children with Cancer UK held at the Royal College of Physicians, London, UK, on 2–3 June 2014.

Executive summary

Prenatal genetic changes are known to be a cause of at least some childhood cancers, yet the mechanisms responsible for initiating cancer generally remain poorly understood. With some rare exceptions, the exposures triggering cancer development are also unclear.

The **placenta** plays a key role in protecting the fetus from noxious insults that might initiate cancer development. However, it is not a total barrier, and is also turning out to have its own biological functions that could influence the risk of cancer development. It is, for example, a significant source of fetal blood cells and produces melatonin, a potentially protective anti-oxidant.

There is good evidence that the 'first hit' triggering development of childhood **acute lymphoblastic leukaemia (ALL)** occurs *in utero*. It is possible that critical genetic changes occur in a specific B-cell precursor population found only during embryonic development.

Genetic analyses are revealing that ALL and other cancers consist of **families of related cell populations** with a common ancestor but with varying combinations of mutations. This has important implications for treatment, as elimination of some populations can create a space in which less-susceptible populations can proliferate, leading to recurrence of disease.

Genetic approaches have also been used to characterise the brain cancer **medulloblastoma**. Four types of medulloblastoma have been distinguished, varying in their cells of origins and genetic abnormalities, reflected in differing prognoses for patients.

Similarly, **embryonal tumours** such as neuroblastoma and Wilms' tumour are genetically diverse. Research is beginning to identify genetic changes associated with the transition from normal to cancerous cell, via proliferatively abnormal but non-malignant intermediates.

Nanoparticles have emerged as a potential source of prenatal damage. Work on nanoparticles of the type used in medical imaging suggests they do have the potential to harm placental cells and to cross the placental barrier.

Metal nanoparticles have been found to damage cells protected by a placental barrier. Notably, such particles do not actually have to pass through the barrier to harm cells, suggesting a 'bystander effect' in which placental cells are induced to release signals that damage other nearby cells.

Radionuclides are a well-known risk factor for cancer. Models have been developed to estimate fetal exposures based on radiation doses received by mothers. A large-scale study in Russia, following up offspring of mothers affected by radioactive pollution released from a plutonium-processing facility, may provide valuable information on exposure risks.

There is some epidemiological evidence that exposure to **road traffic pollution** is associated with a modest increase in risk of some childhood cancers. However, it is difficult to model exposures to airborne pollutants.

In the UK, levels of several traffic-associated airborne pollutants have dropped in recent years. However, there remains a dearth of monitoring information about certain airborne materials, particularly **ultrafine particles**.

Dietary constituents and the **products of food preparation processes** are a further possible source of carcinogenic exposure. The EU-funded NewGeneris programme has identified numerous biochemical signals associated with exposure to harmful products, and documented widespread changes in gene expression following exposure.

Alcohol may have the potential to damage the fetus, through its conversion to toxic acetaldehyde within mothers. Studies in mice have shown that the fetus is heavily reliant on the mother's aldehyde-metabolising system for protection.

Epidemiological studies have shown that **magnetic fields** (such as those generated by power lines) are associated with an increased risk of cancer, but to date experimental studies have not provided an accepted mechanism. A very large study in rats, based on exposure from before birth to natural death, suggests that exposure to magnetic fields in combination with other carcinogenic factors can increase the risk of some cancers.

Arsenic contamination of groundwater is associated with a wide range of long-term health issues, including cancer, and affects many millions of people worldwide, including in the UK. Arsenic's mechanisms of action, however, remain unclear. Studies in Thailand have identified genetic changes following arsenic exposure that could contribute to cancer initiation and/or promotion.

Evidence has emerged of links between disrupted **daily (circadian) rhythms** and cancer, such as a higher incidence of breast cancer in female shift workers. Potentially, disrupted sleep patterns in pregnant women could increase the risk of cancer in offspring through an indirect effect linked to abnormal hormonal control of appetite, excess weight gain and high birth weight (a known risk factor for childhood leukaemia).

In rats, disrupted light–day routines have been found to have a significant impact on **maternal metabolism** and hence the fetus. It is unclear if this has any effect on cancer risk in offspring.

The placenta has been found to be a major source of **melatonin** during pregnancy. This may reflect its anti-oxidant properties, and a role in protecting against oxidative stress. Clinical trials are testing whether melatonin

supplementation can reduce the risk of pre-eclampsia and fetal growth restriction.

Background

Recent decades have seen a steady increase in the number of cases of childhood cancer in the UK and other developed countries. Although this may in part stem from improved detection and diagnosis, it is also thought to reflect a genuine increase in underlying risk.

Like all cancers, childhood cancers reflect dysregulated control of cellular proliferation, due to underlying genetic abnormalities. Evidence is growing that the first mutations that set a cell on the path to childhood cancer (and indeed some adult cancers) occur in the womb: at birth, some infants are already at heightened risk of developing cancer in early life.

Embryonic development in the womb is therefore a period of vulnerability. Indeed, several factors have already been identified that affect the fetus and increase the risk of childhood cancer, particularly ionising radiation and chemical carcinogens such as diethylstilboestrol. Other potentially important factors have been suggested, yet their impact remains uncertain. And for the vast majority of childhood cancers, the initiating triggers remain unclear.

To provide a clearer picture, Children with Cancer UK organised an interdisciplinary international workshop to stimulate discussion on the impact of possible *in utero* exposures. The workshop had several key themes, including the role of the placenta as a protective barrier, the origins and genetic characteristics of key childhood cancers, the impact of potentially important chemical and other exposures and to identify gaps in the knowledge and produce a future research agenda.

In utero origins of cancer

The placenta is a critical protective barrier between mother and fetus. **John Aplin** (Manchester) provided a brief introduction to its basic biology.

The placenta's structure ensures that, although fetal and maternal circulations do not mix, there is a large surface area through which materials can diffuse from one circulation to another. The primary barrier function is the **trophoblast layer**, which consists of two layers of cells – a layer of stem-cell-like **cytotrophoblasts** which act as a source of new cells that fuse to create a single giant syncytial cell, the **syncytiotrophoblast**. All material entering the fetal circulation from the maternal circulation (and vice versa) must cross the syncytiotrophoblast.

The syncytiotrophoblast is clearly not an absolute barrier. As well as the diffusion or active transport of small molecules and metabolites, larger particles and even cells can also cross the trophoblast layer, through gaps in the syncytiotrophoblast.

Perhaps less well appreciated is the fact that the placenta is also a site of **blood cell formation**. In humans, haematopoiesis is known to occur in the yolk sac. In the placenta, blood cell formation can be detected as early as four weeks gestation, at sites known as **haemangioblastic foci (HAF)**. These go on to form elongated structures with one end anchored at the trophoblast. Their primary function appears to be red blood cell formation, with erythrocytes being gradually released into the circulation as the fetal heart starts to beat at around four weeks.

Although macrophages cannot be detected in HAFs, some white blood cells can be distinguished. Moreover, transfer to immune-deficient mice suggests that multilineage precursor cells are present. Hence the placenta appears to contain a population of extra-embryonic mesenchymal stem cells capable of generating erythrocytes and other blood cell lineages, as well as blood vessels. HAFs disappear at around nine weeks but haematopoietic stem cells remain in the placenta throughout pregnancy[1].

