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Review article

Phthalates and their effects on human health: Focus on erythrocytes and the reproductive system

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ABSTRACT

Plastics, long-chain artificial polymers, are used worldwide with a global production of 350 million tonnes per year. Various degradation processes transform plastics into smaller fragments divided into micro, *meso* and macroplastics. In various industries, such as construction, certain plastic additives are used to improve flexibility and enhance performance. Plastic additives include phthalates (PAE), dibutyl phthalate (DPB) and diethyl phthalate (DEP). Due to the use of plastics and plastic additives, these small fragments of different shapes and colours are present in all environmental compartments. For their characteristics, PAEs can be introduced particularly by ingestion, inhalation and dermal absorption. They can accumulate in the human body, where they have already been identified in blood, amniotic fluid and urine. The purpose of this review is to gather the effects that these plastic additives have on various systems in the human body. Being endocrine disruptors, the effects they have on erythrocytes and how they can be considered targets for xenobiotics have been analysed. The influence on the reproductive system was also examined. Phthalates are therefore often overused. Due to their properties, they can reach human tissues and have a negative impact on health. The aim of this review is to give an overview of the presence of phthalates and their hazards. Therefore, the use of these plastic additives should be reduced, replaced and their disposal improved.

1. Introduction

Nowadays, plastic materials can be considered the most globally used material for their versatility, low cost and durable character. However, their continuous use and harmful disposal are persistent pollutants in every ecological niche of the world (Bratovčić et al., 2015). In 2017, plastic usage reached 350 million tonnes (Bratovčić et al., 2015; Gholamhosseini et al., 2023) China can be considered the largest plastics producing country (Bratovcic, 2019). Plastic materials are polymer and are used as a key material in buildings and constructions, transportation, and scientific equipment. A few wastes are found in uncovered and covered landfill sites and will stay there for several years. The primary land-based sources of plastics are bottles and bags, untreated sewage and disposable (Alimba and Faggio, 2019; Schwarz et al., 2019). Plastics buried in covered landfill sites will remain for several decades, posing potential problems in the long run. Additionally, 'landfill mining', where debris is incinerated as fuel to produce energy, has been proposed. Whereas the disposal of plastics in landfills does sequester almost 100 % of the carbon from the atmosphere, this also implies that both the fabric and the energy stored in plastics are lost within the long-term process (Waring et al., 2018). There are different types of plastic, such as polyethene (PE), high-density construct (LDPE) and low-density construct (HDPE), polypropylene (PPL), polyvinyl chloride (PVC) (Lombardo et al., 2022; Multisanti et al., 2022). Photo and thermoxidative processes as well as various degradation processes cause the transformation of plastics into microplastics, mesoplastics, and macroplastics. The term 'microplastics' (MPs) was coined in 2004. Thomson used it to describe microscopic plastic fragments in the marine environment. In 2009, it was decided to call microplastics only fragments smaller than 5 mm (Aliko et al., 2022a; Aliko et al., 2022b; Hodkovicova et al., 2022; Hwang et al., 2020; Martyniuk et al., 2023; Prata et al., 2020).This definition was further refined in 2011. MPs, which are particles of varying sizes, colours, and shapes were detected in freshwater, soil, air, and some food product (Impellitteri et al., 2022). It was observed that within Italian freshwater, microplastic is more concentrated than other kinds of plastic. There is, in particular, 98,1 % microplastic and just 1.4 % of others. Its fragments are resistant to degradation and are difficult to remove. Today, they significantly impact the environment and human

