The dog as a sentinel species for environmental effects on human fertility

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Abstract

Despite the vast body of evidence that environmental toxicants adversely affect reproductive development and function across species, demonstrating true cause and effect in the human remains a challenge. Human meta-analytical data, showing a temporal decline in male sperm quality, are paralleled by a single laboratory study showing a similar 26-year decline in the dog, which shares the same environment. These data are indicative of a common cause. Environmental chemicals (ECs) detected in reproductive tissues and fluids induce similar, short term, adverse effects on human and dog sperm. Both pre- and post-natal stages of early life development are sensitive to chemical exposures and such changes could potentially cause long term effects in the adult. The environmental 'pollutome' (mixtures of ECs) is determined by industrialisation, atmospheric deposition and bioaccumulation and characterises real-life exposure. In Arctic ecosystems, dietary and non-dietary chemical contaminants are detectable in biological tissues and linked with adverse health effects in both dogs and their handlers. In the female, such exposure could contribute to disorders such as ovarian insufficiency, dysregulated follicle development, ovarian cancer, and polycystic ovarian syndrome. In the dog, ovarian chemical concentrations are greater in the testis. In addition, preliminary studies indicate that dietary exposures may influence the sex ratio in the offspring in favour of females. Within this article, we review current knowledge on chemical effects on human reproduction and suggest that the dog, as a sentinel species for such effects, is an essential tool for addressing critical data gaps in this field.

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Sentinels: a historical perspective

In the early twentieth century, coal miners exploited the enhanced sensitivity of the canary as an early warning system to carbon monoxide. As soon as the canary birds fell off their perch, miners had time to respond to ensure their own safety (reviewed [Burton 2014](#)). This highly cited example of the use of an animal as a bio-monitor illustrates the utility of a sentinel species for assessing risk to human well-being when exposed to environmental hazards (Bowser & Anderson 2018). Since the use of canaries in coal mines, a range of other species have been proposed as sentinels for infectious agents, food hazards, or toxic substances present in the immediate environment ([Rabinowitz et al. 2010](#)). Some key examples of these are outlined in [Table 1](#).

While not demonstrating true cause and effect, sentinel species have been used as an index of environmental pollution in soil, air, plants, water, and human habitats. For example, the common earthworm can provide information on the detritus in the soil in

addition to the dietary exposure of birds that prey on such species. Another example is that of sheep, providing an index of the exposure from plant material ingested throughout grazing and any soil swallowed in the process. In the aquatic world, sentinel species include oysters, fish, and animals that obtain their food from the aquatic environment, such as seals, dolphins, and killer whales. With respect to the latter, bioaccumulation of PCBs through the food chain has been associated with a decline in species number, placing the highly contaminated killer whale at a high risk of population collapse ([Desforges et al. 2018](#)) and eliciting severe health consequences ([Haraguchi et al. 2006](#), [Alava & Gobas 2016](#), [Kurt-Karakus et al. 2019](#)).

In the human household, dogs and cats share our environment more than any other species and are exposed to household contaminants similar to that of humans. In the Western world, dogs tend to live and travel with their owners and are thus exposed to not only the same infectious agents, but also to non-infectious environmental factors, such as chemical pollutants, of

Table 1 Animal models proposed as sentinel models for the study of environmental exposure to endocrine disrupting chemicals.

Proposed model	Specific area of study	Evidence	Reference
Companion animals			
Feline	Low-level chronic exposure to PBDEs	Empirical	Dye et al. (2007)
Canine		Empirical	Schilling et al. (1988) , López-Alonso et al. (2007) , Sonne et al. (2010, 2020) , Lea et al. (2016b)
Canine/feline	Metal concentration in tissue		
Production animals	Exposure of chemicals within environment	Empirical	Schilling & Stehr-Green (1987)
Bovine/ovine	2,3,7,8 Tetrachlorodibenzodioxin	Empirical	Wahl & Reif (2009) , Petro et al. (2010) , Lea et al. (2016a)
	Sperm quality		
	Tissue and fluid concentrations of PCB, OCPs, and PBDEs		
Wildlife species			
Primate		Empirical	Mitchell et al. (2008)
	Germ cell differentiation		
	Testicular germ cell tumour		
Mink	Mercury and PCBs	Review	Basu et al. (2007)
Marine mammals	Anthropogenic toxins	Case Study and review	Bossart (2006) , Sonne (2010) , Jepson et al. (2016) , Sonne et al. (2019)
General reports			
Domestic animals	Endocrine disruptors	Review	Majdič (2010)
Mammals	Toxic environmental contaminants	Review	O'Brien et al. (1993)
Animals	ECs	Review	Van Der Schalie et al. (1999)
Companion animals		Review	Schmidt (2009)
	Public health		
	Environmental contaminants		