[1] Robin C et al. Human placenta is a potent hematopoietic niche containing hematopoietic stem and progenitor cells throughout development. Cell Stem Cell. 2009;5(4):385–95.
Dzierzak E, Robin C. Placenta as a source of hematopoietic stem cells. Trends Mol Med. 2010;16(8):361–7.

Acute lymphoblastic leukaemia

In the first of three talks on the *in utero* origins of specific childhood cancers, **Tariq Enver** (UCL) described what is known of the genetics of acute lymphoblastic leukaemia (ALL). With 400 new cases each year in the UK, ALL is the most common cancer in children.

Like all cancers, ALL is genetically diverse, but around a third of cases feature a specific translocation affecting the *TEL* and *AML1* genes, both known to be involved in haematopoiesis, generating a ***TEL–AML1*** fusion gene which appears to be an early or 'first hit' mutation. A wide range of additional genetic aberrations can lead to the development of ALL, though a handful of recurrent mutations predominate.

Retrospective analysis of Guthrie spots has shown that the *TEL–AML1* fusion arises *in utero*. Further evidence for its *in utero* origins have come from studies of identical twins. Genetic analyses suggest that pairs of twins can both carry blood cells with identical *TEL–AML1* fusion genes, which presumably arose *in utero* and populated both twins through a shared blood supply[2].

Additional insight has come from work on a pair of identical twins only one of which developed ALL. A comparison of white blood cells from the two revealed that both had a population of cells carrying the *TEL–AML1* fusion, but only one had acquired additional mutations leading to the development of ALL. This suggests that a population of cells with the potential to generate ALL ('pre-leukaemic stem cells') arose before birth, when the twins shared a blood supply. Indeed, modified versions of these cells could generate pre-cancers or pre-leukaemia when injected into mice[3].

[2] Ford AM et al. Fetal origins of the TEL-AML1 fusion gene in identical twins with leukemia. Proc Natl Acad Sci USA. 1998;95(8):4584-8.

[3] Hong D et al. Initiating and cancer-propagating cells in TEL-AML1-associated childhood leukemia. Science. 2008;319(5861):336-9.

The *TEL–AML1* fusion changes the properties of cells, for example causing them to be less responsive to the suppressive effect that infection typically has on haematopoietic stem cell proliferation, and to be less responsive to inhibitory mediators such as TGF[4]. Although a *TEL–AML1* fusion-containing cell is not itself cancerous, these properties confer a slight survival advantage and increase the likelihood that it receives additional 'hits' that ultimately generate ALL.

Cancer has traditionally been viewed as a stepwise process, in which a cell sequentially acquires mutations that provide it with a growth and survival advantage. Genetic analysis of cancers has revealed a more complex branching picture, in which a progenitor cell generates a diverse family tree of related cancer cell populations (clones), with different combinations of mutations. These populations coexist within individuals, often with one or a small number of dominant clones. One child with ALL, for example, was found to have at least nine different subclones[5]. This complexity has important implications for therapy: even if a dominant subclone is eliminated, a previous minor constituent may proliferate once its competitors have disappeared, leading to relapse.

The heterogeneity of cancers can reflect both genetic and epigenetic changes, which again has implications for therapy. Chemotherapy eliminates actively dividing cells, but has less impact on genetically identical but epigenetically silenced quiescent cells. These surviving cells can act as a source of new proliferating cells, again triggering relapse[6].

Professor Enver also highlighted the importance of the environment or 'niche' in which a cancer cell exists. For example, in a mouse model, leukaemia-propagating cells have been found to recruit mesenchymal stem cells to bone marrow where they generate a protective sheath. These protective cells also

[4] Ford AM et al. The TEL-AML1 leukemia fusion gene dysregulates the TGF-beta pathway in early B lineage progenitor cells. J Clin Invest. 2009;119(4):826-36.

[5] Anderson K et al. Genetic variegation of clonal architecture and propagating cells in leukaemia. Nature. 2011;469(7330):356-61.

[6] Lutz C et al. Quiescent leukaemic cells account for minimal residual disease in childhood lymphoblastic leukaemia. Leukemia. 2013;27(5):1204-7.

release a factor that acts on the leukaemia-propagating cells and renders them resistant to chemotherapy. In patients, the existence of such niches in bone marrow is associated with failure to achieve complete remission[7].

Why is ALL such a problem in children? One possibility, suggested Professor Enver, is that particular B-cell lineage progenitors may be present only in early development and provide a cellular context in which the *TEL–AML1* fusion can exert its effects. Potentially, the first steps on the road to ALL are taken only when a *TEL–AML1* fusion arises in these fetal-specific B-cell progenitors. Indeed, although it is common genetic aberration – one in 100 infants are born with a *TEL–AML1* fusion – only one in 100 of those with the fusion go on to develop ALL.

A key challenge is to identify this embryonic B-cell lineage. Professor Enver suggested that it may arise from yolk sac cells rather than the embryo's haematopoietic stem cells. A potentially exciting opportunity may come from manipulation of human induced pluripotent stem cells (iPS cells). To date it has proved challenging to generate haematopoietic stem cells from iPS cells, but progress is being made and some early B-cell lineages can now be produced *in vitro*. Introducing a *TEL–AML1* fusion into such cells could help to identify the critical population for the later development of ALL. Such cells could also be used to study the processes by which additional changes lead to the development of ALL.

Brain tumours

Silvia Marino (Queen Mary, University of London) described her work on **medulloblastoma**, the most common malignant brain tumour of childhood. Although survival of medulloblastoma patients has improved in recent years, the tumour is still associated with significant morbidity, owing to the severity of the therapies currently used.

[7] Duan CW et al. Leukemia propagating cells rebuild an evolving niche in response to therapy. Cancer Cell. 2014;25(6):778-93.

Medulloblastoma is an example of a cancer resulting from deregulated normal development – in this case of the cerebellum [8]. Unusually for brain development, the cerebellum derives from two germinal layers – the **external granule layer**, which generates granule cells, and the **subventricular zone**, which gives rise to all other cells; cancers can arise from cells in either of these layers. Medulloblastoma also shows considerable heterogeneity, and a major challenge is to relate this heterogeneity to underlying genetic and epigenetic mechanisms.

Molecular characterisation has led to a classification of medulloblastoma into four subgroups [9]. Group 1 tumours show abnormalities in *WNT* pathway signalling, and seem to arise from progenitor cells located in the ventricular zone, while group 2 tumours have changes affecting the *SHH* (sonic hedgehog) pathway and derive from granule cell progenitors. Group 3 tumours are diverse, but mainly involve disruptions to *MYC* signalling. Group 4 tumours are common, but their molecular basis is not well understood, although epigenetic abnormalities seem to predominate in this group.

To gain insight into the role of epigenetic modifications in group 4 tumours, Professor Marino has been examining the role of **Bmi1**, a component of a large multiprotein complex involved in histone modification. Bmi1 is expressed in the external granule layer, particularly when cells are proliferating, and in its absence, granule cells proliferate less and mice develop a smaller cerebellum. Overexpression could therefore lead to excessive proliferation of cerebellar progenitor cells, and indeed high levels of Bmi1 expression have been seen in group 4 tumours[10].

Professor Marino has found that overexpression of Bmi1 in granule cells in mice does not on its own initiate medulloblastoma formation[11]. Rather, it

[8] Taylor MD et al. Molecular subgroups of medulloblastoma: the current consensus. Acta Neuropathol. 2012;123(4):465–72.

