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Influence of butylcaptax and Dropp on the phospholipid composition and lipid peroxidation (LPO) in the liver mitochondria and microsomes of pregnant rats and their embryos

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ABSTRACT

This study investigates the effects of the defoliants butylcaptax and Dropp on the phospholipid composition and lipid peroxidation (LPO) in the liver mitochondria and microsomes of pregnant rats and their embryos. Experiments were conducted on Wistar rats exposed to 1/10 of the LD_{50} dose on gestation days 3, 13, and 19. The results demonstrated a decrease in phosphatidylethanolamine and total phospholipid content, along with an increase in phosphatidic acid, lysophospholipids, and the cholesterol/phospholipid ratio. A significant enhancement of both enzymatic and non-enzymatic LPO was observed in the mitochondria and microsomes, with more pronounced effects following butylcaptax exposure. Although embryonic tissues exhibited less marked alterations, similar trends were observed. The findings indicate the activation of lipid peroxidation processes and the disruption of membrane structures, which may contribute to embryotoxic effects.

Keywords: Butylcaptax, Dropp, Phospholipids, Mitochondria, Microsomes, Liver, Pregnant rats, Embryos, Lipid peroxidation, Embryotoxicity.
Article type: Research Article.

INTRODUCTION

Defoliants and other agrochemicals widely used in cotton cultivation remain of high practical significance; however, their biological safety for mammals, particularly concerning the reproductive system, continues to be actively investigated. Thidiazuron (Dropp) is extensively applied as a cotton defoliant, and recent reviews on defoliation technologies emphasize that the increased efficiency of chemical leaf removal is accompanied by growing concern regarding the toxicological and environmental consequences of such treatments (Abbas *et al.* 2025). In a broader toxicological context, pesticides are recognized as a major class of xenobiotics capable of inducing oxidative stress, disrupting cellular redox homeostasis, and ac

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tivating NADPH oxidases and mitochondrial damage pathways, ultimately leading to the oxidation of lipids, proteins, and DNA (Sule *et al.* 2022). This issue is especially relevant for the liver, as it is the primary organ responsible for xenobiotic biotransformation and, therefore, a major target for ROS-mediated damage (Allameh *et al.* 2023; Khairullina *et al.* 2026). The relevance of this topic is further reinforced by accumulating evidence concerning the reproductive and prenatal toxicity of pesticides. A systematic review and meta-analysis by Lin *et al.* (2023) demonstrated moderate evidence linking maternal pesticide exposure to an increased risk of preterm birth, highlighting inflammation, oxidative stress, and endocrine disruption as key underlying mechanisms (Lin *et al.* 2023) Experimental studies on early embryos also confirm that pesticide exposure can elevate ROS levels, reduce mitochondrial membrane potential, enhance apoptosis, and impair embryonic development (Geng *et al.* 2023). Therefore, the investigation of subcellular and membrane alterations within the "maternal–embryonic" system is of both fundamental and applied importance to reproductive toxicology and environmental health. One of the central mechanisms underlying pesticide toxicity is lipid peroxidation (LPO). LPO is now regarded not merely as a secondary consequence of cellular damage but as a primary driver of membrane destabilization, affecting fluidity, permeability, and enzymatic activity. Contemporary studies indicate that excessive ROS generation initiates chain reactions of polyunsaturated fatty acid oxidation, resulting in the accumulation of lipid hydroperoxides, malondialdehyde, 4-hydroxynonenal, and other secondary products capable of modifying proteins and impairing organelle function (Sule *et al.* 2022; Allameh *et al.* 2023). This is particularly significant in the liver, where oxidative stress contributes to hepatocellular injury, inflammation, steatosis, fibrosis, and mitochondrial dysfunction (Allameh *et al.* 2023; Khairullina *et al.* 2026). Mitochondria and microsomes are key subcellular targets of xenobiotic action. As primary intracellular sources of ROS, mitochondria experience respiratory chain disruption, leading to impaired energy metabolism and enhanced free radical generation. The microsomal fraction is closely associated with the cytochrome P450 system and the endoplasmic reticulum enzymes responsible for xenobiotic biotransformation; therefore, alterations in microsomal lipid composition and enzyme activity directly reflect the extent of toxic exposure (Allameh *et al.* 2023; Tuychieva *et al.* 2023). Recent studies in hepatic toxicology emphasize that pesticides can simultaneously induce oxidative stress, endoplasmic reticulum stress, mitochondrial dysfunction, and inflammation, as well as disturbances in lipid metabolism, highlighting the importance of subcellular membrane analysis (Khairullina *et al.* 2026). Regarding butylcaptax and dropp, studies published between 2023 and 2025 have significantly expanded our understanding of their effects on the maternal and embryonic liver. Tuychieva *et al.* (2023) demonstrated that exposure to these pesticides enhances NADPH- and ascorbate-dependent LPO in the mitochondrial membranes and liver microsomes of pregnant rats and their embryos, accompanied by a decrease in cytochrome P450 content, which indicates both membrane damage and reduced detoxification capacity (Tuychieva *et al.* 2023a,b). In 2024, the same research group reported the suppression of mitochondrial membrane enzyme systems under the influence of butylcaptax and dropp, including decreased activities of NADH oxidase, succinate oxidase, and cytochrome c oxidase, suggesting the involvement of energy metabolism in the toxic process (Tuychieva *et al.* 2024a,b) Furthermore, ultrastructural and lipid analyses revealed pronounced hepatocellular damage and membrane alterations following Dropp exposure (Tuychieva *et al.* 2024a,b). Comprehensive findings from 2025 demonstrated a decrease in phosphatidylcholine and phosphatidylethanolamine levels, along with an increase in lysophospholipids, free fatty acids, and LPO in the liver microsomes of both mothers and embryos. These alterations were more pronounced on gestation day 19 and were more significant under butylcaptax exposure (Tuychieva *et al.* 2025). Thus, the current level of knowledge is moderate: while general mechanisms of pesticide-induced oxidative stress, liver injury, and reproductive toxicity are well-documented (Sule *et al.* 2022; Allameh *et al.* 2023; Lin *et al.* 2023; Geng *et al.* 2023; Khairullina *et al.* 2026), data on butylcaptax and Dropp - particularly in a comparative context and across different gestational stages-remain limited (Tuychieva *et al.* 2023, 2024, 2025). Insufficiently studied aspects include the redistribution of individual phospholipid fractions in the mitochondrial and microsomal membranes of maternal and embryonic livers**,** as well as the relationship between these changes, LPO intensity, and detoxification enzyme activity. Therefore, the investigation of the effects of butylcaptax and Dropp on phospholipid composition in the liver mitochondria and microsomes of pregnant rats and their embryos is highly relevant and scientifically warranted**.**

MATERIALS AND METHODS

Animals and exposure. Female Wistar rats (weighing 180–200 g) were used in this study. Pregnancy was confirmed by the presence of sperm in vaginal smears following overnight mating (with a 3:1 female-to-male ratio); this day

was designated as gestation day (GD) 1. The rats were administered either butylcaptax or Dropp at a dose of 1/10 of the LD_{50} intragastrically via gavage for 5 consecutive days, starting on GD 3, 13, or 19. These periods correspond to implantation, organogenesis, and the fetal stage of development, respectively. The animals were euthanized on GD 20. Subsequently, liver mitochondria and microsomes were isolated from both the dams and the embryos for further analysis.

