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Perfluorooctanoic Acid (PFOA) an Environmental Pollutant: Threat to Human Health

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ABSTRACT

All of us now carry in our body a virtual stew of heavy metals and hundreds of synthetic chemicals: persistent ones, which can have a "half-life" in the body of several years; and non persistent compounds, which may pass through the body in a matter of hours. Research has revealed that Perfluorooctanoic acid (PFOA) is now ubiquitous environmental contaminants which are bioaccumulating in wildlife and humans all over the world and can alter the reproductive system of laboratory animals even at extremely low exposure levels. This is relevant because PFOA is chronically present in our environment with the potential for constant exposure, making it functionally equipment to a persistent compound. This review emphasizes particular outcomes that occur in response to the relevant dose of PFOA exposure that cause developmental effects on reproductive system, and metabolic process, and the male and female germ line. At a specific dose level, PFOA exposure also shows oxidative toxicity and carcinogenic effects.

KEYWORDS Perfluorooctanoic acid, carcinogenic

Introduction

Perfluorooctanoic acid (PFOA) is a member of the perfluoroalkyl acids (PFAAs) family of compounds. These compound are man made synthetic chemicals which have a carbon backbone with hydrogen replaced by fluorine and include a carboxylic functional group. The fluorine content renders PFAA (Perfluoroalkyl acid) stable, inert, oleophobic and hydrophobic, and resistant to high temperatures (Prescher ***et al***., 1985; Key ***et al.,*** 1997). Due to the presence of strong carbon fluoride bonds, it is practically no biodegradable and highly persistent in the environment (Lau ***et al.,*** 2007). Firstly the DuPont chemical plant in Washington, West Virginia, began using PFOA in its manufacturing process in 1951 (Ylinen ***et al.,***1985) which have been produced and used in commercial products and industrial processes for over 60 years. Perfluoroalkyl acids are emerging pollutants of the 21st century and have a global distribution in the environment and wild life (Giesy ***et al.,*** 2001) including humans and have shown adverse effects. It is used in the manufacture of fluoropolymers and fluoroelastomers and is present as a component of some of the top antireflective coating materials in use today. Fluoropolymers are widely used as surfactants in the textile, paints, waxes, polishes and manufacturing such as lubricants, medical equipment, electronics, food packaging and fire resistance due to their oil, stain, grease and water resistant properties which makes them ideal for creating a non-stick surface for cookware and protective coatings on clothing and carpeting and are also valuable to the aerospace industry as well. Fluoroelastomers are a family of synthetic rubbers that can be repeatedly stretched and still return to their original shape, such as Viton (Lindstrom ***et al.,*** 2011).

While they provide societal benefit, there is concern, however, about the potential adverse ecological or human health impacts of PFASs as they are persistent and ubiquitously found in the environment and have been detected in air, surface waters, and soils and in a variety of mammals, birds, and fishes around the world, and have exhibited liver, developmental, immune, and endocrine toxicity in animal models (Castiglioni ***et al.,***2014). Unlike most other persistent and bioaccumulative organic toxicants, PFOA is water-soluble and does not bind well to soil, allowing for easy transportation through and contamination of human drinking water. PFOA has an exceedingly long half-life in humans and, posing harmful effects due to accumulation in organs (Hundley ***et al.,***2006). Consistent median PFOA serum levels of 2–8 ng/ml have been found in various industrial countries around the world (Vestergren and Cousins, 2009). Exposure to PFOA can cause tumor and non tumor effects on the immune and nervous systems and adversely affect hepatic function, reproduction, and development (Post ***et al.***2012; Shi ***et al***. 2013; Yan ***et al.***2014).

Perfluorooctanoic acid (PFOA) (CAS No. 335-671) is a perfluoroalkyl acid (PFAA) having the structure with molecular formula: $C_8HF_{15}O_2$.Synonyms to PFOA 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluorooctanoic acid; perfluoroheptanecarboxylic acid; perfluoro-n-octanoic acid; Fluorad FC-26; perfluorocaprylic acid.