[9] Taylor MD et al. Molecular subgroups of medulloblastoma: the current consensus. Acta Neuropathol. 2012;123(4):465–72.

[10] Leung C et al. Bmi1 is essential for cerebellar development and is overexpressed in human medulloblastomas. Nature. 2004;428:337–41.

[11] Behesti H et al. Bmi1 overexpression in the cerebellar granule cell lineage of mice affects cell proliferation and survival without initiating medulloblastoma formation. Dis Model Mech. 2013;6(1):49–63.

appears to be involved in tumour maintenance, acting in concert with other chromatin remodelling factors.

Embryonal tumours

Keith Brown (Bristol) discussed a third class of childhood cancer, embryonal tumours – a name that reflects their prenatal origins. His focus was on **neuroblastoma** and **Wilms' tumour**, a cancer of the kidney.

In each case, the cells from which cancers originate are well established – neuroblastomas arise from **neural crest cells** and Wilms' tumours from **metanephric blastema cells** in the embryonic kidney. There is a strong heritable component to Wilms' tumour but less so to neuroblastoma. Notably, both show distinctive intermediate pre-malignant stages on the path to full-blown tumours.

Dr Brown focused his discussion on Wilms' tumour, which affects around 1 in 10,000 children. Its roots lie in embryonic kidney development, which takes place between 4 and 36 weeks gestation. Intermediate stages – known as '**nephrogenic rests'** – are far more common, affecting around 1 in 100 fetuses; usually they spontaneously regress.

Much insight has been gained into Wilms' tumour through its association with a developmental condition, WAGR (Wilms' tumour, aniridia, genitourinary abnormalities and retardation) syndrome. A chromosomal deletion eliminates the *WT1* Wilms' tumour gene, as well as other genes leading to the other symptoms of the syndrome. Other predisposing syndromes that do not involve *WT1* include a rare fetal overgrowth condition, Beckwith–Wiedemann syndrome – a genetically complex condition involving the *IGF2* gene, in which the effects of a mutation depend on whether it is inherited from the mother or from the father.

WT1 is the most commonly mutated gene in Wilms' tumour, but the disease remains poorly understood genetically – only 30% of cases have an identified

genetic cause. Multiple genes have been implicated in the condition, alongside epigenetic changes.

An understanding of the evolution of Wilms' tumour pathology, with nephrogenic rests as an important stepping stone towards malignant cancer, has stimulated efforts to link specific genetic changes to particular stages in its development. Mutation of *WT1*, for example, appears to be an early event, while various other genes are more commonly affected later in tumour development. Similarly, early-acting and late-acting epigenetic changes have been identified. Apart from the inherited mutations, the origins of tumour-promoting mutations remain unclear.

Stem cell manipulations may again provide a way to improve understanding of the development of Wilms' tumour. First reports are appearing of embryonic stem cells being converted into metanephric blastema cells, and attempts are being made to generate such cells from iPS cells. One advantage of the latter approach is that iPS cells could be generated from individuals with an inherited predisposition, such as a *WT1* mutation.

The possibility of identifying and characterising early cancer precursor cells raises the possibility of childhood cancer vaccines. However, as Professor Enver pointed out, only 1 in 100 people with a *TEL–AML1* fusion go on to develop ALL, so any treatment would have to be mild as most children would not go on to develop disease.

Immunotherapy-based approaches might hold more promise, with treatments directing the host immune response to specific proteins on cancer cells. Improved technology for detecting circulating cancer cells may also offer the prospect of earlier treatment, though there is little evidence yet that this provides survival benefits.

Xenobiotics: Nanoparticles and the placenta

Returning to the placenta, **Margaret Saunders** (Bristol) discussed recent work evaluating the impact of **nanoparticles** on placental function. Because of its rapid cell division and the immaturity of its detoxification systems, the fetus is potentially vulnerable to noxious insults. The placenta therefore has a critical protective role, regulating the substances to which the fetus is exposed. Furthermore, the fetal origins of adult disease concept – the Barker hypothesis – illustrates how exposures *in utero* can have profound impact on later-life health.

A variety of systems are available to study placenta, each with its own advantages and drawbacks. The guinea-pig placenta is the rodent model most similar to humans, while donated human placenta itself can be used for short periods, although it is not easy to work with. Although not totally physiological, *in vitro* cell systems are convenient and relatively easy to establish. They are typically based on 'transwell' arrangements, with two chambers separated by a porous horizontal platform on which placental cells can be grown, forming a simple trophoblast-like barrier between compartments. Dr Saunders typically uses BeWo cells, from an immortalised placental cell line, which maintain many key features of placental cells, including the ability to form polarised cell layers.

As part of the EU **NanoTEST** programme[12], Dr Saunders has been studying the impact on varying aspects of placenta function and physiology of nanoparticles similar to those used in medical imaging (e.g. magnetic resonance imaging, MRI, angiography and tumour imaging). These particles are typically iron oxide and silica nanoparticles of varying sizes and composition (e.g. coated or uncoated). Such studies have revealed evidence of toxic effects, particularly of iron oxide nanoparticles, in some cases even at relatively low doses[13]. In addition, imaging studies suggest that significant numbers of particles are able to traverse a placental cell barrier.

[12] http://www.nanotest-fp7.eu/

[13] Correia Carreira S et al. The toxicity, transport and uptake of nanoparticles in the in vitro BeWo b30 placental cell barrier model used within NanoTEST. Nanotoxicology. 2013. [Epub ahead of print]

The extent to which pregnant women might be exposed to nanoparticles is unclear. MRI is not used in women during the first trimester, and would only be used under special circumstances at other stages of pregnancy. Accidental exposure at work might be another potential route of exposure. Motor vehicle pollution is another known source of potentially damaging ultrafine particles.

Radionuclides, described by **John Harrison** (Public Health England), are another potential source of damage to the fetus. He described models developed by the International Commission on Radiological Protection (ICRP), which are used to generate dose coefficients (factors used to convert radionuclide intake to exposure or dose measures) and provide a tool for deriving risk estimates.

For each radioactive element, the ICRP models provide an estimate for how radionuclides become distributed through the body. In ICRP publications 88 and 95, the models estimate how radionuclides taken in by the mother are transferred across the placenta to the fetus (and to various compartments within the fetus) or via mother's milk to an infant.

Models are validated in experimental systems and by analysing the results of natural exposure, for example from fall-out, and are used to develop dose coefficients. These are generally smaller for the fetus than the mother, except for those elements that can be incorporated into skeletal structures.

From a practical perspective, the ICRP models are used by regulatory authorities to provide guidance on exposure limits. Regulation is based on the concept of an 'effective dose', a risk-adjusted dosimetric quantity that sums exposures across all tissues. Dr Harrison was at pains to point out that effective dose was a simplified practical tool, and should not be applied to risk estimates for particular groups or individuals, not least because of the high degree of uncertainty associated with low exposures.

Dr Harrison suggested that the ICRP models were unlikely to be underestimating risk. As an example, he pointed to the fact that major spikes in annual radionuclide exposures in the mid-1960s due to atmospheric weapons testing were not associated with noticeable subsequent increases in the incidence of childhood leukaemias.

Dr Harrison is also coordinating a major EU-funded project (**SOLO**)[14], which is assessing the long-term impact of a significant release of radioactive material into the Techa River from the Mayak plutonium production plant in the former Soviet Union during the 1950s. Follow up of workers from the plant itself and populations along the river will provide more insight into the impact of exposure, including cancer following *in utero* exposure, and help to refine models. The project has identified some 11,500 offspring exposed *in utero* along the river and 3400 exposed at Mayak.