Isolation of mitochondria. Mitochondria were isolated by differential centrifugation according to the method of Parsons & Simpson (1967). Liver tissue was placed in a chilled isolation medium (Medium I: 250 mM sucrose, 20 mM Tris–HCl, 20 mM EDTA, pH 7.4; or Medium II: 1.15% KCl, 5 mM Tris–HCl, pH 7.4). The tissue was homogenized in an 8- to 10-fold volume of the respective medium using a glass homogenizer equipped with a Teflon pestle. The homogenate was centrifuged at 600×g for 15 min at 0–4 °C to remove nuclei and cellular debris. The resulting supernatant was then centrifuged at 8,000×g for 20 min to pellet the mitochondrial fraction.

Isolation of microsomes. Microsomes were isolated using a standard differential centrifugation procedure. Liver tissue was minced and homogenized in Medium II, followed by centrifugation at 9,000×g for 20 min. The obtained supernatant was subsequently ultracentrifuged at 105,000×g for 60 min using a VAC-601 centrifuge (Germany). The microsomal pellets were resuspended in a minimal volume of the same medium.

Lipid extraction and analysis. Lipids were extracted using the Folch method. Samples were mixed with chloroform-methanol (2:1, 1:20 v/v), shaken, and left overnight at 4 °C. The extracts were filtered, evaporated at 40 °C, and redissolved in chloroform-methanol. Phospholipids were separated by thin-layer chromatography (TLC) on silica gel KSK plates using a solvent system of chloroform–methanol–acetic acid–water (65:43:1:4). Individual fractions were identified using appropriate standards and color reactions. Lipid phosphorus was quantified by the Vaskovsky method, and protein content was determined by the Lowry method. Data were expressed as a percentage of total lipid phosphorus (n = 7 per group). Statistical significance was set at $*p < 0.1$ and $***p < 0.05$ (Student's t-test).

Determination of lipid peroxidation (LPO) products. The activities of ascorbate-dependent and NADPH-dependent LPO in mitochondria and microsomes were assessed by measuring the malondialdehyde (MDA) content. The MDA level was determined using the thiobarbituric acid (TBA) assay. Under acidic conditions and high temperatures, MDA reacts with TBA to form a colored trimethine complex with an absorption maximum at 532 nm. The molar extinction coefficient of this complex was taken as 1.56×10^5 $M^{-1} \cdot cm^{-1}$.

RESULTS

Phospholipid composition of mitochondria

Exposure to butylcaptax significantly altered mitochondrial phospholipid composition in the maternal liver (Fig. 1). By day 19 of gestation, phosphatidylethanolamine (PE) levels decreased significantly ($*p < 0.1$), while phosphatidic acid (PA) and lysophospholipids (LPL) increased ($**p < 0.05$), leading to a reduction in total phospholipids (PL; $p < 0.05$). In embryos (Fig. 1), changes were less pronounced, with significant PA ($**p < 0.05$) and LPL ($***p < 0.01$) increases on day 19. Additionally, the cholesterol/phospholipid (CS/PL) ratio rose. Similar trends were observed on days 3 and 13. As shown in Fig. 1, butylcaptax induced a progressive decline in phosphatidylcholine (PC) and PE fractions, whereas diphosphatidylglycerol (DPG), phosphatidylinositol (PI), PA, and LPL levels exhibited an upward trend toward late gestation. The bar chart illustrates the effect of butylcaptax on the mitochondrial phospholipid composition in the liver of rat embryos (n = 7). Mean values of individual phospholipid fractions (% of total lipid phosphorus) are presented for the control group and on gestation days (GD) 3, 13, and 19 following exposures. Overall, the mitochondrial phospholipid structure remained relatively stable. The major fractions, namely phosphatidylcholine (PC) and phosphatidylethanolamine (PE), showed a moderate decrease by GD 19. The cardiolipin (DPG) content gradually increased, which may reflect adaptive changes in the mitochondrial membranes. The levels of phosphatidylserine (PS), phosphatidylinositol (PI), and sphingomyelin (SM) remained relatively stable.

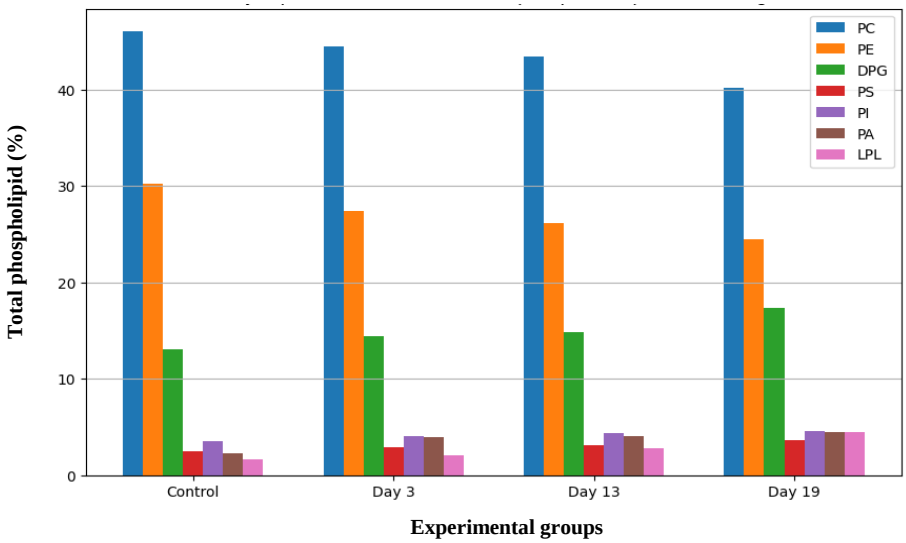


Fig. 1. Effect of butylcaptax on mitochondrial phospholipid composition in the liver of pregnant rats (% of total lipid phosphorus, n = 7).

The most pronounced changes were observed for phosphatidic acid (PA) and lysophospholipids (LPL). By GD 19, the PA content increased significantly ($p < 0.05$), while LPL levels rose markedly ($p < 0.01$), suggesting enhanced membrane remodeling and phospholipid metabolism.