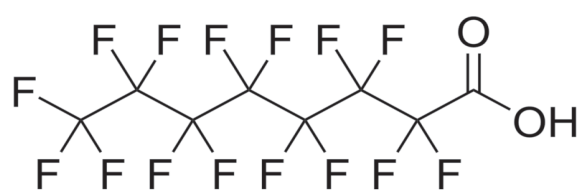

FIG. 1 - STRUCTURE OF PFOA

PFOA has been detected in the blood of more than 98% of the general US population in the low and sub parts per billion ranges, and levels are higher in chemical plant employees and surrounding subpopulations (Nicole, 2013) and has been identified in human tissue samples, including liver, kidney, adipose tissue, brain, basal ganglia, hypophysis, thyroid, gonads, pancreas, lung, skeletal muscle, and blood from non occupationally exposed subjects (Kato ***et al.,*** 2011). It is easily absorbed via the gastrointestinal tract and binds to serum albumin and can cross the blood-placenta border in a facilitated way and enter the fetus where it is mainly found in the liver (EFSA, 2008).

By far studies summarizes that PFOA exposure results into many health problems like it may act as a carcinogen, liver toxicant, an immune system toxicant, and also exerts harmful hormonal effects (Lau ***et al.,*** 2007), in addition with a developmental toxicity that reduces birth size, physical developmental delays, endocrine disruption (Betts, 2007). Concern has been raised regarding overall adverse health effects of $PFAA_s$ including effects on the reproductive system (Olsen ***et al.,***2009).

Exposure assessment

The existence of PFAAs (Perfluoroalkyl acids) in the human body was first suspected in the late 1960s when fluoride in blood samples was found to be partially bound to organic compounds

of unknown structure (Taves, 1968). For the assessmemt of the human exposure to PFOA, different pathway have to be considered but in general there are two important sources of exposures to humans ingestion of contaminated food and drinking water and inhalation of contaminated air and house dust and to a lesser extent dermal absorption is also observed. The uptake of PFOA in children on a body weight basis is higher compared to adults because of a higher relative uptake from food and hand- mouth transfer from treated carpets and ingestion of dust. It was also detected in breast milk and amniotic fluid (NCM, 2013) and essentially non-volatile, the general public would not be expected to be exposed via inhalation (COT, 2005). PFOA can enter in the environment from direct and indirect sources. Direct sources include the production and use of PFOA, or containing products, whereas indirect sources are reaction impurities or biodegradation of related compound, for example- N-methyl perfluorooctane sulfonamide ethanol (N-MeFOSE), perfluoro sulfonamides, and fluorotelomer raw materials (Prevedouros ***et al.,*** 2006).

Direct emission or accidental escape of PFOA to the environment can occur during their manufacture and application to consumer articles like furniture, insulation of electrical wires, etc. (Stock ***et al***., 2004, Prevedouros ***et al.,*** 2006). An important source of PFOA into the environment is thought to be the discharge of waste water from sewage treatment works, as the cleaning and care of surface-treated products (from clothing to carpets) by consumers and use in industrial processes are believed to release these compounds to municipal wastewater treatment systems (Boulanger, 2005; Higgins ***et al.,*** 2005). PFOA can then enter the aquatic environment and find their way into aquatic food webs. Discarded consumer articles containing PFOA may also contribute to the environment by leaching from landfills (Stock ***et al***., 2004; Boulanger, 2005). Sinclair ***et al***.,(2007) found that measurable PFOA released from several brands of nonstick cookware when heated which suggests that residual PFOA from the manufacturing process may remain on the surface and can be off-gassed when heated at normal cooking temperatures and also present in the vapors that released from prepackaged microwave popcorn bags (Olsen ***et al.,*** 2003).

Perfluoroalkyl compounds are considered to be environmentally persistent chemicals and resistant to biodegradation, direct photolysis, atmospheric photooxidation, and hydrolysis. The carbon atoms of the perfluoroalkyl chain are protected from attack by the shielding effect of the fluorine atoms; furthermore, environmental degradation processes generally do not possess the energy needed to break apart the strong fluorine-carbon bonds (ATSDR, 2009). So, due to chemical stability, PFOA is not metabolized and is eliminated slowly in humans with an estimated elimination half-life of 3 to 4 years and < 24 h in female and < 9 days in male rats, of 21 – 30 days in Cynomolgus monkeys have been estimated (Olsen ***et al.,*** 2007; Bartell ***et al.,*** 2010).