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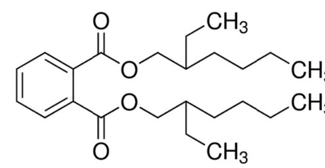
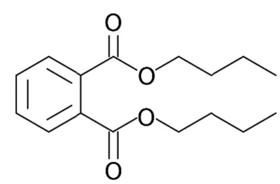
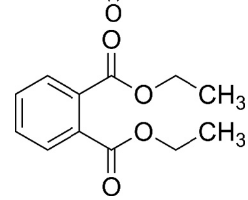
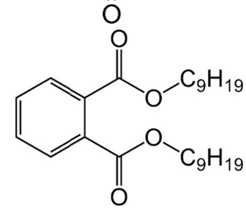
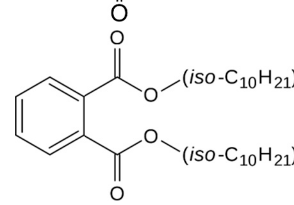
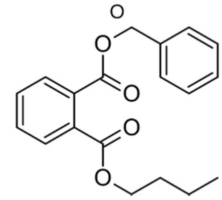
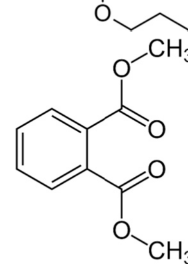
health and there is a serious concern about their effects in the literature. The toxicity of microplastic is determined by its physical nature, like its size and by its chemical nature such as the polymer of which they are composed (Cole et al., 2011). Microplastics' physical-chemical properties (e.g., size, specific density, charge and chemical composition), hydrodynamic factors and environmental characteristics (e.g., the velocity of water currents, turbidity, the density of water mass, temperature and wind) can influence their transport dynamics and consequently the distribution and accumulation of those in numerous marine areas. Plastic waste and microplastics accumulate in the water column and seabed sediments (Pastorino et al., 2023; Zhang et al., 2022). The fragments by marine organisms significantly impact this habitat and can cause physiological damage, energy deficiencies, and death (Zimmermann et al., 2020). MPs can be translocated along the food chain and enter relationships with terrestrial and marine animals, invertebrates and plant pollinators. Absorption of MPs occurs through ingestion, inhalation and skin contact (Savuca et al., 2022; Zicarelli et al., 2023). The absorption of microplastics is mainly due to the ingestion of many marine organisms such as mussels, fish and oysters (Savoca et al., 2019). For example, mussels are important filter feeders and bioaccumulators that play an important role in the food chain (Fabrello et al., 2022; Pagano et al., 2022; Tresnakova et al., 2023). Microplastics enter the food chain precisely because humans eat contaminated organisms, so that, after ingestion, the fragments are absorbed by specialised M-cells of the intestine and insoluble particles penetrate the intestinal mucus and are then transported to the lymphatic system (Stock et al., 2019). Another mechanism of microplastic adsorption is inhalation. MPs are released into the air at different concentrations. The size and quantity of the particles influence their accumulation in the respiratory system, and some fragments are translocated by macrophages, the circulatory system, and the lymphatic system. Moreover, smaller particles accumulate in the deepest parts of the lungs and cause chronic inflammation (Amato-Lourenço et al., 2020). Absorption, retention, and translocation into tissues are related to the shape and size of the fragments due to the exposed organisms' characteristics. The workers of synthetic textile, the vinyl chloride or polyvinyl chloride industries, are subject to risk, and high concentrations of the microplastic in the air could cause damage to respiratory systems (Prokić et al., 2021). Dermal contact is considered less dangerous, even if microplastics could cause inflammation and strange body reactions. The skin is exposed to microplastics when cosmetics containing microbeads are used since microspheres are among the main ingredients in personal care products such as facial wash, shaving creams, wrinkle creams, facial scrubs, toothpaste, deodorants, soaps, and shampoos (Strungaru et al., 2019).