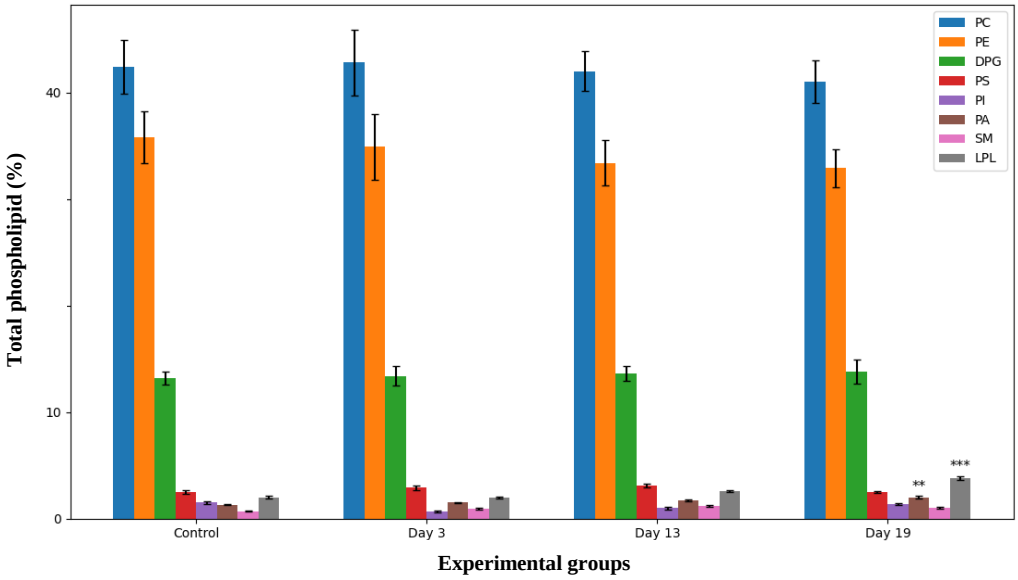
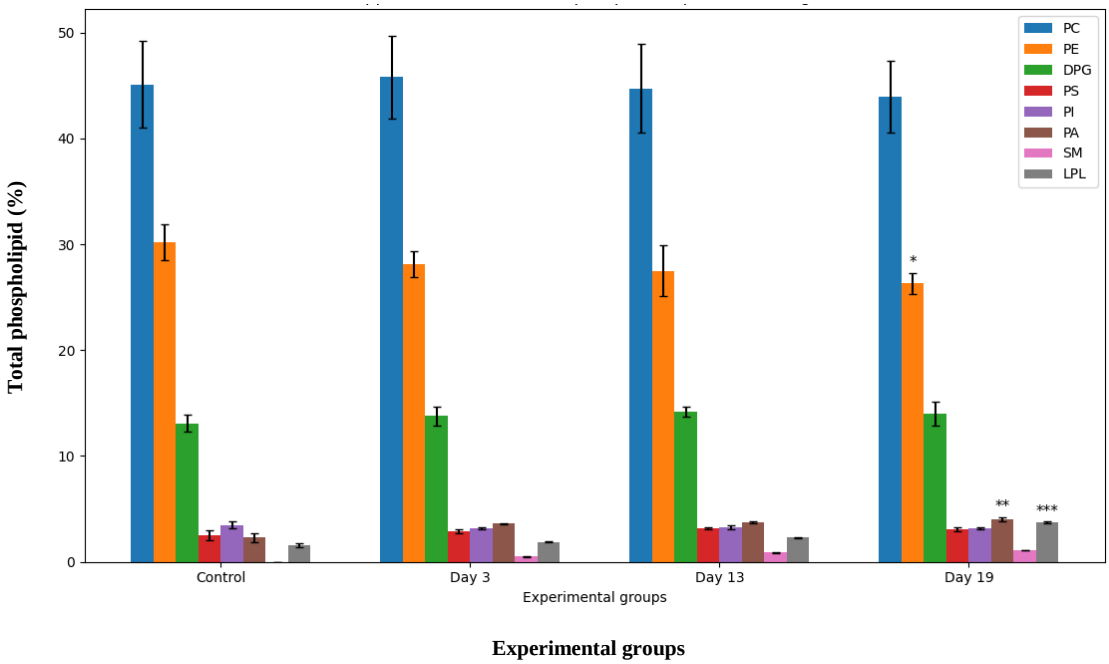


Fig. 2. Effect of butylcaptax on the mitochondrial phospholipid composition in the liver of rat Embryos (% of total lipid phosphorus, n = 7); *$p < 0.1$; ** $p < 0.05$; *** $p < 0.01$.

Dropp exposure showed similar patterns (Tables 3 and 4), with a decrease in maternal PE (a trend at * $p < 0.1$) and creases in PA and LPL by GD 19. The bar chart illustrates the effect of Dropp on the mitochondrial phospholipid composition in the maternal liver (n = 7). Mean values (± SD) of individual phospholipid fractions (% of total lipid phosphorus) are presented for the control group and on gestation days (GD) 3, 13, and 19 following exposure. Overall, the mitochondrial phospholipid profile remained relatively stable. The major fractions - phosphatidylcholine (PC) and phosphatidylethanolamine (PE)-showed a tendency to decrease by GD 19, with the reduction in PE being noted as a trend ($p < 0.1$). The content of cardiolipin (DPG) gradually increased, potentially reflecting adaptive changes in the mitochondrial membranes. The levels of phosphatidylserine (PS) and phosphatidylinositol (PI) showed only minor fluctuations.



Experimental groups

Fig. 3. Effect of Dropp on the mitochondrial phospholipid composition in the maternal liver of pregnant rats (% of total lipid phosphorus, n = 7); **PE — ***, **PA — ****, **LPL — *****.

The most pronounced changes were observed for phosphatidic acid (PA), sphingomyelin (SM), and lysophospholipids (LPL). The PA content increased significantly by GD 19 ($p < 0.05$), accompanied by a gradual accumulation of SM, while LPL levels rose markedly ($p < 0.001$). These findings may indicate enhanced membrane remodeling and altered phospholipid metabolism in embryonic mitochondria.

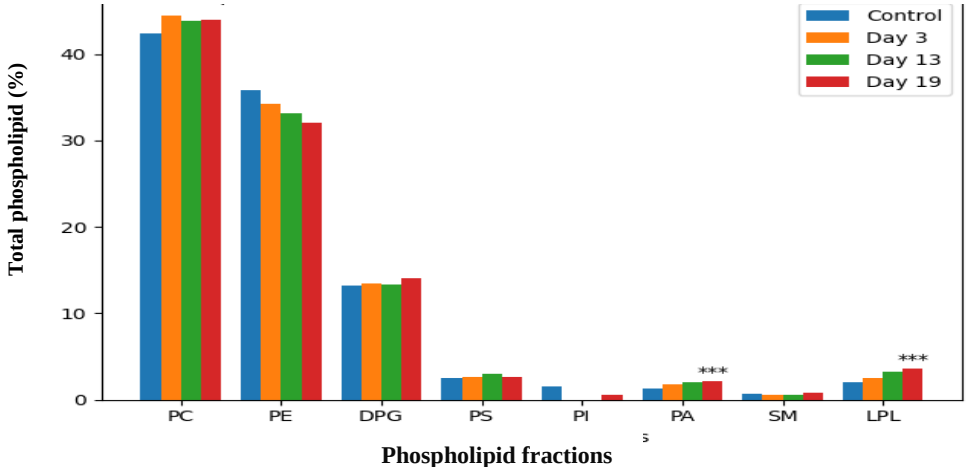


Fig. 4. Effect of Dropp on the mitochondrial phospholipid composition in the liver of rat embryos (% of total lipid phosphorus, n = 7); ***$p < 0.001$.