ECs, Environmental chemicals; OCPs, Organochlorine pesticides; PBDEs, Polybrominated diphenyl ethers; PCBs, Polychlorinated biphenyls.

household or recreational origin. It follows therefore that environmentally linked health conditions in the dog may be mirrored in the owners. Sentinel species typically provide a range of tissues for chemical analysis post-slaughter. In contrast, the routine surgical neutering of hundreds of thousands of dogs and cats provides easy access to surplus reproductive tissues which could be used for this purpose. Furthermore, after routine neutering, the male reproductive tract can be further utilised to provide sperm from the tail of the epididymis ([Ponglowhapan et al. 2006](#)). Another method by which the dog may be used as a sentinel is through the provision of sperm as part of routine fertility monitoring. This is a procedure tolerated by many species ([Hermansson & Forsberg 2006](#)) and provides a further, cost effective, resource for monitoring contaminants and the quality of the ejaculate. For these reasons, our recent work has focussed on the domestic dog as an index of the reproductive health of humans, particularly as it shares the same environment.

Temporal trends in human reproductive and metabolic health

In humans, perturbed reproductive potential is characterised by reports of declining semen quality over several decades, an increase in incidence of testicular cancer, malformations of male babies at birth, precocious female puberty, premature menopause, and

ovarian cancer. These temporal changes are not limited to reproductive parameters, since a striking increase in global obesity, other cancers, and metabolic syndrome have occurred over the same period ([Borch-Johnsen 2007](#)).

Although the precise drivers behind these temporal trends remain uncertain, ECs have been implicated as playing a role alongside Westernised lifestyles and gene-environment interactions ([Ellulu & Jalambo 2017](#)). In support of this hypothesis, many chemicals have been described as endocrine disrupting, obesogens, and metabolic disrupting, raising considerable concern over their effects on animal and human health ([Landrigan et al. 2018](#)).

Male semen quality and testicular dysgenesis syndrome

There is an increasing body of published evidence to indicate that human male fertility and/or reproductive health has declined over the last 40 to 60 years ([Levine et al. 2017](#)). Meta-analytical studies suggest an approximate 50% decline in sperm concentration over the past 70 years, averaging a 2% decline per annum ([Carlsen et al. 1992](#), [Swan et al. 2000](#), [Levine et al. 2017](#)). Alarming, this rate of decline appears to show no 'levelling off' in more recent years and an increasing proportion of men with less than 40 million sperm per mL of ejaculate has been reported ([Sharpe 2012](#)).

Other parameters of sperm function, such as motility and morphology, have been less extensively reported. There are reports indicative of a decline in percentage morphologically normal sperm along with a decline in sperm count over a 17-year period limited to France (1989–2005) (Rolland *et al.* 2013). More recently, two separate studies have reported a temporal decline in total motile sperm count at fertility centres in Europe and North America from 2002 to 2017 (Chang *et al.* 2018, Tieggs *et al.* 2019). Such adverse temporal trends have been linked with additional temporal global increases in reproductive perturbations, such as testicular cancer (TCa), and genitourinary abnormalities, such as cryptorchidism and hypospadias.

Collectively, the adverse reproductive trends present today are termed testicular dysgenesis syndrome (TDS) (Skakkebaek *et al.* 2016). Over a 10-year period, the global total incidence rate of TCa has increased by 5.6%, with higher incidences in developed vs undeveloped countries (Park *et al.* 2018). Similarly, subtypes of TCa, including seminoma and nonseminomas, increased annually by 3.26% and 1.15%, respectively, with significant variation between racial and ethnic populations (Ghazarian *et al.* 2018). TCa incidence appears to have increased incidence at a younger age, adding further evidence of a possible environmental aetiology (Pishgar *et al.* 2019).