PFOA and Human Health

Any acute and chronic changes are the results of slow and long-term exposure of PFOA. Although PFOA affects human differently under various doses, we have considered the following criteria as major effects of long term exposure.

Reproductive system

We discuss here the effects of PFOA on male and female fertility. Studies reported that, both male and female reproductive systems are affected by the PFOA.

Exposure of PFOA may cause reduced testosterone levels and increased estradiol levels (Lau ***et al***., 2007). Joensen ***et al.,*** 2009 reported the effects on testicular function and decrements in sperm count and number of morphologically normal sperm with higher exposure to the combined level of PFOA and PFOS in humans lower sperm quality have a harder time conceiving children. A study on sexually mature mice indicated that PFOS exposure might affect testicular signalling, causing reduced serum testosterone and decreases in epididymal sperm counts (Wan ***et al.,*** 2011).

It also disrupt the blood testis barrier (BTB) which is a potential reason for reproductive dysfunction because BTB prevents the entry of harmful endogenous substrates and exogenous contaminants, thereby providing a suitable environment for spermatogenesis and but PFOA exposure disrupted BTB integrity and caused immune privilege and harm to the reproductive system, resulting in reproductive dysfunction (Yin, 2015). PFCs at environmentally relevant concentrations were associated with differences in sperm head, morphology, and DNA characteristics, including differences indicative of higher and lower semen quality. These exploratory findings suggest some deleterious differences in sperm morphology (e.g., immature, bicephalic) but await corroboration. Follow-up investigation of the impact of semen changes on male reproductive health or couple fecundity is needed, including in-depth semen analyses (Germaine ***et al.,*** 2015).

PFOA and PFOS affects sex hormones, homeostasis, increases the incidence of pregnancy loss and decreases the number of regular estrous cycle (Case ***et al***., 2001; Austin ***et al***., 2003; Lau ***et al***., 2007; Wolf ***et al***., 2007). The association between PFOA level and irregular menstrual cycle was found and the fetal developmental disruptions which may have adverse consequences for health later in life (Barker, 2004; Crain ***et al.,*** 2008; Barouki ***et al***., 2012).

A recent UCLA (University of California, Los Angles) study found that women with higher serum levels of PFOA and PFOS have increased risk of infertility. Women who required greater than 12 months to achieve pregnancy had median PFOS concentrations of 38.3±13.0 (ng/ml plasma), while women who achieved pregnancy in less than one month had median concentrations of 35.5±12.8 (ng/ml plasma). A similar trend was seen for PFOA concentrations, with concentrations of 6.3±2.7 (ng/ml plasma) and 5.6±2.6 (ng/ml plasma) respectively. Women in the higher PFC category were also more likely to have irregular menstrual cycles (Fei ***et al.,*** 2009). Animal toxicology studies conducted by various researchers reports its effects on female reproduction, including altered ovarian function, histopathologic changes in the reproductive tract and delay in vaginal opening and in development of mammary gland tissue (Yang ***et al.,*** 2009; White ***et al***., 2011; Dixon ***et al***., 2012; Zhao ***et al***., 2012). Fei ***et al.,*** 2012 used data from the Danish National Birth Cohort and observed an association between higher serum levels of PFOA and PFOS in pregnant women and a longer waiting time to pregnancy. Moreover, exposure to PFOA and PFOS in levels found in the general population may reduce fecundity.

Developmental Toxicity

Over the last several decades, hundreds of experimental studies have been conducted, mostly with rats and mice, on the potential reproductive and developmental toxicity of PFOA. Gestational exposure to PFOS decreased prenatal and postnatal survival of offspring, and developmental effects included reduced fetal body weight, increased liver weight, cleft palate, edema, delayed maturation of the lung, delays in ossification of bones, and cardiac abnormalities (Lau ***et al.,*** 2003). PFOA readily crosses the placenta and is secreted in milk. In the rat, PFOA and PFOS have been detected in placenta, fetus, amniotic fluid, and milk, and these chemicals have also been found in human breast milk (Kennedy ***et al.,*** 2004; Kuklenyik ***et al***., 2004; Hinderliter ***et al.,*** 2005; So ***et al.,*** 2006).