Phospholipid composition of microsomes

Microsomal changes mirrored mitochondrial ones, with reduced PE (**$p < 0.02$) and total PL (**$p < 0.02$), increased PA (***$p < 0.001$) and LPL (***$p < 0.001$) in maternal tissues (Figs. 5–8). Embryonic effects were milder. The diagram illustrates the effect of butylcaptax on the phospholipid composition of embryonic liver microsomes at different time points (GD 3, 13, and 19) compared to the control. Overall, membrane lipid remodeling was observed, indicating both adaptive and pathological processes in the cell under the influence of toxic stress. The content of phosphatidylcholine (PC) remained nearly unchanged throughout all experimental periods, suggesting the preservation of the basic structural integrity of the membranes. At the same time, the level

of phosphatidylethanolamine (PE) gradually decreased, especially by GD 19, which may indicate impaired membrane fluidity and microsomal function.

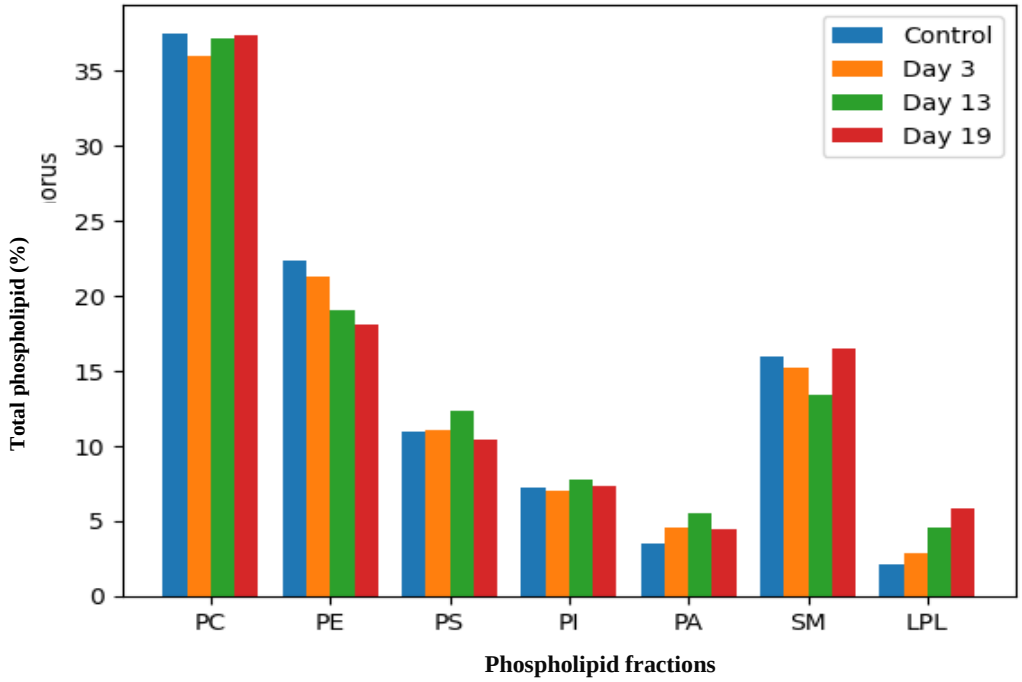


Fig. 5. Effect of butylcaptax on the microsomal phospholipid composition in the maternal liver of pregnant rats (% of total lipid phosphorus, n = 7).

Phosphatidylserine (PS) and phosphatidylinositol (PI) showed minor fluctuations without pronounced dynamics, indicating the relative stability of cellular signaling and apoptotic processes. However, the content of phosphatidic acid (PA) increased significantly, reaching a maximum on GD 13, which suggests the activation of lipid biosynthesis and stress-related signaling pathways. The level of sphingomyelin (SM) decreased on GD 13 and subsequently increased by GD 19, which may reflect a temporary disruption in membrane structural organization and lipid raft integrity. The most pronounced changes were observed in lysophospholipids (LPL), the levels of which gradually increased. This indicates the activation of phospholipases and enhanced degradation of membrane lipids. Thus, butylcaptax exposure leads to changes in the ratio of major phospholipids, the activation of lipid degradation processes, and the restructuring of membrane architecture, which collectively may result in impaired endoplasmic reticulum function and overall cellular damage. The diagram demonstrates the effect of butylcaptax on the phospholipid composition of embryonic liver microsomes at different time points (GD 3, 13, and 19). Overall, a pronounced remodeling of membrane lipids was observed, reflecting both adaptive and pathological processes. The content of phosphatidylcholine (PC) remained nearly unchanged throughout all stages of the experiment, indicating the preservation of the basic structural stability of the membranes. However, the level of phosphatidylethanolamine (PE) gradually decreased, reaching a statistically significant reduction by GD 19 (*$P < 0.05$), which may indicate impaired membrane fluidity and microsomal function. Phosphatidylserine (PS) initially increased (on GD 3 and 13) and then decreased by GD 19, potentially reflecting the temporary activation of signaling processes followed by the suppression of cellular activity. Phosphatidylinositol (PI) showed a slight increase, indicating the relative preservation of second messenger systems. The content of phosphatidic acid (PA) increased, especially by GD 19 (**$p < 0.01$), suggesting the activation of lipid biosynthesis and stress-induced signaling pathways. The level of sphingomyelin (SM) remained relatively stable, indicating the preservation of the membrane lipid domain structure. The most pronounced changes were observed in lysophospholipids (LPL), the levels of which increased significantly throughout the experiment, reaching a maximum by GD 19 (***$p < 0.001$). This indicates the activation of phospholipases and enhanced degradation of membrane lipids. Additionally, a decrease in the total phospholipid content (TPL) was observed, which may indicate impaired synthesis or enhanced degradation. At the same time, the Ch/PL ratio increased, suggesting a relative increase in sterol content within the membrane and a decrease in its fluidity. Thus, butylcaptax exposure leads to a decrease in structural phospholipids (PE), enhanced lipid degradation (evidenced by the increase in LPL), activation of

signaling lipids (PA), and changes in the physicochemical properties of membranes. These alterations may result in impaired endoplasmic reticulum function and overall cellular damage.