The human sperm quality debate

Adverse temporal trends in male sperm quality have raised much controversy ever since the concept was first proposed by Carlsen *et al.* (1992) (Pacey 2013). This is predominantly a result of limitations in the heterogeneity of meta-analytical publications, in addition to the inclusion of historical data sets (Carlsen *et al.* 1992, Swan *et al.* 2000). Developments in semen analysis have led to a shift towards the use of haemocytometer techniques as opposed to the Makler Chamber. Indeed, when applying modern technologies with increased precision, sperm count values have been reported to be 1.5–2.7 times lower, thus giving the impression of a decline in sperm quality (Pacey 2013). In subsequent studies, the exclusion of those that did not use a haemocytometer and the utilisation of standardised PRISMA and MOOSE meta-analytical protocols still continued to show a decline in fertility (Levine *et al.* 2017). Furthermore, a decline in total motile sperm count between 2002 and 2017 has recently been reported. This was based on the analysis of data generated from just two laboratories (Tieggs *et al.* 2019).

The dog as a sentinel for human environmental chemical exposure

The dog lives in close proximity to humans, where individuals are exposed to similar environmental

factors. Parallel temporal trends of semen quality have been reported in the dog sentinel model, with an overall decline of 30% in progressive sperm motility over a period of 26 years (Lea *et al.* 2016b). In contrast to the human meta-analyses, the dog study used semen quality parameters collated from a single laboratory by staff with standardised training, promoting consistent analytical methodologies. Consequently, the confounders that complicate the interpretation of meta-analytical studies in the human do not apply and this in turn adds to the weight of evidence for a decline in semen quality in both species.

Genetic, epigenetic, or lifestyle influences on these reproductive trends cannot be ruled out, particularly with respect to the many potential confounders that account for the noise in the human studies. In addition to changing methodologies, tight underwear has been linked with poor sperm quality and other factors such as smoking, alcohol, physical activity, adiposity, and cell phone usage have also been suggested as possible factors (Mínguez-Alarcón *et al.* 2018, Kaya *et al.* 2020). In the dog study, the list of possible alternative causes for declining sperm quality is smaller, mostly due to the ‘non meta-analytical approach’ used. In addition, within the population of stud dogs studied, heritability measures for a range of sperm quality parameters, including reduced motility, were low (England *et al.* 2010). Given that there was no influence of breed, body weight, or sire, an environmental aetiology would appear to be the most likely cause.

Over the same period as the adverse trends in dog sperm motility, an increased incidence rate of cryptorchidism in male pups was observed from the same population of stud dogs (Lea *et al.* 2016b). Preliminary evidence suggests that the incidence of testicular tumours in the dog has increased over a 40-year period and that the histological characterisation of such tumours is similar to that reported in the human (Grieco *et al.* 2008a). In total, these studies suggest that the dog exhibits the same range of reproductive abnormalities as reported in the human (Grieco *et al.* 2008b, Ghazarian *et al.* 2018). Such trends demonstrate the importance and worth of the dog as a sentinel species for environmental influences on human reproductive health. However, in contrast to the ‘canary in the coalmine scenario’, the mechanism underlying these reproductive temporal trends remains uncertain. Although we cannot be certain of a common trigger in both species, there is a clear commonality in the temporal reproductive trends observed and thus the proposal is that the underlying aetiology is environmental and consistent in both the dog and human.

The environmental ‘pollutome’

Despite the ongoing debate surrounding reproductive perturbations, the weight of evidence suggests a common

aetiology involving the interaction of a complex group of anthropogenic ECs (Bellingham *et al.* 2009, Cabrera-Rodríguez *et al.* 2019, Sumner *et al.* 2019). There exist more than 14,000 classified environmental pollutants inclusive of, but not limited to, organic chemicals, heavy metal ions, and trace elements (Jeon *et al.* 2015, Daoud *et al.* 2017, Dusanov *et al.* 2018). A multitude of *in vitro* studies have sought to investigate the pathogenesis and mechanisms by which ECs disrupt endocrine and metabolic functionality, resulting in a number of reproductive and non-reproductive perturbations. However, such research is limited in its extrapolation to the human population due to the complexity of chemical mixtures involved in real-life exposure. This further emphasises the importance of alternative models including the utilisation of a sentinel species.