CD-1 mice treated with PFOA daily during pregnancy from day 1 until birth by oral gavage (1, 3, 5, 10, 20, 40 mg/kg bw per day) and enlarged liver in treated dams at all dosages, but did not alter the number of implantations or malformations. The 40 mg/kg bw per day group resorbed their litters, the 20 mg/kg bw per day group had a reduced percentage of live foetuses and their weights were significantly lower. Post natal survival was significantly reduced in the 5, 10 and 20 mg/kg bw per day group. Dose dependent growth deficits were noted in all dose groups except in the 1 mg/kg bw per day dose group. Significant delays in eye opening were noted at 5 mg/kg bw per day and at higher dosages but not in the 1 mg/kg bw per

day dose group PFOA and PFOS to affect fetal growth and development (Lau ***et al***., 2006). PFOA exposures also lead to delayed and reduced body weight and eye opening and body hair growth in pups (Wolf et ***al.,*** 2007). ***In utero*** exposure to PFCs is associated with a range of nonspecific adverse developmental outcomes in mouse, rat, and rabbit models (Lau ***et al.*** 2007; Olsen ***et al***.,2009), including reduced fetal weight and increased neonatal mortality.

Oxidative Stress

Various in vivo and in vitro models have been used to assess the potential bio effects of PFOA over the past decades. It was found that exposure to PFOA could arrest cell cycle distribution, alter peroxisomal and MAP Kinase-related signalling pathways, and induce oxidative DNA damage in mammalian cells (Takagi ***et al.,*** 1991;Shabalina ***et al.,*** 1999; Yao ***et al.,*** 2005; Fang ***et al.,*** 2009).

Cultured freshwater tilapia (***Oreochromis niloticus***) hepatocytes were exposed to PFOS or PFOA which resulted in a significant induction of reactive oxygen species (ROS) accompanied by increases in activities of superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR), demonstrating that PFOA produced oxidative stress and induced apoptosis with involvement of caspases in primary cultured tilapia hepatocytes(Liu ***et al***., 2007). PFOA increases the levels of 8-hydroxydeoxyguanosine (8- OHdG), an indicator of oxidative DNA damage, in the liver of Ppar (peroxisome proliferator activated receptor-) null mice but did not elevate 8-OHdG levels in the liver of wild-type mice (Minata ***et al.,*** 2010). Orally administration of PFOA in mice developed serious hepatocellular injury and inflammatory cell infiltration. In addition to malondialdehyde formation and hydrogen peroxide generation, indicators of oxidative stress, were significantly increased. Furthermore, hepatic levels of interleukin- 6, cyclooxygenase-2, and C-reactive protein, markers of inflammatory response, were also markedly increases. So that PFOA induce hepatic toxicity may be involved in oxidative stress and inflammatory response in mice (Yang ***et al***., 2014).

Genotoxicity

From several studies genotoxic effect of PFOA have a controversial results. Several studies reported that PFOA is not directly genotoxic and does not induce mutations but some reported that, at higher dose it is genotoxic. Studies conducted for the United States Environmental Protection Agencies by an independent laboratory concluded that PFOA and APFO does not induce mutations with or without metabolic activation in AMES tests, in human lymphocytes or in Chinese Hamster Ovary (CHO) cells (Lawler ***et al***., 1995, 1996). Another study reported that APFO was able to induce both chromosomal aberrations and polyploidy with the presence of metabolic activation in human lymphocytes (Hazleton ***et al.,*** 1995; Murli ***et al***., 1996a, 1996b).

Yao ***et al***., 2005 using human hepatoma cell line HepG2 found a significant increase in the tail moment in the single cell gel electrophoresis assay in HepG2 cells exposed to PFOA which indicates that PFOA was able to induce DNA strand breaks in HepG2 cells and also induced a dose dependent increase in the frequency that a micronucleus was found in binucleated HepG2 cells, which indicates that chromosome breaks occurred in HepG2 cells after PFOA treatment**.** The generation of ROS leading to the activation of initiator caspase-9 which activated caspase-3/7, inducing the apoptotic pathway. Oxidative stress might decrease the DNA repair capacity and thus mutagenicity was induced (Narayanan ***et al.,*** 1997).