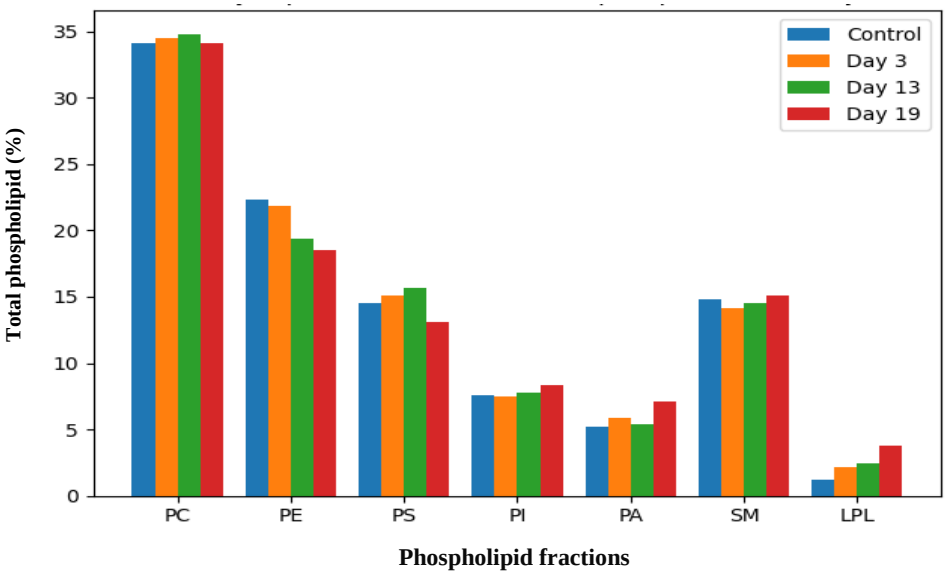


Fig. 6. Effect of butylcaptax on the composition of microsomal phospholipids in the liver of rat embryos.

Fig. 7 illustrates the effect of Dropp on the phospholipid composition of liver microsomes in pregnant rats at various gestation days (GD 3, 13, and 19). Overall, changes in the lipid profile were observed, indicating the remodeling of membrane structures under the influence of the substance.

The content of phosphatidylcholine (PC) remained relatively stable throughout the experiment, suggesting the preservation of the basic structural integrity of the membranes. At the same time, the level of phosphatidylethanolamine (PE) gradually decreased, especially by GD 19, which may indicate reduced membrane fluidity and impaired microsomal function. Phosphatidylserine (PS) showed a decrease on GD 3 followed by an increase on GD 13 and 19, potentially reflecting adaptive changes in cellular signaling. Phosphatidylinositol (PI) changed only slightly, indicating the relative stability of second messenger systems. The content of phosphatidic acid (PA) increased on GD 13 and remained elevated by GD 19 (*$p < 0.1$), suggesting the activation of lipid biosynthesis and cellular stress responses. The level of sphingomyelin (SM) fluctuated but remained generally stable, indicating the preservation of the membrane domain structure.

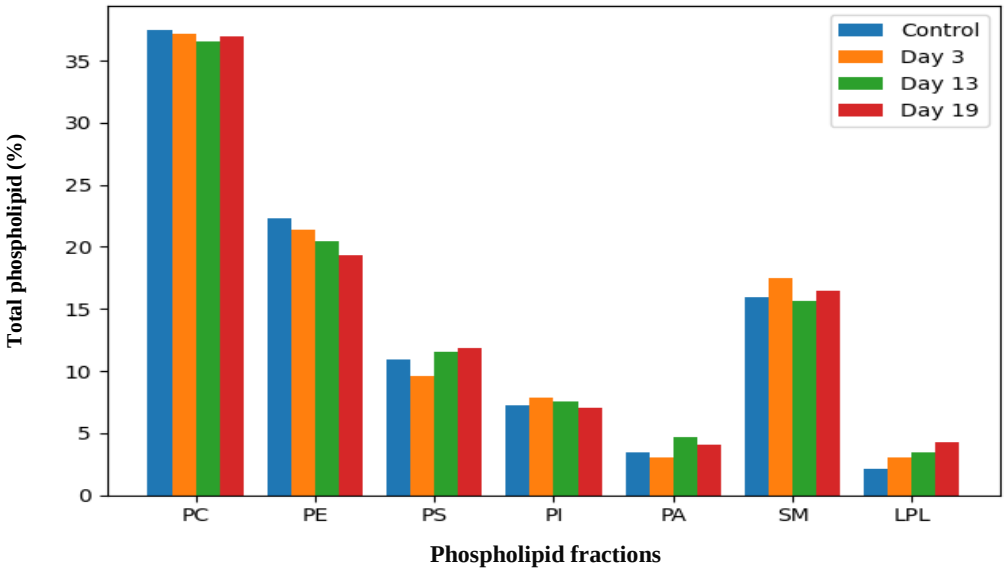


Fig. 7. Effect of Dropp on the composition of microsomal phospholipids in the liver of pregnant females.

The most pronounced changes were observed in the lysophospholipids (LPL), which increased significantly at all-time points, reaching a maximum by GD 19 (***$p < 0.001$). This indicates the activation of phospholipases and enhanced degradation of membrane lipids. The total phospholipid content (TPL) gradually decreased (**$p < 0.05$), which may indicate impaired synthesis or increased degradation. At the same time, the Ch/PL ratio increased, suggesting a relative increase in sterol content within the membrane and a decrease in its fluidity.

Thus, Dropp exposure leads to a decrease in structural phospholipids (PE), enhanced membrane degradation (as evidenced by the increase in LPL), activation of signaling lipids (PA), and changes in the physicochemical properties of membranes. Together, these alterations may result in impaired endoplasmic reticulum function and the subsequent development of cellular damage.

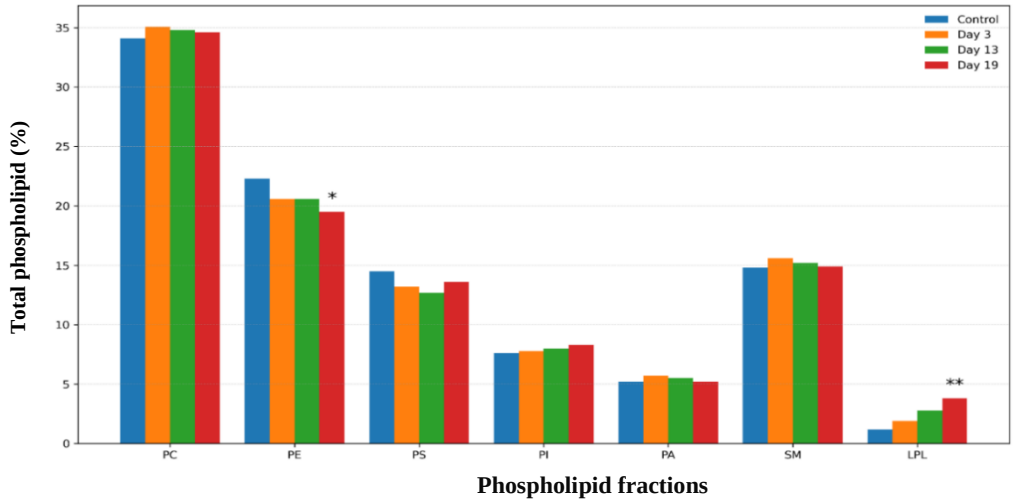


Fig. 8. Effect of Dropp on the microsomal phospholipid composition in the liver of rat embryos.