Organic ECs are typically utilised for industrial and agricultural processes due to their properties as plasticisers, pesticides, and solvents (Chen *et al.* 2019). Dependent on the congener, ECs are present in a variety of industrialised resources (Fig. 1). ECs are released into the environment either through direct emission from the production process or migration from products themselves (Wania 2003). The latter is possible due to the inability of many ECs to chemically bond to the matrices in which they originate, leaching into the surrounding environment (Lenoir *et al.* 2016). Present in air, water, soil, and vegetation, ECs are consistently available for uptake by humans and other species on a global scale (Terzaghi *et al.* 2018). In reference to the agricultural industry, the use of EC contaminated sewage sludge for pasture fertilisation and organochlorine pesticides is a primary concern due to studies demonstrating detectable levels of repro-toxic ECs including phthalates and Bisphenol-A (BPA) (Rivera *et al.* 2009, Tran *et al.* 2015).

Industry plays a major role in global pollution. The global distribution of chemicals facilitates the spread and atmospheric deposition of ECs in remote non-industrialised areas of the world, thus EC contamination is ubiquitous. This is particularly true for persistent organic pollutants (POPs) – ECs including PCBs, PBDEs, organochlorine pesticides and dioxins – that are

categorised by extended half-lives and high lipophilicity, making them bio-persistent in nature (Wania 2003). Numerous chemical congeners have thus been detected in a range of biota and in ecosystems in areas once considered isolated from anthropogenic influence including ant cuticles from the deep Amazonian rain forest; Amazonian caymen and fish; polar zones including the Arctic ecosystems; and endemic arthropods in the Mariana and Kermadec trenches, the two deepest oceanic trenches (Wania 2003, Lenoir *et al.* 2016, Jamieson *et al.* 2017). Shockingly, EC levels have also been detected in Arctic ecosystems. In coastal areas, the Arctic fox has been identified as a sentinel for contaminant monitoring in humans, particularly in relation to the element mercury from a marine diet. In addition, sled dogs living with their handlers have been identified as sentinels to both dietary and non-dietary environmental contaminants (Harley *et al.* 2016). The concept of the sled dog as a sentinel model to monitor arctic ecosystems has recently been highlighted by Sonne and colleagues (Sonne *et al.* 2020).

Marine ecosystems are the final destination for many persistent ECs. Aquatic species are thus exposed to ECs and these tend to accumulate within the adipose tissue (Jeong *et al.* 2019). Elevated bodily burdens of lipophilic chemicals have been found to increase at each trophic level, resulting in bio-magnification in species with higher blubber content and longer life spans, such as apex predators. Consequently, such apex predators are of great scientific interest for further understanding the effect of chronic EC exposure and substantial levels of contamination. An interesting comparison is that of three aquatic species displaying high blubber content with differing diets, from the herbivorous Dugong dugon (otherwise known as the sea cow), to the finless porpoise (surviving mainly on a fish based diet), and finally the killer whale (with a main dietary source of contaminated seal blubber). The Dugong dugon has reported bodily burdens of PBDE of approximately 120 ng/g lipid weight (lw) (Weijs *et al.* 2019). In Korean coastal line populations, the finless porpoise, an endangered marine mammalian species, is reported to have PBDE concentrations at around 294 ng/g lw (Jeong *et al.*

Figure 1 Overview of sources giving rise to environmental chemicals. This figure illustrates sources in which chemicals present within the environmental 'pollutome' could potentially contribute to reproductive and developmental perturbations.

2019). In the male southern Pacific resident killer whale, PBDE levels are reportedly eight times greater than that of the Dugong dugon (Rayne *et al.* 2004). Although geographical variation in the environmental 'pollutome' of each habitat will vary, higher concentrations in killer whale populations are likely due to the consumption of contaminated seal blubber leading to the bio-magnification of these lipophilic ECs. The reported concentrations in each of these species demonstrate the ability of ECs to bio-magnify at each trophic level, but also highlight exposure routes other than the consumption of contaminated meat, raising additional concern of reproductive health in herbivorous species. Given that the three species discussed are endangered populations, concerns of further population decline due to the repro-toxic nature of ECs have been raised.

Due to EC congeners being utilised for specific industrial or agricultural purposes, it follows that the profile of contaminants constituting the 'pollutome' could reflect this variation (Fig. 1). To our knowledge, present research is restricted to geographical variation in EC levels, with limited understanding of contaminant profiles in relation to specific industry processes. Assessing ecotoxicological risks associated with exposure to a multitude of ECs are essential due to their synergistic toxicity (Jeong *et al.* 2019). To assess industrially associated contaminants, ants have been proposed as a valuable bio-monitoring species for environmental contamination and the chemical profiling of differing geographical regions due to the abundance of ant populations on a global scale (Wania 2003). While research in this area has the ability to advance understanding in specific EC profiles and potentially predict the subsequent interactions of ECs within humans, there is a distinct need for a sentinel that closely shares our environment. It is for these reasons that we propose the dog as a sentinel species for better assessing human exposure to ECs.