Carcinogenicity

A carcinogen is any substance, radionuclide, or radiation that is an agent directly involved in causing cancer. Biegel ***et al.,*** 2001 performed a 2-year study in which 300ppm APFO/PFOA was introduced into the diet of male CD rats. Liver adenomas were induced in 13% of the PFOA treated group versus 3% of the control group. Leydig cell adenoma was induced in 11% of the testes of the PFOA treated group versus 0% of the control group with Leydig cell hyperplasia present in 46% of the animals exposed to PFOA in their diet. Acinar cell adenoma was induced in 9% of the PFOA treated animals versus 0% of the control group. Numerous studies reported that, PFOA is distributed predominantly in the liver and plasma in humans and animals (Kudo ***et al.,*** 2003) and induces tumors of the testicles, liver, and pancreas in rodents via dietary intake, and mammary gland tumors (Sibinski, 1987; Biegel ***et al***., 2001; USEPA, 2005). Specially in acinar cells of pancreas and Leyding cells of testis (Lau ***et al.,*** 2007).

Immunotoxicity

Due to PFOA exposure, immunosuppression has been reported in adult animal models manifesting with B and T cell depletions, thymus atrophy, splenic atrophy, suppression of inflammatory responses, and decreased de novo antibody production, decreased thymocyte and splenocyte counts, decreased immunoglobulin response, and changes in specific populations of lymphocytes in the spleen and thymus at relatively high doses (Yang ***et al***., 2001; Dewitt ***et al.,*** 2008). It was shown to be immunosuppressive in both in vivo and ex vivo systems (Yang ***et al.,*** 2002a). The primary humoral response to horse red blood cell immunization was prevented by PFOA pretreatment while ex vivo spleen cell proliferation in response to both T- and B-cell activation was attenuated by the fluorochemical. This may decrease the body's ability to respond to bacterial invasion and infection. Exposure to PFOA may also enhance the immune response

to environmental allergens, which increases the severity of allergies (Fairley ***et al***., 2007). Weakening the immune system may be the one of the mechanisms of PFOA carcinogenicity in people and decrease the levels of serum immunoglobulins IgG, IgA and IgE, key proteins that help the body fight pathogenic microorganisms and suppress tumor development (Frisbee, 2008). PFOA suppressed and disrupted immune systems in PFOA-exposed people linked with death of immune cells and weakening of the body's ability to protect itself (Dewitt ***et al.,*** 2009).

Experimental studies of PFOA and PFOS in laboratory animals have also demonstrated exposure-related suppression of the antibody response among other immune changes including altered inflammatory response, cytokine signaling, and measures of both innate and adaptive immunity (Dewitt ***et al.,*** 2012) and elevating the expression of proinflammatory cytokines tumor necrosis factor and interleukin-1 and IL-6 in the spleen and mast cells (Thoudam ***et al.,*** 2012). Suppression of the antibody response to vaccines and increased incidence of autoimmune ulcerative colitis have been reported in adults living in an area of Ohio and West Virginia where public drinking water had been contaminated with PFOA (Looker ***et al.,***2014).

Endocrine Disruption

PFOA inhibited genes responsible for thyroid hormone biosynthesis and significantly induced estrogen-responsive genes. These findings implicate PFOA in endocrine disruption (Yanhong ***et al.,*** 2007). It may also cause reduced testosterone levels and increased estradiol levels (Lau ***et al***., 2007). It is developmentally toxic in mice, with broad and varied health consequences that may include long lasting effects in reproductive tissues and metabolic reprogramming. To date, the only demonstrated mode of action by which the health effects of PFOA are mediated is via the activation of the peroxisome proliferator activated receptor alpha (PPAR) and alter steroid hormone production or act indirectly, via ovarian effects, as a novel means of endocrine disruption (White ***et al***., 2011) and effect on the function of growth and sex hormones, including activation of the estrogen receptor (Benninghoff ***et al.,***2011; White ***et al***., 2011; Du ***et al.,***2013).