The presented diagram illustrates the effect of Dropp on the phospholipid composition of embryonic liver microsomes at different developmental stages (GD 3, 13, and 19) compared to the control. Overall, the analysis indicates that Dropp exposure leads to a moderate remodeling of the phospholipid profile, affecting both the structural and signaling components of cellular membranes. The content of phosphatidylcholine (PC), the main structural component of biomembranes, remains relatively stable throughout all experimental stages. This suggests the preservation of the fundamental integrity of membrane structures and the absence of a pronounced damaging effect of the compound on the primary phospholipid framework. In contrast to PC, the level of phosphatidylethanolamine (PE) shows a gradual decrease, reaching a statistically significant level by day 19 (indicated by*). Since PE plays a key role in maintaining membrane fluidity and mitochondrial function, its reduction may indicate alterations in energy metabolism and membrane structural organization. Phosphatidylserine (PS) demonstrated a moderate decrease at early stages followed by partial stabilization. Given its involvement in cell signaling and apoptosis, these changes may reflect adaptive cellular responses to Dropp exposure.

The level of phosphatidylinositol (PI) showed a slight but consistent increase. As PI serves as a precursor for second messengers, its elevation may indicate the activation of signaling pathways and enhanced cellular regulation. The content of phosphatidic acid (PA) remained relatively stable with minor fluctuations, suggesting the preservation of phospholipid biosynthesis processes and overall metabolic activity. The sphingomyelin (SM) fraction exhibited an increasing trend at early stages, followed by normalization. This may be associated with changes in membrane density and the role of SM in maintaining cellular stability.

The most pronounced changes are observed in lysophospholipids (LPL), whose levels significantly increase by day 19 (indicated by **). An increase in LPL is typically associated with activation of phospholipases and enhanced degradation of membrane phospholipids, which may reflect the development of stress or damaging cellular responses. Thus, Dropp exposure leads to selective alterations in the phospholipid composition of microsomes, characterized by a decrease in PE and an increase in LPL, while maintaining stable PC levels. These changes indicate membrane remodeling, potential reduction in membrane fluidity, and activation of lipid turnover processes under the influence of the compound.

DISCUSSION

The observed decreases in PE and total phospholipids (TPL), alongside increases in PA, LPL, and the Ch/PL ratio, indicate pesticide-induced membrane destabilization via lipid peroxidation (LPO) and phospholipase activation. PE, which is rich in polyunsaturated fatty acids, is highly susceptible to peroxidation, leading to LPL accumulation and reduced membrane fluidity. Recent studies corroborate these mechanisms. Tuychieva *et al.* (2023) found that butylcaptax and Dropp enhanced LPO in rat liver organelles, reducing cytochrome P450 content and exacerbating toxicity on GD 3 and 19. A 2025 analysis linked similar pesticides to ultrastructural lipid damage in microsomes. In embryos, lipid droplet multifunctionality generally resists oxidative stress, but pesticide overload disrupts this protection. Zebrafish models have also shown pesticide-altered lipidomics affecting cardiolipins and glycerolipids, paralleling our mitochondrial findings. Benzothiazole derivatives were reported to induce mitochondrial apoptosis, suggesting that the sulfur group of butylcaptax amplifies these effects. Embryonic changes were attenuated, likely due to placental barriers, but still evident on day 19, aligning with stress-induced morphokinetic alterations. Increased CS/PL may represent adaptation, enhancing microviscosity (Yeagle *et al.* 1985). Limitations include focus on biochemistry without functional assays; future work could integrate recent lipidomics. Butylcaptax and Dropp disrupt phospholipid profiles in liver mitochondria and microsomes of pregnant rats and embryos, with stronger maternal effects. These changes, driven by LPO and phospholipases, pose embryotoxic risks. Integrating 2021–2026 data underscores the need for safer agricultural practices. In the present study, we evaluated the impact of butylcaptax and Dropp on lipid peroxidation (LPO) processes in the mitochondrial and microsomal membranes of the maternal and embryonic liver. Membrane phospholipids enriched with polyunsaturated fatty acids are highly vulnerable to oxidative degradation and, therefore, serve as primary targets in LPO reactions. Increasing evidence suggests that LPO plays a central role in the development of various pathological conditions. The intensification of oxidative processes results in the depletion of unsaturated fatty acids and a relative accumulation of saturated fatty acids, ultimately disrupting membrane organization and functional integrity. To assess oxidative damage, we measured the content of malondialdehyde (MDA), a widely recognized end product of LPO, in the liver tissues of pregnant rats and embryos (Figs. 9 and 10). Our findings demonstrate that the administration of butylcaptax on GD 3 markedly stimulated both NADPH-dependent and ascorbate-dependent LPO in the maternal liver, increasing these parameters by 61% and 75%, respectively. In the microsomal fractions, LPO products increased by 60% and 72%. Exposure on GD 13 similarly enhanced both enzymatic and non-enzymatic LPO, although the magnitude of change was less pronounced compared to GD 3. By GD 19, LPO levels remained elevated, with NADPH-dependent and ascorbate-dependent processes increasing by 62% and 58% in the mitochondria, and by 67% and 62% in the microsomes. Notably, the microsomal fractions consistently exhibited higher levels of LPO than the mitochondrial fractions, indicating the greater susceptibility of these membranes to oxidative damage (Taha *et al.* 2021; Dohl *et al.* 2025). Comparable trends were observed in the embryonic tissues, although the magnitude of the changes was less pronounced. On GD 3, LPO increased substantially in both the mitochondria and microsomes. By GD 13 and 19, elevated MDA levels persisted, confirming that oxidative stress extends to the embryonic tissues. These findings support the hypothesis that the placental barrier provides only partial protection against xenobiotic-induced oxidative damage (Zhang *et al.* 2025). The elevated LPO levels in the microsomes can be explained by their structural and functional organization. In NADPH-dependent systems, polyunsaturated fatty acids located near the redox-active components of the electron transport chain are particularly prone to oxidation. In contrast, ascorbate-dependent LPO involves alternative lipid substrates. Additionally, oxidative stress may trigger the activation of phospholipases, leading to the formation of lysophospholipids, which further destabilize the membrane structure and amplify cellular damage (Engel *et al.* 2021; Prabutzki *et al.* 2024). These observations are consistent with recent studies demonstrating that pesticide exposure induces oxidative stress through increased ROS production, mitochondrial impairment, and enhanced lipid peroxidation (Janoš *et al.* 2023; Dohl *et al.* 2025). Furthermore, experimental evidence indicates that butylcaptax and Dropp can reduce cytochrome P450 levels while promoting oxidative damage in hepatic membranes, thereby exacerbating their toxic effects (Tuychieva 2023). Taken together, the results indicate that both butylcaptax and Dropp act as potent inducers of oxidative stress, leading to the structural disruption of membrane systems, particularly within the microsomal fractions. These alterations may contribute to the development of hepatotoxicity and embryotoxicity through the combined mechanisms of lipid peroxidation and membrane remodeling. As illustrated in the diagram, the control group demonstrated a relatively stable level of lipid peroxidation (LPO) in the mitochondria throughout gestation, with a slight increase