Geographical differences in chemical exposure

As discussed, EC concentrations present within the environment fluctuate depending on the geographical location, level of industrialisation, and population density. Given the contribution of industry to pollution, distinct regional variation in reproductive perturbations are reported, with higher incident rates in industrialised areas (Lin *et al.* 2017). Sperm samples collected from sub-fertile men in Middle Eastern and North-African regions have been reported to have lower sperm motility and morphology, alongside increased DNA damage (Elbardisi *et al.* 2018). In the Western world, a temporal decline in sperm quality includes North America, Europe, Australia, and New Zealand (Levine *et al.* 2017). In the same meta-analysis, no such trends were found in South America, Asia, and Africa. Reports had previously shown higher semen quality and a lower incidence

of TCa in Finland, compared to other neighbouring countries such as Denmark (Znaor *et al.* 2014, Virtanen & Toppari 2015). More recent studies now indicate that the incidence of TCa in Finland is increasing and that sperm quality is converging to the lower quality levels of Danish men (Rodprasert *et al.* 2019). By contrast sperm quality and TCa rates in Denmark have remained relatively stable (Priskorn *et al.* 2018). While geographical variations in sperm quality and TCa have been reported, data demonstrating a causal relationship with ECs are contentious (Foster *et al.* 2008). In this regard, the dog constitutes an appropriate sentinel model for monitoring geographical differences in seminal or testicular EC concentrations. Figure 2 illustrates that, in the dog, the testicular chemical profiles vary across different areas of the United Kingdom.

Factors influencing tissue profiles of chemicals include diet, obesity, environment, and even genetics. Increased numbers of Westernised fast food chains in these areas have led to a diet consisting of products higher in fatty foods and animal products (Elbardisi *et al.* 2018). Since persistent organic pollutants are lipophilic, it follows that diet may be a key source of exposure and that this, in turn, may impact on reproductive health (Pratt *et al.* 2012). Since the number of studies carried out in more remote areas is limited, it remains uncertain

Figure 2 Geographical variation of dog testicular ECs across three regions of the United Kingdom. As shown, PBDE47, a flame retardant, was greater in samples from West England (A). PCB-153 was consistently expressed across England, even though this chemical was banned from the 1970s (B). Finally, DEHP was observed consistently in the majority of samples, other than at a greater concentration in a testicular sample from South of England (C). Data obtained as part of the data published in Lea *et al.* (2016b). Scientific report articles are published under a CC BY license allowing for maximum dissemination where users are free to adapt data. Error bars represent ± 1 S.E.M.

if fertility related perturbations are a primary problem of the industrialised world.

Chemical profiling of reproductive tissues and fluids

Returning to the dog sentinel, the availability of tissue from neutering procedures provides an opportunity to determine the profile of chemicals in testis and ovary. A total of 12 chemicals across three chemical types have been detected in both testis and ovary (Lea *et al.* 2016b, M.E. Van der Mescht, unpublished observation). These comprise PCBs, PBDEs, and the phthalate DEHP. These chemical types have also been detected in the dog ejaculate and from the limited studies carried out in the human. Such chemicals are likely reflective of what is present in the human. Since the basis of the sentinel paradigm is that ECs have similar effects in both species, two chemicals predominant in testis and seminal fluid, DEHP and PCB153, have been shown to have similar effects on dog and human sperm (Sumner *et al.* 2019). Gonad-relevant concentrations of these chemicals reduced sperm motility and increased DNA fragmentation in both species. This work demonstrates that the development of this research field could be achieved by integrating the use of a sentinel model in combination with *in vitro* experiments.

Real-life exposure to chemical mixtures

Real-life exposure to chemical contaminants constitutes exposure to complex mixtures of chemicals, which inevitably interact, thus influencing the overall biological or toxicological effect. Since the 1950s, it is estimated that more than 140,000 new chemicals have been produced and many of these are released into the air, sea, and soil (Landrigan 2017). Geographic variability will inevitably reflect different degrees of regional industrial activity. Since many chemicals are non-biodegradable and exhibit bioaccumulation and bio-magnification, this constitutes a concern of global magnitude. For these reasons, determining biological responses to real-life mixtures of chemicals presents a challenge to the establishment of a link between environmental contaminants and altered fertility or reproductive function.