2. Phthalates

Phthalates (PAEs) are esters of phthalic acid; in particular, they act as plastic additives and add plasticity to industrial polymers. The reaction between phthalic anhydride and alcohol causes the formation of phthalates. Their chemical structure consists of a benzene ring and two carboxylic acids (Mankidy et al., 2013). The bonds are covalent and this characteristic allows phthalates to be released in a process known as lyeing in soil, fresh water, ocean and sediments. PAEs have been widely used in occupational settings, mainly as plasticizers in various applications (Dutta et al., 2020). According to some data (Burgos-Aceves et al., 2021) around 7.5 million tonnes of plasticisers are consumed each year in particular industries such as building products, leather goods, medical devices, perfumes, and cosmetics. They help lubricate and soften other substances, increase spread ability, and enhance absorption. Some phthalates are in polyvinyl chloride plastics, which are used to make products such as plastic packaging, garden hoses, and medical tubing (Przybylińska and Wyszkowski, 2016). Phthalates are easily released into the environment even if they do not generally persist due to rapid biodegradation, photodegradation, and anaerobic degradation. Occupational exposure to different phthalates has been studied in several occupational settings using human biomonitoring (HBM) (Fréry et al., 2020). This family of chemicals is widely used to soften plastics and has a strong performance in terms of durability and stability. Due to their characteristics and use, phthalates are present in, for example, dairy products, fish and shellfish (Serrano et al., 2014). It is assumed that food is the main source of exposure to phthalates in the population, with fatty foods, such as milk, butter and meat, being the main sources. There are different types of phthalates: Di(2-ethylhexyl) phthalate (DEHP), dibutyl phthalate (DBP), diethyl phthalate (DEP), di-isononyl phthalate (DiNP), di-isodecyl phthalate (DiDP), benzyl butyl phthalate (BBP) and dimethyl phthalate (DMP) (Table 1). Among them, DBP is classified as

Table 1
List of the main phthalates with chemical structures and their common.

Name	Structure	Use
Di(2-ethylhexyl) phthalate (DEHP)		Building products (wallpaper, wire and cable insulation), car products, clothing (footwear, raincoats), food packaging, children's products (toys, grip bumpers), medical device.
Di-n-butyl phthalate (DBP)		PVC plastics, latex adhesives, cosmetics, personal care products, cellulose plastics, solvent for dyes.
Diethyl phthalate (DEP)		Personal care products, cosmetics.
Di-isononyl phthalate (DiNP)		Garden hoses, pool liners, flooring tiles, tarps, toys.
Di-isodecyl phthalate (DiDP)		PVC plastics, covering on wires and cables, artificial leather, toys, carpet backing, pool liners.
Benzyl butyl phthalate (BBP)		Traffic cones, food conveyor belts, and artificial leather.
Dimethyl phthalate (DMP)		Insect repellents, safety glass, and lacquer coatings.

dangerous to the environment, but especially to aquatic organisms (Zhang et al., 2021). Haemocytes are the main cells of the immune system of these invertebrates and have various functions such as phagocytosis or ROS production. An increase in haemocytes may indicate that the organism has been exposed to pathogens or environmental pollutants (Burgos-Aceves and Faggio, 2017). The negative influence of phthalates affects both aquatic invertebrates such as mussels, the environment and human health, as already mentioned, phthalates have been detected in the environment, the reason is that weathering degrades plastics and other materials consisting of various types of phthalates and these are released. In particular, their composition favours their release at all stages such as production, use and disposal. In addition to the aquatic environment, phthalates have also been detected in the atmosphere (Abdel daiem et al., 2012). In rats, DEHP has been noted to have toxic effects on both sexes, specifically, together with its metabolite mono-2-ethylhexyl phthalate (MEHP) it has Sertoli cells within the testis and granulosa cells in the ovary as target organs. After rats were subjected to DBP, testosterone levels decreased and Leyding cells increased. A special focus is on phthalates and their metabolites that cause toxicity in male development and reproduction (Latini, 2005). Furthermore, in rats, DEHP and co-exposure to other phthalates cause an increase in systolic blood pressure. DEHP causes a decrease in thyroid hormone values, thus an alteration in energy homeostasis and consequently an increase in body fat. In addition, DEHP can induce an imbalance in endothelial nitric oxide synthase phosphorylation and a dependence of cardiac cells on fatty acids (Zhang et al., 2018). In some studies that used data from the National Health and Nutrition Examination Survey (NHANES), phthalates can determine an increase, as mentioned above, in oxidative stress and thus inflammation, particularly bilirubin, absolute neutrophil count, alkaline phosphatase and ferritin levels, C-reactive protein, gamma-glutamyltransferase (Gao et al., 2017; Lin et al., 2017) Phthalates were found in rainwater because of their ability to move from industrial products into the air. Through irrigation, phthalates also reach plants, and from the roots, they reach the stems and seeds. This shows that phthalates are everywhere and can get everywhere. Any part of our body can absorb phthalates through food, inhalation, drugs, cosmetics and perfumes. In the body, phthalates induce significant endocrine disruption; they are absorbed into the blood and fluids and impair physiological mechanisms (Miodovnik et al., 2011).