observed on GD 13, followed by a decrease by GD 19. In contrast, the microsomal fractions exhibited more pronounced fluctuations, characterized by a reduction in LPO on GD 13 and a partial restoration by GD 19.

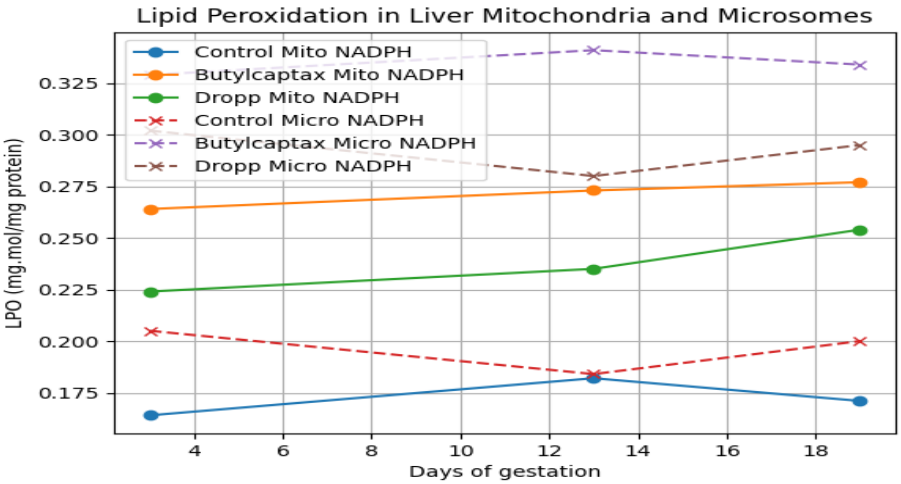


Fig. 9. Lipid peroxidation (LPO) product content in the liver mitochondria and microsomes of pregnant rats treated with butylcaptax and Dropp (nmol MDA/ *mg.mol/mg protein*).

These changes may reflect the activation of compensatory antioxidant mechanisms aimed at maintaining membrane integrity. The administration of butylcaptax induced a significant elevation of LPO levels in both the mitochondrial and microsomal fractions at all examined stages of pregnancy. In the mitochondria, the increase occurred progressively, suggesting an intensification of free radical-mediated reactions and potential structural damage to membrane components. In the microsomes, LPO reached a maximum on GD 13, followed by a slight decline, although levels remained substantially higher than the control values. This pattern indicates the pronounced pro-oxidant activity of butylcaptax and its capacity to disrupt redox homeostasis. In the Dropp-treated group, an increase in LPO was also observed relative to the control; however, the magnitude of this effect was lower than that induced by butylcaptax. Mitochondrial LPO demonstrated a gradual elevation toward GD 19, suggesting a cumulative effect of the compound over time. In the microsomes, LPO levels remained consistently elevated with moderate variations, indicative of persistent disturbances in lipid metabolism and membrane stability. Overall, the findings indicate that both butylcaptax and Dropp act as potent inducers of oxidative stress, enhancing lipid peroxidation processes in hepatic organelles. Notably, butylcaptax exhibited a more pronounced cytotoxic potential compared to Dropp. The observed increase in LPO is likely to compromise the structural and functional integrity of cellular membranes, particularly in metabolically active organelles such as the mitochondria, as well as in microsomal systems involved in detoxification processes. The effect of Dropp was studied at the same stages of pregnancy and embryonic development. On GD 3, enzymatic and non-enzymatic LPO increased by 36% and 43% in the mitochondria, and by 60% and 50% in the microsomes, respectively. Intoxication on GD 13 resulted in an increase of MDA levels in the mitochondria and microsomes by 29% and 45%, and by 50% and 37%, respectively. Exposure of pregnant rats on GD 19 led to an increase in MDA levels in the mitochondria by 50% and 79%, and in the microsomes by 47% and 45%. Similar changes in oxidation products were observed in the mitochondria and microsomes of the embryonic liver exposed to Dropp on GD 3, 13, and 19 (see Fig. 10). On GD 3, enzymatic and non-enzymatic LPO in the embryonic liver mitochondria increased by 66% and 42%, and in the microsomes by 60% and 50%, respectively. On GD 13, the increase was 41% and 50% in the mitochondria and 44% and 37% in the microsomes. On GD 19, enzymatic LPO increased in the mitochondria and microsomes by 57% and 47%, respectively, while ascorbate-dependent LPO increased by 52% and 33%. The levels of enzymatic LPO in the mitochondria, as well as ascorbate-dependent LPO in both the mitochondria and microsomes, were significantly lower than those observed after butylcaptax exposure. However, NADPH-dependent LPO in the microsomes remained at the same level as in the liver of pregnant rats intoxicated with butylcaptax. As described above, in the mitochondrial fraction of the control liver, the level of lipid peroxidation (LPO) remains relatively stable, corresponding to the physiological level of reactive oxygen species (ROS) generation during cellular respiration (Chaudhary *et al.* 2023). Mitochondria are primary intracellular sources of ROS, making them key participants in lipid peroxidation processes (Feng *et al.* 2022). Under the

influence of butylcaptax, a consistent increase in LPO was observed at all stages of gestation, indicating a pronounced pro-oxidant effect and the impairment of mitochondrial function. A similar but less pronounced trend was observed with Dropp exposure. From a biochemical perspective, LPO is a free-radical chain reaction comprising the stages of initiation, propagation, and termination, during which the polyunsaturated fatty acids of membrane lipids undergo oxidation (Dragoev *et al.* 2024). The intensification of these processes is associated with the accumulation of lipid hydroperoxides and secondary toxic products that disrupt the structure and function of biological membranes (Liu *et al.* 2025).