Responses to chemicals may be dependent on whether chemicals in a mixture, as well as their respective metabolites, share similar or differing modes of action (Chen *et al.* 2019). Exposure to multiple ECs is reported to have a greater adverse effect compared to exposure to a single contaminant (Woodruff *et al.* 2011). Some *in vitro* studies have utilised commercially available mixtures of chemicals in toxicological *in vivo* studies. For example, in the mouse, the 50-day administration of a commercial mixture of PCBs (Aroclor 1254) by oral lavage every 3 days induced a significant decrease in testicular weight and increased degenerative testicular alterations.

Interestingly no changes were seen at lower doses (0.5–50 µg/kg) (Cai *et al.* 2011). Environmental chemicals have been shown to mimic hormones such as prostaglandin and progesterone to activate CatSper channels invoking a Ca²⁺ ion increase. In particular, a metabolite of the pesticide Dichlorodiphenyltrichloroethane (DDT) has been reported to activate CatSper channels on sperm membranes (Schiffer *et al.* 2014), inducing hyper-activation of sperm. While this report demonstrates a potential mechanism of action for ECs to influence sperm quality, not all chemicals investigated invoked effects in this manner. It has been suggested that the observed chemical effects might be specific to human sperm. Some ECs that induced an influx of Ca²⁺ ions in human sperm had no effect in a mouse model (Schiffer *et al.* 2014). In combination, the ECs assessed by Schiffer and colleagues on human sperm evoked greater Ca²⁺ ion responses like that of progesterone *in vivo* (Diamanti-Kandarakis *et al.* 2009). The determination of EC mechanism of action is difficult due to the lack of an appropriate animal model or sentinel.

One approach, more meaningful from a biological perspective, is to use chemical mixtures relevant to everyday exposure to see if chemicals act in synergistic or additive manners. In this regard, the dog has been used to evaluate chemical mixture effects administered in a diet composed of minke whale blubber, contaminated with a cocktail of bio-accumulated chemicals. The feeding of blubber to male sled dog pups elicited direct and trans-generational effects, resulting in significantly reduced testes weight. In addition, the offspring of mothers fed that a total of 20 kg of blubber from 2 months postpartum to time of weaning had reduced testes weight and reduced IgG antibody concentration in blood (Kirkegaard *et al.* 2010, Sonne *et al.* 2010, 2017). These effects correlated with contaminant concentrations.

In addition to the dog, the exposure of pregnant ewes to mixtures of chemicals contained within a sewage sludge derived fertiliser (bio-solids) is widely considered as a real-life model of human exposure to chemical mixtures. A wide range of reproductive and other biological effects have been reported in developing foetuses, in addition to the offspring of mothers exposed during pregnancy. These include effects on the foetal testis, ovary, hypothalamus, and pituitary (Paul *et al.* 2005, Bellingham *et al.* 2009, Fowler *et al.* 2009, Lea *et al.* 2016a).

Timing of exposure

Exposure to chemicals is likely to occur throughout an individual's life, beginning *in utero*, continuing postpartum, throughout adolescence, adulthood, and gametogenesis (Fig. 3). The intrauterine environment is where the foetus is most susceptible to the exposure of endocrine disruptive ECs, due to an endocrine mediated period of growth and development (Kot *et al.* 2019).

Figure 3 Exposure to chemicals can occur throughout all points in life. Each point of exposure can have a significant impact on developmental processes further in life.

The placenta is an interface at which substrate transport between mother and foetus is mediated, although some ECs have the ability to cross the placental barrier through active transplacental transfer or passive diffusion, depending on their chemical composition (Bommarito *et al.* 2017, Cabrera-Rodríguez *et al.* 2019). A recent report measuring the concentrations of trace elements and heavy metal ions found higher concentrations of lead (Pb) in the foetal membrane and umbilical cord than in the placenta (Kot *et al.* 2019). This is suggestive of an accumulative nature of Pb and demonstrates the ability of Pb, and other chemicals, to cross the placental barrier. Mechanisms underlying this passage, however, remain unidentified (Bommarito *et al.* 2017). In mice, the exposure to particulate air pollution from industrialised areas during pregnancy has been shown to induce morphological changes to the placenta (Veras *et al.* 2008). Changes to the integrity of the placenta could lead to a higher rate of chemical diffusion from mother to foetus, although this remains speculative at this time.