3. Effects of phthalates on human health

3.1. Phthalates as endocrine disruptors

Among the phthalates, the most widely used and most worrying is DEHP; studies have shown that it causes adverse effects in humans and laboratory animals by acting as an endocrine disruptor (Palacios-Arreola et al., 2022; Huang et al., 2022; Dong et al., 2019). Endocrine disruptors (EDs) are artificial or natural molecules such as steroid hormones and include, in fact, phthalates. The latter can bind to nuclear hormone receptors and can mimic or influence the effect of physiological hormones introduced into the human body (Benjamin et al., 2017). Clinical studies show numerous abnormalities such as endometriosis, precocious puberty, overweight, asthma and obesity (Durmaz et al., 2018). Phthalates are on the increase and their effects are unavoidable at any time of life, although the most sensitive periods are the foetal, perinatal and early childhood phases, during which their effect affects physiological parameters throughout life (Lucaccioni et al., 2020).

Endocrine disruptors have been shown to preferentially target hypothalamic-pituitary-gonadal system receptors, negatively affecting the proper production of sex hormones such as androgens and progesterone. Therefore, they can influence normal hormonal functions by binding to steroid hormone receptors, activating the response of the receptor-carrying cell (Csaba, 2018). Endocrine-disrupting chemicals are positively associated with insulin resistance syndrome, evidenced by worse prognoses among individuals with higher levels of exposure (Ore et al., 2022). They also alter the production of sex hormones, negatively interfering with children's sexual development and adult sexual behaviour. Endocrine disruptors can directly affect sex organ development, as teratogens, during foetal life (cryptorchidism, hypospadias, micropenis), and during late sexual events (pubertal, adult) as defective imprinting (functional teratogens, when exposure occurs perinatally or later) (Hlisníková et al., 2020).

3.2. Effects of phthalates on erythrocytes

Red blood cells (RBCs) can be considered excellent target cells and are used to understand the toxicity of xenobiotics. Exposure to xenobiotics causes haemolysis or programmed eryptosis, which is meant cell death, for the contraction and shrinkage of cells and the shuffling of the cell membrane and thus the exposure of phosphatidylserine on the cell surface (Arrigo et al., 2023; Briglia et al., 2015; Eberhard et al., 2010). Haemoglobin is the protein in red blood cells that provides oxygen and carbon dioxide transport. Synthesis of haemoglobin begins when the RBC is within the proerythroblast stage and continues into the reticulocyte stage (Briglia et al., 2017; Pagano and Faggio, 2015). Phthalates, due to their liposolubility, enter the erythrocyte membrane and interact with haemoglobin, altering its structure and function and, inducing accelerated erythrose, decreased erythrocytes and anaemia (Sicińska et al., 2020). Phthalates, including DEHP, affect the plasma membrane of erythrocytes, causing osmotic fragility. Exposure to phthalates makes erythrocytes more flexible and deforms them due to the decrease in membrane surface area (Labow et al., 1987). Some data collected, report that DEHP causes changes in membrane conformation after blood has been stored in polyvinyl chloride (PVC) bags plasticised with DEHP. It was noted that during this exposure, which was evaluated for approximately 5 days, the total membrane proteins of the erythrocytes tended to increase and cause haemolysis (Serrano et al., 2016).