Air pollutants

In the first of two talks on air pollution, **Julia Heck** (University of California Los Angeles) discussed the epidemiological evidence connecting exposure to **air pollution** with the development of childhood cancer. Some 25 studies have examined such links, mainly in North America and Europe, focusing primarily on leukaemia, CNS cancer or all cancers collectively; there is little information on individual types of cancer, particularly rare ones.

As Dr Heck pointed out, it is challenging to assess individual levels of exposure. The main source of exposure is from road traffic, so simple proxy measures have traditionally been used to estimate exposure levels, such as distance from roads or local traffic densities. This has generally been based on residential address. Since individuals move, complications include when to assess exposure (at birth, at death or at diagnosis?), and a full residential history is not usually available. Parental workplace location is also rarely taken into account.

[14] http://www.increast.eu/en/1200.php

More recent studies have been based on actual measurements of pollutant levels in particular environments, combined with models to extrapolate from such data and provide estimates of more localised exposures. Models such as CALINE4 look at how pollutants mix, react and disperse from a roadway, to provide estimates of air quality in surrounding areas. Land use models take account of roadways but also whether surrounding land is being used for residential, industrial, agricultural or other purposes.

Pooled data from six studies using simple proximity measures found a small correlation between exposure and the incidence of ALL and/or leukaemia (odds ratio of 1.21). A larger number of studies have examined traffic density, finding only a small increase in risk (odds ratio 1.04). Modelling-based studies, 11 in total, again found a modest increase in risk of ALL/leukaemia (odds ratio 1.07).

Studies have provided little evidence for increased risk for acute myeloid leukaemia or for CNS tumours, but an increased risk was seen for retinoblastoma (odds ratio 1.08) and germ cell tumours (odds ratio 1.11). No dose-response effects were seen, and use of lifetime residential history had no impact, possibly because exposure in a specific developmental window rather than lifetime exposure is the critical factor.

A recently published meta-analysis[15] (which does not include some of the studies discussed by Dr Heck) found an increased risk for leukaemia based on exposure in childhood (odds ratio 1.53) but not prenatally. Indeed, Dr Heck suggested that leukaemia risk was significantly higher in the under fives.

In California at least, levels of many (but not all) traffic-associated pollutants have been declining in recent years. However, industrial activity is another important source of potentially harmful pollutants. Interest in this area was

[15] Boothe VL et al. Residential traffic exposure and childhood leukemia: a systematic review and meta-analysis. Am J Prev Med. 2014;46(4):413–22.

stimulated by Knox's landmark (and controversial) work in the 1990s on clusters of leukaemia near industrial sites in the UK. In California, air-monitoring stations are providing a source of data on air quality at numerous sites across the state. These will provide an important source of data for examining links between levels of a wide range of chemicals and cancer development. Studies of parental exposure may also generate useful information, though this has tended to focus on paternal employment and more work is needed on maternal exposure.

Although Dr Heck concentrated on California, Mathuros Ruchirawat pointed out that the incidence of leukaemia had risen markedly in Bangkok in recent years. Given the city's problems with traffic congestion, one possible reason might be increased exposure to chemicals such as benzene. DNA damage and impaired repair capacity has been documented in children exposed to urban air pollution and correlates with levels of exposure to benzene[16] [17].

Returning to the UK, **Matthew Wright** (Bristol) described some of the challenges of measuring air pollution and hence in assessing exposure. Air pollution was as a complex mix of gases and particulates. Moreover, there are multiple interactions between the two, complicating attempts to model their behaviour and hence exposures.

Multiple sources of pollutants exist, with road traffic being a particularly important source in urban areas. EU regulation sets limits for levels of many airborne pollutants (but not all). Notably, levels of several pollutants, including sulfur dioxide and PM10 and PM2.5 particulates (particles with diameters less than 10□m and 2.5□m, respectively), have been in decline since the 1970s, thanks to Clean Air Acts and changes in areas such as power generation and car exhaust design.

[16] Buthbumrung N et al. Oxidative DNA damage and influence of genetic polymorphisms among urban and rural schoolchildren exposed to benzene. Chem Biol Interact. 2008;172(3):185-94

[17] Ruchirawat M et al. Assessment of potential cancer risk in children exposed to urban air pollution in Bangkok, Thailand. Toxicol Lett. 2007;168(3):200-9.

Dr Wright has a particular interest in **particulates**. Although particles exist in a range of sizes, they are regulated only in terms of mass. Hence regulation does not take into account potentially important factors such as particle number concentration, total particle surface area and their oxidative potential. Indeed, he suggested, size is critical: one particle of 1 □m diameter has the same mass as one million 10 nm particles. Although their biological impact might be quite different, regulatory frameworks do not take account of these differences.

Additional complexity comes from the chemical interactions between pollutants. One result of these reactions is the production of ground-level ozone, a powerful oxidant that can itself react with organic pollutants and generate reactive chemical species.

Particles can also be affected by nucleation, as new particles are created from, for example, exhaust gases within the atmosphere. Particles can also increase in size as pollutants condense onto existing particles. Furthermore, particles may also adhere to one another or coagulate, creating larger and often irregularly shaped particles. All these factors affect how chemicals and particles interact with body tissues such as the lungs.

Pollutants become less concentrated as they disperse from their source, and in fact distance from road can be a reasonably accurate way of assessing likely exposure [18]. However, the situation is more complicated in urban environments, which show complex patterns of air movements that can be difficult to model[19]. There are also temporal complexities, with pollutant levels varying according to traffic levels – in cities, typically high in rush hours and lower at weekends. Indeed, pointed out Dr Wright, roadside levels can vary enormously over very short time frames, depending on what kind of vehicles happen to be passing.

[18] Zhu Y et al. Concentration and size distribution of ultrafine particles near a major highway. J Air Waste Manag Assoc. 2002;52(9):1032-42.
[19] Kumar P et al. Dynamics and dispersion modelling of nanoparticles from road traffic in the urban atmospheric environment - a review. J Aerosol Sci. 2011;42(9):580-603.

In terms of monitoring equipment, a wide range of equipment is available, some suitable for local monitoring and others for laboratory analysis. There is in general a trade off between cost and convenience. Portable devices tend to be cheaper but less able to carry out the sophisticated analyses possible in laboratory set-ups.

Through modelling, measurements can be related to exposures. Although the accuracy of models is improving, it is difficult to capture the impact of daily variations in pollutant levels. Moreover, it is challenging to incorporate the daily behaviour patterns of individuals, which will obviously influence exposure, and the impact of indoor exposures (where people will actually spend most of their time).

Dr Wright drew attention to important gaps in knowledge about ultrafine particles, which are not yet covered by EU regulation and are subject to minimal monitoring. In the long term, he suggested, monitoring strategies should be designed so they take account of the needs of epidemiological research into childhood cancer.

A further potential source of exposure is from **dietary carcinogens**, a topic covered by **Jos Kleinjans** (Maastricht). Since childhood cancers are rare, it is difficult to identity potential risk factors by epidemiological methods. An alternative approach, adopted by the EU-funded **NewGeneris** programme[20], is to focus on biomarkers – potentially harmful chemical or other changes – that reflect exposure to dietary carcinogens.