Although susceptibility to the adverse effects of ECs remains high *in utero*, new-borns are still at risk of endocrinological alterations through EC exposure (Wineland *et al.* 2019). In humans, mini-puberty occurs 1 to 12 weeks postpartum and incorporates a hormonal surge, inducing gonocyte development (Hadziselimovic *et al.* 2005). Exposure to ECs with endocrine disrupting properties during this time, such as via fatty breast milk, could have additional adverse effects on sexual development and maturation (Pajewska-Szmyt *et al.* 2019).

Breast milk is suggested as a main EC exposure route to new-borns (reviewed in Pajewska-Szmyt

et al. 2019). Due to the lipophilic properties of some ECs including PCBs, Polychlorinated dibenzo-dioxins (PCDDs), and polychlorinated dibenzo-furans (PCDFs), primary deposition occurs in lipid droplets in adipose tissue, including in mammary tissue (Lee *et al.* 2017). During milk production, free fatty acids and lipoprotein stores are utilised, along with the release of ECs that have accumulated there (Pratt *et al.* 2012). Despite the lower affinity of chemicals such as BPA and other bisphenol analogues to adipose tissue, concentrations are detected in breast milk samples (Deceuninck *et al.* 2015). Detectable concentrations in breast milk have decreased following more stringent regulations of chemical usage (Pratt *et al.* 2012, Fång *et al.* 2013). Geographical variation is also present within breast milk samples where significant differences in POP congener type and concentration have been reported evident between countries (Antignac *et al.* 2016). Ingestion of chemicals by breastfed infants during mini-puberty could induce adverse effects on endocrinology and genitourinary development, having both immediate and chronic effects.

ECs are reported to adversely affect reproduction through endocrinological interactions, compromising gametogenesis (Daoud *et al.* 2017, Ianos *et al.* 2018). It has also been suggested that EC exposure may, in some cases, manifest long after initial exposure due to epigenetic modifications originating from exposure at the critical window of genitourinary development in the foetus and new-born (Thankamony *et al.* 2016, Bommarito *et al.* 2017). By having the potential to interact with DNA transcription, and more specifically the molecular intricacies involved in gametogenesis, adverse effects will be present throughout an individual's life, with potential multi- and even transgenerational effects. Consequently, EC-induced adverse trends in semen quality and male fertility, as highlighted previously, could result from a combination of epigenetic modifications originating from early life exposure, cumulative concentrations of high lipophilic substances, and acute exposure to chemicals at the point of spermatogenesis, although this is only speculative.

A clear difference between the dog and human is the period of exposure due to differences in longevity. Although this may contribute to the noise observed in the human data, chemical effects are also influenced by diet, adiposity, the physico-chemical properties of the contaminants, mixture effects, metabolism, and possibly other factors that are less understood. It is therefore noteworthy that despite these many complex influences, the parallel between-species trends in reproductive function remain.

Female reproductive development and function

Historically, concerns over declining trends in male reproductive health have preceded those in the female

and this purely reflects the relative ease of empirical study in the male. Since there are similarities in the early ontogeny of the developing male and female reproductive tracts, it has been suggested that early developmental perturbations in the female may manifest as impaired fecundity and/or altered reproductive function in later life (Buck-Louis *et al.* 2011). A key reproductive process that has particular sensitivity to environmental change is folliculogenesis. In the human, this highly regulated process that follows meiotic arrest of the oocyte occurs *in utero*, and the number of oocytes that determine female reproductive lifespan is set prior to birth. In contrast, a majority of follicle development in the dog and mouse occurs after birth, thus providing experimental access to the very earliest stages of development that are difficult to access in the human (Reynaud *et al.* 2012, Sarraj & Drummond 2012).

In the human, the dysregulation of foetal ovarian development due to maternal smoking has been linked with early menopause (Tawfik *et al.* 2015) and there is increasing evidence of a link between premature ovarian insufficiency and exposure to environmental toxicants (reviewed in Vabre *et al.* 2017). In addition, widespread environmental pollutants, such as Bisphenol A, have been reported as potential contributors to the pathogenesis of polycystic ovarian syndrome (PCOS) (Palioura & Diamanti-Kandarakis 2015, Hu *et al.* 2018, Soave *et al.* 2020). These observations provide support for the concept of 'ovarian dysgenesis syndrome (ODS)' with additional linkages to fertility, ovarian cancer, and PCOS.