Phthalates affect the body differently depending on age, sex, and lifestyle. As mentioned, phthalates can get inside food and beverages, but also some drugs are made up of these plastic additives in various excipients (Brassea-Pérez et al., 2021). After DMP exposure of erythrocytes, haemoglobin levels are decreased due to increased concentrations of dimethyl phthalate. DMP can cause iron release, and haemoglobin will not be able to be oxidized by potassium ferricyanide to form haemoglobin (Chi et al., 2021). Some studies report a decrease in the number of RBCs and white blood cells (WBCs) as the concentration of DMP increases. In addition, a reduction in haemocyte circulation has been shown due to what could be an effect of DMP on erythrocyte maturation and formation in the bone marrow. Phthalate concentrations in blood were measured, but the experiment was conducted at higher levels of exposure (Chi et al., 2022). After erythrocytes were exposed to phthalates, it caused an increase in methaemoglobin (MetHB) and reactive oxygen species (ROS) levels. The increase in ROS causes changes in enzyme activity, apoptosis, and necrosis. This could cause the activation of transient receptor potential (TRPs) channels, which are sensitive to oxidative stress and will cause secretion of Calcitonin Gene-Related Peptide (CGRP), which will have a strong negative impact on the inflammation (Zhou et al., 2020). A decrease in superoxide dismutase was found regarding enzyme activity after exposure to DBP and BBP. In addition, after in vivo exposure to xenobiotics such as phthalates, there is an increase in catalase (CAT) activity. Mono-butilftalato (MBP) metabolite of phthalates causes a disturbance in the redox balance of erythrocytes (Sicińska et al., 2020). Exposure to phthalates also leads to increased externalization of phosphatidylserine erythrocytes. The translocation of phospholipids into the cell membrane is mediated by the enzyme scramblase, which is activated by the increase in cytosolic calcium levels. The increase in cytosolic calcium causes an increase in calpain, a type of cysteine protein, which determines a protocollination of cytoskeleton proteins, disrupting the structure of the cell membrane, which hinders microcirculation and leads to the removal of red blood cells. The main and important intrinsic factors that trigger erythrosis are the increase of calcium ions within the cell, activation of calpain, stimulation of caspases, ceramide formation, reduction of ATP levels, osmotic shock or oxidative stress (Sicińska et al., 2020). Phthalates also cause a decrease in glutathione peroxidase (GSH) levels in the blood. A relationship has been observed between GSH levels and malate dehydrogenase (MDH) and phthalates. DMP in erythrocytes also causes a decrease in immune complexes (IC) and the C3b complex, which are important for the immune adhesion of erythrocyte membranes because phthalates are soluble and easily enter the membrane and diffuse into the cytosol (Sicińska, 2018). Complement immune complexes tend to cause tissue damage and vasculitis as they accumulate on the walls of blood vessels. The immune function of erythrocytes is estimated by the concentration of red blood cells C3b receptor rosette and RBC-IC. After exposure to DMP the activity of the receptors is diminished, which causes the immune function of the erythrocytes to be compromised. Enzyme activity increases even at low DMP concentrations and decreases when phthalate concentrations increase. This process is known as osmosis. After erythrocytes have been subjected to low phthalate concentrations (100 mg kg^{-1} d^{-1}), CAT tends to increase, whereas when phthalate doses are higher (200 or 400 mg BCP kg^{-1}), SOD and CAT enzyme activity decrease. The level of GSH increases as DMP increases. The change in SOD and CAT concentrations following exposure to phthalates may indicate a difficulty in eliminating free radicals, which leads to lipid peroxidation and hampers immune defence (Sicińska, 2018) The concentration of MDH, a product of lipid peroxidation, was also evaluated in order to understand the damage caused by phthalates on antioxidant activity. The concentration of MDH increases following exposure to phthalates (Li et al., 2019). In addition to the increase in MDH, due to the exposure of erythrocytes to DEHP, a decrease in glutathione, vitamin E and vitamin K was also found. In addition, a reduction in haemoglobin release by erythrocytes was noted (Devi et al., 1998).

In addition to the effects on erythrocytes reported in the next chapter, the impact of DBP on other blood cells such as granulocytes was also evaluated. These data report that DBP decreases the expression of surface markers such as CD24 on eosinophils and neutrophils. In addition, this phthalate caused a reduction in tumor necrosis factor (TNFα) and interferon-gamma (IFNγ) (Maestre-Batlle et al., 2018).

3.3. *Effects of phthalates on the reproductive system of men and women*

Among the targets of phthalates is certainly the reproductive system.