The NewGeneris programme, based on materials collected at 10 birth cohorts and biobanks across Europe, analysed cord blood samples for exposure to a wide range of organic compounds, including established pollutants, food preparation byproducts and compounds found naturally in the gut. In all, samples from some 1100 participants were analysed. As well as looking for

[20] Merlo DF et al. NewGeneris: a European study on maternal diet during pregnancy and child health. Cancer Epidemiol Biomarkers Prev. 2009;18(1):5-10.

the presence of potential dietary carcinogens in samples, the programme also analysed 'response biomarkers', such as modifications to DNA and haemoglobin, that provide evidence of damage induced by dietary compounds.

Studies with placenta explants suggested that these compounds can readily cross the placenta, though at varying rates[21]. The programme also found good evidence for chemical modifications indicative of exposure to a wide range of potentially hazardous compounds. In the UK, the Born in Bradford cohort showed particularly high levels of acrylamide-based modifications[22], which Professor Kleinjans attributed to a diet high in deep-fried foods.

Notably, the programme also looked for the presence of **micronuclei**, genomic debris resulting from severe chromosomal damage, which in adults have been strongly associated with carcinogenesis. The frequency of micronuclei showed significant associations with levels of exposure to dietary toxins[23].

The programme also analysed genome-wide patterns of gene expression associated with exposure to dietary toxins, which revealed interest sex-specific differences. Some of the genes differentially affected have been implicated in childhood cancers, providing a possible explanation for sex biases in the incidence of diseases such as ALL.

The programme followed up a subset of genes, to identify those associated with both micronuclei formation and an exposure biomarker. Several such genes have previously been implicated in control of cellular proliferation or apoptosis. They represent genes that could potentially be important in mediating the damaging effects of dietary toxins.

[21] Mose T et al. Meta-analysis of data from human ex vivo placental perfusion studies on genotoxic and immunotoxic agents within the integrated European project NewGeneris. Placenta. 2012 May;33(5):433-9.

[22] Pedersen M et al. Birth weight, head circumference, and prenatal exposure to acrylamide from maternal diet: the European prospective mother-child study (NewGeneris). Environ Health Perspect. 2012;120(12):1739–45.

[23] Merlo DF et al. Micronuclei in cord blood lymphocytes and associations with biomarkers of exposure to carcinogens and hormonally active factors, gene polymorphisms, and gene expression: the NewGeneris cohort. Environ Health Perspect. 2014;122(2):193–200.

The programme also carried out an analysis to identify genes whose expression was both altered by prenatal exposure to dioxin-like chemicals and affected vaccination responses at age 3[24]. Such genes may potentially be mediating the detrimental impact of dioxin exposure on vaccination responses. The programme also identified detrimental impacts of acrylamide exposure and dioxin-like compounds on birth weight, head circumference and birth outcomes.

Mechanisms of *in utero* damage

Returning to the impact of nanoparticles on the placenta, **Patrick Case** (Bristol) presented data suggesting that they may be able to damage fetal tissues without even crossing the placenta.

There is evidence that metal nanoparticles can cause cytogenetic damage to bone marrow cells, including aneuploidy[25]. However, it is unclear whether they might affect a developing fetus.

To explore whether there is any evidence for this theoretical possibility, Dr Case established a transwell tissue culture system, using BeWo cells to create a multilayer placental barrier. He then assessed the potential of metal (cobalt–chromium) nanoparticles (or larger micrometre-sized particles) added to the upper transwell chamber to cause DNA damage in fibroblasts grown in the bottom chamber.

Notably, DNA damage could be detected in fibroblasts, primarily tetraploidy – even though the nanoparticles did not seem to traverse the placental cell barrier[26]. Dr Case likened the phenomenon to the 'bystander effect', whereby cells not directly affected by radiation are harmed by their proximity to radiation-damaged cells.

[24] Hochstenbach K et al. Toxicogenomic profiles in relation to maternal immunotoxic exposure and immune functionality in newborns. Toxicol Sci. 2012;129(2):315–24.

[25] Raghunathan VK et al. Influence of particle size and reactive oxygen species on cobalt chrome nanoparticle-mediated genotoxicity. Biomaterials. 2013;34(14):3559–70.

[26] Bhabra G et al. Nanoparticles can cause DNA damage across a cellular barrier. Nat Nanotechnol. 2009;4(12):876–83.

The effect was dependent on communication through gap junctions connecting placental cells, and there was some evidence for the involvement of ATP-based signalling and for calcium waves. Hence, suggested Dr Case, nanoparticles interacting with the placental cell barrier may trigger a series of intracellular and intercellular signalling events that ultimately lead to the release of an agent (possibly ATP) from the far side of the placenta barrier that can induce damage in nearby cells.

Notably, this effect was only seen with multilayer barriers – single-cell barriers, though physically smaller, seem to form a more effective barrier[27].

Dr Case has gone on to show that human embryonic stem cells, as well as fibroblasts, suffer similar damage, but only when they are dividing. *In vivo* studies have also revealed that fetal blood cells and liver cells can be damaged following infusion of nanoparticles into pregnant mice even though the particles themselves are not detectable in the fetus, again pointing to an indirect effect.

Alcohol and aldehydes

Another potentially significant genotoxic agent is **alcohol**, through its conversion in the body into **acetaldehyde**. **Nina Oberbeck** from K J Patel's laboratory in Cambridge presented results from studies in mice suggesting that maternal aldehyde metabolism is critical to the health of the developing fetus.

Dr Oberbeck's work has focused on mouse models of **Fanconi anaemia**, a condition caused by mutations affecting components of a DNA repair enzyme complex. The consequent impairments in DNA repair leave cells vulnerable to chromosomal aberrations, which ultimately promote the development of cancer.

[27] Sood A et al. Signalling of DNA damage and cytokines across cell barriers exposed to nanoparticles depends on barrier thickness. Nat Nanotechnol. 2011;6(12):824–33.

Aldehydes are among the factors known to induce DNA damage requiring repair. They are produced naturally in the body as well as being a product of alcohol metabolism. Normally, aldehydes are rapidly removed by a family of **aldehyde dehydrogenases**, most notably **Aldh2**. Although *Aldh2* knockout mice show no obvious phenotype, *Aldh2*/*Fancd2* double knockout animals, which lack a key component of the DNA repair complex as well as aldehyde dehydrogenase, die before birth.

However, a double knockout fetus will survive if its mother retains a single working copy of the *Aldh2* gene, highlighting the importance of maternal aldehyde breakdown to fetal survival. Even so, with their own aldehyde breakdown and DNA repair impaired, double knockout offspring show haematopoietic stem cell defects and rapidly develop leukaemia[28].

Dr Oberbeck has gone on to demonstrate similar results with a knockout of a second Fanconi anaemia gene, *Fanca*. Indeed, double *Fanca*/*Aldh2* knockouts hardly develop at all in *Aldh2*-lacking mothers, and show curtailed growth in mothers with a single copy of the *Aldh2* gene.

As well as aldehydes produced by natural metabolic processes, Aldh2 also metabolises acetaldehyde generated after alcohol consumption. Excessive alcohol consumption in pregnancy causes a well-known clinical condition, fetal alcohol syndrome. In mice, alcohol given to pregnant females exacerbates the phenotype of *Fanca* knockout fetuses, presumably by generating DNA-damaging acetaldehyde. Consistent with this idea, an additional copy of the *Aldh2* gene in mothers protects *Fanca* knockout fetuses from alcohol-related harm.