In the dog, although there is little evidence of an environmental impact on female fertility, easy access to ovaries removed at spaying does provide a means of assessing chemical contaminants directly in the gonad. In this regard, our own studies have shown that known environmental toxicants are present in the dog ovary, in some cases at higher concentrations than detected in the testis. Similar contaminants have also been detected in milk from lactating bitches (Fig. 4). Furthermore, contaminants comprising PCB congeners, PBDE congeners, and plastic-derived phthalates have been detected in commercial dog food (Lea *et al.* 2016b).

This raises the possibility that the perturbation of female reproductive development may occur through exposure to contaminants in the diet delivered in milk to the pups and/or via food to the mature bitch. As alluded to earlier, the partitioning of maternal lipophilic chemicals into her own high fat content milk provides a natural mechanism by which her chemicals in adipose tissue are off-loaded (Lehmann *et al.* 2015). The problem is that the primary source of nutrition for the neonate also becomes a source of exposure to the 'maternal legacy' of lipophilic chemical contaminants. For example, in the polar bear, PCB concentrations in the suckling cubs are higher than in the adults and the concentration found in the milk exceeds maternal dietary intake (Polischuk

Figure 4 Contaminants detected in the milk from lactating bitches. While all chemicals present are of concern, PBDE 47 (A) is the predominant flame retardant congener. Each value represents a different analysed sample.

et al. 2002, Bytingsvik *et al.* 2012, Sonne *et al.* 2020). In the killer whale, greater concentrations of ECs have been found within the calves of lactating females, indicative of transfer from mother to offspring (Haraguchi *et al.* 2009). Our data showing that both PCB and PBDE congeners are detectable in the milk of whelping bitches supports this concept and these same chemical types have been detected in human milk (Needham *et al.* 2011, Garí *et al.* 2019, Pajewska-Szmyt *et al.* 2019).

With regard to maternal environment, it has been reported that litters of Arctic maternal sled dogs fed seal blubber as a source of environmental toxicants exhibit a skewed sex ratio in favour of females (Sonne *et al.* 2010). In a separate study, we have reported that pups generated in a breeding programme of assistance dogs also exhibit a temporal trend towards an altered sex ratio in favour of females. Since stud dogs from the same programme exhibit a temporal decline in semen quality that is thought to reflect exposure to chemical

contaminants, it is possible that the feminisation of the litters may be similarly mediated (Lea *et al.* 2016b). Although direct chemical effects on the dog ovary have not been determined, chemicals detected in the ovary do appear to perturb early follicle development in a post-natal mouse ovary explant culture model *in vitro* (M.E. Van der Mescht, unpublished observation). Furthermore, in the sheep bio-solids model described previously, late gestation female lambs exposed to chemical mixtures via the mother exhibit a dysregulation in early stage follicle (primordial/transitional) development (Lea *et al.* 2016a).

Conclusion and future remarks

Environmental pollution remains a critical issue of global concern. There is now an overwhelming body of evidence to suggest that both male and female reproductive health is being adversely affected through exposure to mixtures of environmental contaminants. Understanding the mechanisms that underlie these effects is fraught with complexity, particularly in terms of selecting the appropriate tools to investigate environmentally relevant 'chemical cocktail' effects on the human. An invaluable experimental strategy is to identify a species in which environmental chemical exposures induce biological effects which approximate those reported in the human. In this regard, the domestic dog that shares our everyday household environment constitutes a valuable species for evaluating such effects on human reproductive health. We postulate therefore that, in combination with *in vivo* models, such as bio-solid exposed sheep, *in vitro* rodent studies, and human biomonitoring, the sentinel household dog provides an invaluable contribution to our understanding of toxicant effects on human male and female reproductive well-being.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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Author contribution statement

R G L conceived and designed the review article. R N S, I T H, and R G L contributed equally to the writing of this manuscript.

R N S and R G L created the figures for the manuscript. M V D M, A B, and G C W E provided information, data, and in-depth comments that developed this review. R N S, I T H, and R G L edited the manuscript for grammar and content.

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