Specifically, in the male reproductive system, phthalates affect Sertoli cells and Leydig cells. Some studies report that after exposure to phthalates, a decrease in sperm motility and concentration has been observed. Certain types of phthalates detected in seminal fluid appear to cause an abnormal number of heads and flagella in spermatozoa. As mentioned, since phthalates affect testicle cells, can cause alteration of the external genitalia and weight loss of the organ (Hlisníková et al., 2020). In addition, phthalates cause certain testicular malformations, again at the foetal level, such as hypospadias and cryptorchidism, which are typical of a condition such as testicular dysgenesis syndrome (Earl Gray et al., 2006). Another demonstration of the effects of phthalates on the male reproductive system is related to mothers' exposure to plastic additives. The newborns again presented cryptorchidism (Mariana et al., 2016). In several studies, in which rats were used as model organisms, exposure to phthalates caused altered structures as androgen-dependent, resulting in a decrease in testosterone biosynthesis in the foetal testes. The foetal testes also showed malformed seminiferous cords and the formation of cell aggregates. Malformations of certain structures such as the prostate, seminal vesicles were also noted (Foster et al., 2006). In adults, on the other hand, there is less differentiation of germ cells, which appear giant and multinucleated (Martino-Andrade and Chahoud, 2010). Certainly, given what has just been said, we can find a correlation with what some studies report about the toxicity of phthalates to human sperm. The concentration of phthalates in sperm has been evaluated and appears to cause sperm alteration; moreover, phthalates have higher concentrations in men found to be infertile (Rozati et al., 2002). This is also confirmed by other studies (Duty et al., 2003; Hauser et al., 2006) in which phthalate concentrations were assessed in the urine samples of some sub-fertile patients; again, the levels of phthalates detected corresponded to a decrease in sperm motility.

With regard to the female reproductive system, it was shown that young women exposed to phthalates suffered premature puberty (Mesquita et al., 2021). Certain phthalates such as DBP, DEP and DEHP were detected in 68 % of the serious samples taken for analysis (Colón et al., 2000). In urine, phthalate metabolites, including mono-methyl phthalate (MMP), were evaluated in another study. MMP was significantly higher in girls with early puberty than in the control (Chou et al., 2009). The correlation between endometriosis and exposure to phthalates was also evaluated. Endometriosis refers to the spread of tissue outside the uterine cavity. In some serum samples, increased concentrations of certain phthalates such as DBP and DEHP were noted in women with endometriosis (Reddy et al., 2006). In plasma samples from women with polycystic ovary syndrome and endometriosis, concentrations of metabolites such as MBzP and mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) were detected (Pednekar et al., 2018). Phthalates have been detected in the urine of some women, causing pregnancy complications such as anaemia, toxaemia and pre-eclampsia. In female rats, used as a model organism, exposure to DEHP can cause polycystic ovaries and inhibit steroidogenesis of ovarian granulosa cells. MEHP, which we know as the active metabolite of DEHP, on the other hand, causes a decrease in oestradiol production (Ventrice et al., 2013).

4. Conclusions

The reports on phthalates' impact on human health, particularly on erythrocytes and the reproductive system, are significant. Although phthalates are considered very useful for their properties and are used in various fields, their disproportionate use needs to be reduced. In fact, phthalates are found in all environments and, as mentioned above, can penetrate the body and cause damage to various organs and tissues. It would be important to continue research to understand the individual effects of each class of phthalates on specific blood cells, such as granulocytes and isolated red blood cells (anucleated and nucleated), and thus on other organisms in addition to mammals. There is more scientific research on some specific classes of phthalates, such as DBP, DMP and DEHP, because they are more widely used and present in the environment. The other classes are also considered hazardous and it is important that their concentrations in the environment are understood and tested. It is indeed worrying that the body is exposed to these plastic additives in various ways from the time it is a foetus. It would be important to continue to investigate whether phthalates have other targets as endocrine disruptors in addition to those tested and whether they therefore affect the production of other hormones in addition to those tested, including sex hormones. Unfortunately, what has been tested so far shows that there is a need to reduce the use of plastics, and therefore these additives, and to promote better disposal of them.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors do not have permission to share data.

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