Interestingly, Aldh2 detoxification of acetaldehyde appears to occur predominantly in the liver – there is no evidence for activity in the placenta.

[28] Langevin F et al. Fancd2 counteracts the toxic effects of naturally produced aldehydes in mice. Nature. 2011;475(7354):53-8.

Collectively, the evidence suggests that aldehydes produced in the mother, from endogenous metabolic processes or generated after alcohol consumption, can be transferred to and harm the developing fetus. Strong evidence in support of this idea has come from embryo transfer experiments, in which *Aldh2*/*Fanca* double knockout fetuses at very early stages of development were transferred to a wild-type mother. Although double knockouts normally die before birth, in this maternal environment they survive to birth. (The animals still show haematopoietic stem cell problems, because of aldehyde production within the fetus itself).

The results indicate that maternal alcohol consumption could be a risk factor for DNA damage in the fetus. At particular risk might be those with the 'Asian flushing' mutation, common in the Far East, which causes striking blushing after alcohol consumption. The effect is linked to genetic changes affecting the human *ALDH2* gene, which lead to elevated levels of acetaldehyde.

A further potential source of aldehydes may be the artificial sweetener aspartame. Metabolism of aspartame can lead to the formation of formaldehyde, via methanol.

Electric and magnetic fields

Another potentially important contributor to childhood cancers could be exposure to **electric and magnetic fields**, for example from power lines. **Morando Soffritti** (Bentivoglio, Italy) described a large-scale animal study designed to shed light on this area.

In particular, evidence that power frequency magnetic fields could be harmful has accumulated from epidemiological analyses over recent decades. An initial connection between exposure and leukaemia in children was proposed in 1979[29], while links to leukaemia in adults was made in 1982[30]. In 2000, a

[29] Wertheimer N, Leeper E. Electrical wiring configurations and childhood cancer. Am J Epidemiol. 1979;109(3):273-84.

[30] Milham S Jr. Mortality from leukemia in workers exposed to electrical and magnetic fields. N Engl J Med. 1982;307(4):249.

pooled analysis identified a significant increased risk of childhood leukaemia at exposures in excess of 0.4 □T[31], while various meta-analyses have since reported a twofold increased risk of childhood leukaemia at exposures of 0.3–0.4 □T. The epidemiological evidence led the International Agency for Research on Cancer (IARC) to classify extremely low frequency (incorporating power frequency) magnetic fields as a possible carcinogen.

Despite this convincing epidemiological evidence, experimental studies have failed to provide conclusive evidence. Experimentally, it has not been possible to identify a carcinogenic effect of magnetic fields, and no accepted mechanism by which they might cause cancer has been described.

However, pointed out Dr Soffritti, studies to date have typically lacked the scale to identify rare events and have not been long enough to track disease in later life. Critically, they have also not taken into account *in utero* exposure.

To address these issues, Dr Soffritti has run a large-scale, long-term experimental project on exposure to magnetic fields with and without co-exposure to other potential carcinogens. The study involved some 7000 rats, exposed to magnetic fields from before birth to death. The animals were all kept in a 900 m^2 room designed to simulate exposure to power lines, and outcomes were compared with a concurrent control group of over 1000 male and female rats and those from 18,000 historical controls.

The study compared the effects of magnetic fields alone and in combination with chronic formaldehyde administration, a single shot of gamma-radiation and nine doses of aflatoxin.

Although magnetic fields alone did not increase the incidence of cancer, effects were seen with combinations of carcinogens. Magnetic fields plus formaldehyde led to an increase in some cancers (e.g. thyroid cancer) in

[31] Ahlbom A et al. A pooled analysis of magnetic fields and childhood leukaemia. Br J Cancer. 2000;83(5):692-8.

males, while the combination of magnetic fields and gamma-radiation was associated with an increased incidence of cancers in males and of mammary gland cancers in females.

Arsenic and cancer

Arsenic is an environmental exposure of growing concern. **William Suk** (National Institute of Environmental Health Sciences, USA) presented a global overview of the threat to health posed by arsenic, and described an innovative programme he is heading that aims to combat it.

Dr Suk suggested that between one-third and one-quarter of ill-health could be attributed to environmental factors, many of them linked to human activities. Children are particularly vulnerable and less-developed countries bear the greatest burden of disease.

Arsenic is just one of many potentially harmful environmental exposures, but globally it is a major problem. Some ten million people in the USA are exposed to arsenic in water supplies, while globally hundreds of millions of people are exposed to damaging levels of arsenic. 'Hotspots' of arsenic exposure are distributed throughout the world, reflecting natural geologic processes leading to arsenic contamination of aquifers as well as human industrial processes, particularly mining.

The mechanisms of arsenic toxicity are unclear, though multiple possible mechanisms have been proposed. The element is a metalloid with unusual chemical properties, able to form complexes with many other compounds. A lack of understanding of mechanisms of toxicity has made it hard to establish 'safe' levels of exposure. While 50 □g/l was used as an arsenic water standard for many years in the USA, this has recently been reduced to 10 □g/l (although adverse health effects have been reported at even lower levels).

Chronic arsenic exposure is associated with damage to numerous organ systems and causes many health conditions, including skin abnormalities (keratosis), cardiovascular disorders and cancer[32]. Animal studies suggest that it can cross the placenta, and prenatal exposure is associated with a wide range of health defects in later life (including childhood cancer).

One of the most striking impacts of arsenic exposure has come from the Chilean city of **Antofagasta**, when residents began using a heavily contaminated water source. From 1958 to 1970, locals were exposed to high arsenic levels on a daily basis, providing a natural experiment for assessing the long-term impact of exposure *in utero* and early life on health and development. One striking finding has been a significant incidence of lung cancer (and other lung abnormalities) and other conditions[33].

Chile is one site of work funded through the Superfund Research Program[34], run by Dr Suk. This global interdisciplinary initiative spans a wide range of research, including risk assessment, biological responses to arsenic, technology development and remediation – all with the ultimate aim of improving public health. The programme is active at sites across Asia and Central and South America, encouraging international as well as interdisciplinary collaboration and sharing of information and expertise. Projects encompass basic and clinical scientists, behavioural researchers and engineers; projects are housed jointly within schools of public health and engineering.

Looking forward, Dr Suk suggested there was a need for concerted action across a number of areas. These include improved data collection to provide a clearer picture of the global burden of arsenic-related disease, a strategic global approach to epidemiology to tie together exposure and its impacts on health, a need for effective new interventions and preventive measures, and

[32] Naujokas MF et al. The broad scope of health effects from chronic arsenic exposure: update on a worldwide public health problem. Environ Health Perspect. 2013;121(3):295–302.

[33] Smith AH et al. Mortality in young adults following in utero and childhood exposure to arsenic in drinking water. Environ Health Perspect. 2012;120(11):1527–31.

[34] https://www.niehs.nih.gov/research/supported/dert/programs/srp/

research to clarify mechanisms of toxicity and contributions to arsenic-associated disease and disease susceptibility.

One country affected by arsenic contamination is Thailand. **Mathuros Ruchirawat** (Chulabhorn Research Institute, Bangkok, Thailand) described how work on another arsenic-exposed population had revealed some of its potentially important mechanisms of action.