Effect on Neuroendocrine System

Animal studies have indicated that PFOA or PFOS may interfere with normal neuromuscular development by inhibiting choline acetyl transferase (ChAT)

activity (Lau ***et al.*** 2003; Johansson ***et al***. 2008).ChAT activity is involved in many behavioural phenomena and cognitive functions, but it has been found slightly reduced only in the prefrontal cortex of rat pups exposed ***in utero*** to PFOS, not in the hippocampus (Lau ***et al***. 2003). USEPA (United State Environmental Protection Agency) 2006 found little evidence to support influence of prenatal PFOA and PFOS levels on motor or mental developmental milestones in early childhood at plasma concentrations that have been reported in the general population.The behavioral effects of PFOA exposure, implicating negative impact on memory, learning, and motor functions, may involve structural changes in brain or affect neuronal plasticity as a result from effects on several neurochemical targets. Neonatal exposure of PFOS and PFOA at specific time points, at the period of high neuronal growth, was shown to induce behaviour effects in adult mice. The exposure involves an effect on the development of the cholinergic system (Johansson ***et al.,*** 2008). It also affects thyroid system, influence the calcium homeostasis, protein kinase C, synaptic plasticity and cellular differentiation (Mariuseen, 2012).

Effect on metabolism

PFOA elimination, tissue distribution, and metabolism were examined in male and female rats for 28 days after a single intra peritoneal dose. A sex difference in urinary elimination of PFOA-derived 14C was observed. Female rats eliminated PFOA derived radioactivity rapidly in the urine with 91% of the dose being excreted in the first 24 hr and in the same period, male rats eliminated only 6% of the administered 14C in the urine. The sex-related difference in urinary elimination resulted in the observed difference in the whole body elimination half-life (t1/2) of PFOA in males (t1/2 = 15 days) and females (t1/2 less than 1 day) (Vanden ***et al.,*** 1991). Several studies demonstrate that PFOA induces lipid accumulation in the liver and increases beta oxidation of fatty acids, several cytochrome P-450 enzymes, inhibition of the secretion of very low density lipoproteins and cholesterol from liver (Yeung ***et al***., 2006). PFOA exposure on developing mouse fetuses altered expression of multiple genes, including genes associated with lipid transport, ketogenesis, glucose metabolism, lipoprotein metabolism, cholesterol biosynthesis, steroid metabolism, bile acid biosynthesis, phospholipid metabolism, retinol metabolism, proteosome activation, and inflammation (Mitchell ***et al.,*** 2007).

A study reported that low dose exposures of PFOA induced elevated leptin, insulin and body weight, while higher PFOA exposures resulted in mixed effects including significantly decreased body weight and spleen weight, and significantly increased brown adipose weight in adulthood (Hines ***et al***., 2009). It is not only affects fatty acid metabolism, but also interferes with other metabolic pathways, particularly glucose metabolism, in the liver and reduced the levels of metabolites including fructose, mannitol, galactose, fumaric acid, malic acid and citric acid. The reduction of these metabolites correlated well with the down-regulation of several glucose metabolism genes (Tan ***et al.,***2013). USEPA (2014a) reported that PFOA increases serum cholesterol, increased liver enzymes, decreased bilirubin, increase of chronic kidney disease.

Conclusion

The global presence and environmental persistence of PFOA, warrants careful consideration as to the proper handling, use and disposal of this chemical. Research shows that level of PFOA in wildlife range from 0.05 ng/ml in the blood of cod collected from European water to 8.14ng/ml in the plasma of loggerhead sea turtles from North America and total Daily Intake value of PFOA in humans is 1.5 µg/kg bw per day. Several epidemiological studies conducted, most of which have been performed by industries that manufacture PFOA, and given that PFOA is accumulating in not only the occupationally exposed, but also in the general population is of concern. The ubiquitous presence of PFOA in the environment with the possibility that viable carcinogenic pathways exist in experimental animals that are mirrored in human carcinogenesis reflect a need to further evaluate the carcinogenic potential of PFOA in humans and the need for continued epidemiological studies of the human population. Not only causes carcinogenicity but also have other critical toxic effects; it contributes in many severe health issues. Being a part in manufacturing of furniture, medical equipments, textile industry and also its use in stain, grease and making nonstick surface for cookware and protective coating on clothing and carpeting, its entry in the human body cannot be ruled out. Its entry in the human body via, inhalation, dermal absorption and ingestion and cannot metabolize up to 3-4 years which may leads to health problems and can be responsible for reproductive toxicity, liver toxicity, immune system toxicity and developmental toxicity which reduce birth size, physical developmental delays and endocrine disruption. We assume that PFOA is one of the serious players of these conditions and thus, it should be scrutinized carefully for all the adverse outcomes expected.

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