It has been estimated that some 140 million people worldwide are exposed to arsenic levels in excess of 10 □g/l. One in ten people experiencing long-term exposure to very high levels (>500□g/l) are likely to die from cancer, and even 10 □g/l is thought to be associated with an increased risk of the disease.

Inside the body, arsenic combines with glutathione, forming arsenic triglutathione, which can be modified by the addition of one or more methyl groups. Variation in the efficiency of these metabolic reactions between individuals could underlie differences in susceptibility to arsenic.

Chronic exposure to arsenic leads initially to skin lesions and progressively to non-malignant and then malignant tumours (among other health impacts). Exposure in the womb has both immediate impacts (such as low birth weight) and longer-term consequences, including impaired mental function in childhood.

Between the 1960s and 1990s, tin mining activities within the **Ron Pibul** area of southern Thailand led to significant arsenic contamination of groundwater, providing an opportunity to examine the impact of prenatal exposure to arsenic on offspring. Gene expression studies in offspring revealed widespread changes in expression (up or down) across several hundred genes. In particular, changes were seen in sets of genes associated with key biological processes, including inflammation and the development of cancer[35].

[35] Fry RC et al. Activation of inflammation/NF-kappaB signaling in infants born to arsenic-exposed mothers. PLoS Genet. 2007;3(11):e207.

Analysis of the 447 genes whose expression was found to be changed by arsenic exposure led to the identification of more than 100 proteins organised into three interacting networks, each associated with inflammatory responses. The set of genes that was identified had high accuracy in predicting arsenic exposure and thus could be potential biomarkers of exposure. In addition, inflammatory responses could be an important mechanism for promoting the development of cancer.

Arsenic exposure was also associated with a slight increase in methylation of the p53 promoter and small changes to global methylation patterns[36]. Follow up studies of the offspring at ages 5–7 have revealed altered arsenic metabolism as well as higher levels of DNA damage and impaired DNA repair and higher levels of inflammatory cytokines in exposed children[37]. Such factors could be contributing to an increased risk of cancer in arsenic-exposed young people.

The growing realisation of the dangers posed by chronic arsenic exposure raises questions about the safety of the water supply in the UK. A surprisingly large number of people – one in 100 – obtain water from ground wells. Arsenic could pose a danger in locations such as Cornwall, where past tin mining activities may have increased groundwater contamination.

Circadian rhythms and melatonin

The final session of the meeting touched upon the intriguing idea that disruption of daily (circadian) rhythms could be a factor in the development of childhood cancer. As **Richard Stevens** (University of Connecticut Health Center, USA) pointed out, there is growing evidence of the harmful impact of circadian rhythm disruption, generally due to increased exposure to light at night. Indeed, as far back as 1987, Professor Stevens was warning of the potential link between light exposure and breast cancer[38] [39], and

[36] Intarasunanont P et al. Effects of arsenic exposure on DNA methylation in cord blood samples from newborn babies and in a human lymphoblast cell line. Environ Health. 2012;11:31.

[37] Hinhumpatch P et al. Oxidative DNA damage and repair in children exposed to low levels of arsenic in utero and during early childhood: application of salivary and urinary biomarkers. Toxicol Appl Pharmacol. 2013;273(3):569-79.

[38] Stevens RG. Electric power use and breast cancer: A hypothesis. Am. J. Epidemiol. 1987;**125**:556-561.

epidemiological studies have gone on to identify a correlation between shift work and the development of breast cancer. At a previous Children with Cancer UK meeting, Professor Stevens had marshalled evidence to support the contention that altered light exposure in pregnant women, neonates and young children could contribute to childhood cancer[40].

The key issue, suggested Professor Stevens, was a mismatch between the environment in which we evolved – characterised by light days and dark nights – and the environment most of us are now exposed to in industrialised countries, with plenty of artificial light at night and less exposure to light during the day if we work inside. The most extreme disruption is likely to be experienced by those undertaking shift work. Indeed, in light of the considerable evidence of the adverse health effects of such disruption, the International Agency for Research on Cancer, IARC has classed night shift work as a probable carcinogen[41].

The human body shows numerous daily rhythms attuned to environmental night–day cycles. The sleep–wake cycle is one obvious example, but our core body temperature also fluctuates on a daily basis, as do our physical activity patterns, hunger and appetite, and many metabolic functions. Deep within cells, many genes show distinctive daily cycles of activity.

The key advantage of having an endogenous body clock, and one that is responsive to environmental light cycles, is that it enables key transitions – sunrise and sunset – to be anticipated. It is such an advantage that nearly all organisms tested have been found to have an endogenous clock.

Human endogenous circadian rhythms were studied extensively by Rutger Wever and colleagues between 1969 and 1984 in an underground chamber – the Andechs bunker – in Germany. These studies suggested that the human

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[39] Stevens RG et al. Electric power, pineal function, and the risk of breast cancer. FASEB J. 1992;6(3):853-60

[40] Stevens RG. Does electric light stimulate cancer development in children? Cancer Epidemiol Biomarkers Prev.2012;21(5);701-4.

[41] IARC Monographs of the Evaluation of Carcinogenic Risks to Humans, volume 98, 2010: Painting, Frefighting and Shiftwork. Published by the International Agency for Research on Cancer, 150 cours Albert Thomas, 69372 Lyon Cedex 08, France. International Agency for Research on Cancer. ISBN 978 92 832 1298 0

daily cycle was naturally around 25 hours and varied widely between individuals. However, noted Professor Stevens, these studies were seriously flawed – participants were able to control their own lighting levels, leading to phase shifts. In more carefully controlled studies at Brigham and Women's Hospital in Boston, the natural daily cycle was found to be just slightly over 24 hours, with little inter-individual variation.

At the heart of circadian rhythms lies a genetic circuit consisting of a series of feedback loops that generate a regular oscillating output. A core set of 10 highly conserved 'clock genes' have been identified. Strikingly, some 10% of the genome shows regular daily patterns in gene expression, including genes known to be involved in cell cycle progression, apoptosis and other processes relevant to cancer.

A structure within the brain, the **suprachiasmatic nucleus**, acts as the body's master clock. Input signals from the eyes provide its link to the outside world. The suprachiasmatic nucleus itself signals to numerous peripheral clocks regulating cyclical activity in particular tissues and organs.

A key marker of circadian rhythms is melatonin, a hormone produced by the pineal gland. It is produced at night, and its release is rapidly curtailed by exposure to light. Melatonin is a powerful natural antioxidant which has multiple functions, and has been shown to have some anti-cancer properties[42] [43].

Epidemiological studies have identified a link between disrupted sleep patterns and cancer. One possible mechanism might be via metabolic changes. Reduced sleep is known to be associated with increased body weight, possibly through an effect on the appetite-controlling hormone leptin; in turn, increased maternal body weight is associated with higher birth weight

[42] Reiter RJ. EDITORIAL NOTE: Mechanisms of cancer inhibition by melatonin. J. Pineal Res. 2004; 37:213–214

[43] Tan DX, et al. One molecule, many derivatives: A never-ending interaction of melatonin with reactive oxygen and nitrogen species? J. Pineal. Res. 2006;42:28-42.

babies, who are also known to be at increased risk of leukaemia[44]. Of potential significance, it has also been reported recently that melatonin production in children is particularly sensitive to suppression by light[45].

Professor Stevens has argued for environmental planners to pay more attention to the impact of artificial light. He also suggested that night-lights, or other forms of exposure to light at night, should be avoided. The type of light makes a difference, with frequencies at the red end of the spectrum having less impact on melatonin production.

A key question is whether disruption of circadian rhythms during pregnancy has any effect on the developing fetus that might increase the risk of childhood cancer (or other conditions). **Tamara Varcoe** (Adelaide, Australia) described work in rats touching on this important issue.

The fetus is exposed to circadian physiological rhythms governed by the mother's endogenous clock. It also has its own endogenous rhythms. It has no direct access to the outside world, so entrainment of the fetal clock is governed by maternal signals. Indeed, if the maternal suprachiasmatic nucleus is ablated, fetal rhythms are disrupted. Potentially important maternal signals could include cyclical changes in melatonin or feeding behaviour.

Shift work, Dr Varcoe notes, is a significant part of modern life – in Australia it is undertaken by 17% of the working population (1.4m people). As well as short-term harmful impacts linked to fatigue, it may also have significant long-term consequences. Notably, shift work during pregnancy is associated with a range of poor outcomes, including pre-term birth and spontaneous abortion. However, the type of shift work undertaken is rarely recorded and there has been little follow up to assess impact on offspring.

[44] Caughey RW, Michels KB. Birth weight and childhood leukemia: a meta-analysis and review of the current evidence. Int J Cancer. 2009;124(11):2658–70.

[45] Higuchi S et al. Influence of Light at Night on Melatonin Suppression in Children. J Clin Endocrinol Metab. 2014:jc20141629. [Epub ahead of print]

Since it is difficult to model shift work directly in animals, Dr Varcoe and colleagues have adopted the strategy of disrupting the circadian rhythms of pregnant rodents and searching for abnormalities in their offspring. Animals were exposed to a series of chronic phase shifts, in which day–night cycles were periodically flipped. Among the most notable changes was excess weight gain in female offspring and associated changes in glucose metabolism[46].

Other studies exploring circadian rhythm disruption, including constant exposure to light during pregnancy, have also identified changes to glucose metabolism in offspring. Removal of the melatonin-producing pineal gland in mothers also led to impaired glucose metabolism in offspring, an effect that could be reversed by maternal administration of melatonin[47].

Interestingly, Dr Varcoe found few changes to normal melatonin rhythms in phase-shifted mothers, though normal cycles in stress hormone (corticosterone) levels were almost completely lost (overall corticosterone production was not altered). Food consumption behaviour changed markedly, with periodic eating giving way to almost constant grazing.
Changes were also seen in the expression of clock genes in offspring. Potentially, phase-shifting treatment of mothers could be driving epigenetic changes affecting fetal clock genes (and genes affecting metabolic processes).

Other studies have also identified a range of changes in the offspring of mothers whose circadian rhythms have been disrupted. These include changes in corticosterone rhythms[48], altered newborn temperature rhythms

[46] Varcoe TJ et al. Chronic phase shifts of the photoperiod throughout pregnancy programs glucose intolerance and insulin resistance in the rat. PLoS One. 2011;6(4):e18504.

[47] Ferreira DS et al. Maternal melatonin programs the daily pattern of energy metabolism in adult offspring. PLoS One. 2012;7(6):e38795.
[48] Mendez N et al. Timed maternal melatonin treatment reverses circadian disruption of the fetal adrenal clock imposed by exposure to constant light. PLoS One. 2012;7(8):e42713.

(in primates)[49], and effects on learning and memory (and molecular processes associated with them)[50].

There is no evidence of an increased risk of cancer in offspring – but this has not been systematically assessed. Interestingly, although the numbers are small, Dr Varcoe did see an increase in the numbers of rats from treated mothers developing tumours. Potentially, circadian rhythm disruption could drive epigenetic changes in offspring having long-term impacts, or changes to clock gene activity could alter the expression of genes known to be involved in cell cycle progression. Moreover, metabolic changes, such as increased obesity, could indirectly have an impact on cancer development.

Concluding the meeting, **Cathy Vaillancourt** (Quebec, Canada) brought the meeting full circle, identifying important links between melatonin and the placenta. The villous trophoblast is subject to constant turnover of cells, through generation of new cells by cytotrophoblasts and recycling of cellular material through apoptosis. It is sensitive to oxidative stress, and an excess of pro-oxidants can lead to enhanced apoptotic turnover and compromised placental function. This can result in conditions such as pre-eclampsia and fetal growth restriction.

Given this sensitivity to oxidative stress, Professor Vaillancourt and colleagues hypothesised that the placenta might have evolved protective mechanisms. With its known direct free radical scavenging activities and indirect effects on cellular anti-oxidant processes, melatonin was one potential protective agent. Indeed, in the 1990s it was discovered that bloodstream melatonin levels rise during pregnancy, peaking at term[51].

Serum melatonin levels were found to oscillate on a daily basis, the amplitude of oscillations increasing through pregnancy. Notably, though, placental melatonin levels were significantly higher than those in the bloodstream (and

[49] Serón-Ferré M et al. Impact of chronodisruption during primate pregnancy on the maternal and newborn temperature rhythms. PLoS One. 2013;8(2):e57710.

[50] Vilches N et al. Gestational chronodisruption impairs hippocampal expression of NMDA receptor subunits Grin1b/Grin3a and spatial memory in the adult offspring. PLoS One. 2014;9(3):e91313.

[51] Kivelä A. Serum melatonin during human pregnancy. Acta Endocrinol (Copenh). 1991;124(3):233-7.

showed no daily rhythms). Professor Vaillancourt went on to show that the placenta was expressing melatonin-synthesising enzymes and melatonin receptors, and was indeed making its own melatonin[52].

Of significance for disease, serum melatonin levels were markedly reduced in both pre-eclampsia and fetal growth restriction. Furthermore, the activity of melatonin-producing enzymes and melatonin production itself were both found to be lower in women with pre-eclampsia[53].

In *in vitro* studies using primary trophoblast cells, melatonin had a protective effect, reducing the impact of hypoxia–re-oxygenation on oxidative stress within cells, as well as lowering inflammatory responses and reducing mitochondrial apoptosis[54]. The findings emphasise the potential value of melatonin in protecting against complications of pregnancy such as pre-eclampsia and fetal growth restriction, and indeed trials of melatonin supplementation have now begun in Australia in attempt to reduce the risk of pre-eclampsia and growth restriction[55, 56].

[52] Lanoix D et al. Human placental trophoblasts synthesize melatonin and express its receptors. J Pineal Res. 2008;45(1):50–60.

[53] Lanoix D, Guérin P, Vaillancourt C. Placental melatonin production and melatonin receptor expression are altered in preeclampsia: new insights into the role of this hormone in pregnancy. J Pineal Res. 2012;53(4):417–25.

[54] Lanoix D, Lacasse AA, Reiter RJ, Vaillancourt C. Melatonin: the watchdog of villous trophoblast homeostasis against hypoxia/reoxygenation-induced oxidative stress and apoptosis. Mol Cell Endocrinol. 2013;381(1-2):35–45.

[55] Alers NO et al. Antenatal melatonin as an antioxidant in human pregnancies complicated by fetal growth restriction--a phase I pilot clinical trial: study protocol. BMJ Open. 2013;3(12):e004141

[56] Hobson SR et al. Phase I pilot clinical trial of antenatal maternally administered melatonin to decrease the level of oxidative stress in human pregnancies affected by pre-eclampsia (PAMPR): study protocol. BMJ Open. 2013;3(9):e003788.