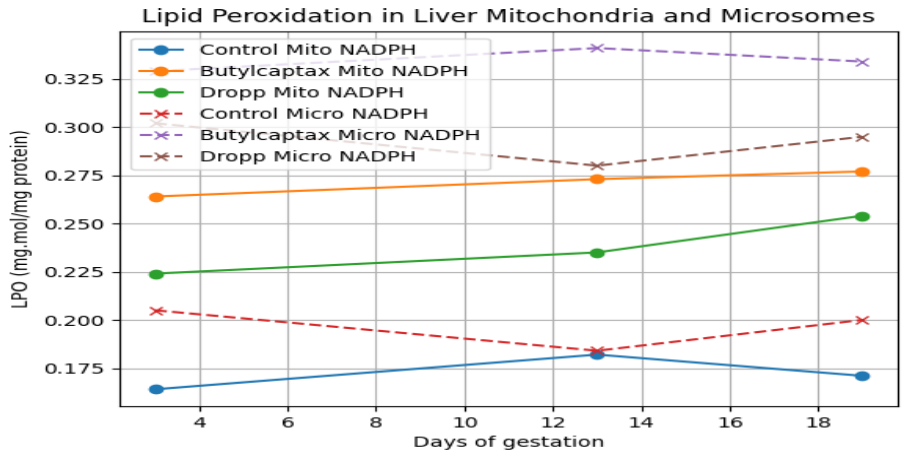


Fig. 10. Lipid peroxidation (LPO) product content in the fetal liver mitochondria and microsomes under the influence of butylcaptax and Dropp (*mg.mol/mg protein*).

In the microsomal fraction of the liver, the LPO intensity in the control group showed moderate fluctuations, reflecting adaptive processes in the endoplasmic reticulum. Microsomes are known to possess high enzymatic activity, particularly involving cytochrome P450 systems, which contribute to the NADPH-dependent generation of free radicals (McCay *et al.* 2020). Exposure to butylcaptax resulted in the highest LPO levels among all groups, especially on GD 13, indicating a significant enhancement of oxidative stress. Dropp also induced an increase in LPO; however, its effect was less pronounced. Additionally, NADPH-dependent systems, including NADPH oxidase, play a crucial role in ROS generation and the initiation of lipid peroxidation processes (Feng *et al.* 2022). An imbalance between pro-oxidant and antioxidant systems leads to the damage of lipids, proteins, and nucleic acids, ultimately resulting in cellular dysfunction (Zhang *et al.* 2024). Comparative analysis showed that the microsomal fraction is more susceptible to LPO intensification, which is associated with a high content of polyunsaturated fatty acids and active oxidative enzyme systems (McCay *et al.* 2020). Overall, the obtained data indicate that both studied compounds induce oxidative stress, with butylcaptax exerting a more pronounced pro-oxidant effect than Dropp, potentially leading to the disruption of the structural and functional integrity of cellular membranes during pregnancy. The present data indicate that butylcaptax and Dropp significantly intensify lipid peroxidation (LPO) in both the mitochondrial and microsomal fractions of the maternal and embryonic liver, as evidenced by elevated malondialdehyde (MDA) levels under both NADPH-dependent and ascorbate-dependent conditions. This pattern is consistent with current concepts of pesticide-induced hepatotoxicity, in which excessive reactive oxygen species (ROS) formation, mitochondrial dysfunction, and the oxidative degradation of membrane lipids represent key mechanisms of toxic injury (Rauf *et al.* 2024; Zhang *et al.* 2024). Recent studies have demonstrated that pesticide exposure promotes oxidative liver damage through ROS overproduction, the disruption of redox homeostasis, and the activation of cell death–related pathways (Rauf *et al.* 2024). A mechanistic explanation for the observed increase in MDA may be linked to the high susceptibility of membrane phospholipids containing polyunsaturated fatty acids (PUFAs) to oxidative degradation. Phosphatidylethanolamine and phosphatidylcholine species enriched with arachidonic acid are considered preferential substrates for peroxidation; their excessive oxidation leads to alterations in membrane architecture, reduced membrane stability, and the propagation of oxidative damage (Chen *et al.* 2024; Chen *et al.* 2025). In the mitochondria, lipid peroxidation plays a particularly critical role, as the oxidation of membrane phospholipids, including cardiolipin-associated pools, is directly linked to increased membrane permeability, impaired bioenergetics, and the activation of apoptotic signaling pathways (Huan *et al.* 2024; Chen *et al.* 2024). The more pronounced increase in lipid peroxidation products in the microsomes compared to the mitochondria may be

explained by the biochemical organization of the microsomal membrane system. Microsomal membranes contain phospholipid acyl chains located in close proximity to redox-active components, which facilitates ROS-dependent initiation and the propagation of lipid oxidation. This interpretation is supported by recent studies demonstrating that pesticide-induced oxidative damage in hepatic tissues is closely associated with disturbances in electron transport chains, enhanced ROS generation, and subsequent membrane destabilization (Rauf *et al.* 2024). In addition, the observed increase in lipid peroxidation can be interpreted in the context of phospholipase-mediated membrane remodeling. Contemporary research indicates that lysophospholipids accumulate in oxidatively damaged tissues through both direct ROS-mediated phospholipid cleavage and the activation of phospholipase A2 (Zheng *et al.* 2024; Chen *et al.* 2025). These lysophospholipids act not only as markers of membrane damage but also as bioactive mediators capable of further destabilizing lipid bilayers and amplifying inflammatory and oxidative responses. Therefore, the reductions in total phospholipids (TPL) or phosphatidylethanolamine (PE), accompanied by increased lysophospholipid (LPL) fractions, strongly support the hypothesis that pesticide exposure induces both peroxidative degradation and the enzymatic remodeling of membranes. The present findings are in agreement with previous studies addressing the toxicological effects of butylcaptax and Dropp. Experimental evidence from 2023 demonstrated that these pesticides enhance lipid peroxidation in the mitochondrial and microsomal membranes of pregnant rats and their embryos, which is associated with a decrease in cytochrome P450 content, further supporting the role of oxidative membrane damage in pesticide toxicity (Zhang *et al.* 2024; Wang *et al.* 2026). Although contemporary data specifically focused on these compounds remain limited, broader toxicological studies published between 2020 and 2025 consistently describe a common pathogenic cascade involving ROS overproduction, phospholipid peroxidation, membrane destabilization, and subsequent mitochondrial and hepatocellular dysfunction (Rauf *et al.* 2024; Kanner *et al.* 2025). The embryonic response observed in the present study was qualitatively similar but quantitatively less pronounced compared to the maternal liver tissues, which may reflect the partial protection provided by the placental barrier. However, the persistence of increased lipid peroxidation in embryos, particularly at later developmental stages, suggests that this protective mechanism is incomplete and that oxidative damage may nevertheless contribute to embryotoxic effects. This is biologically plausible, as the oxidative modification of membrane phospholipids can disrupt mitochondrial function, intracellular signaling, and metabolic adaptation during embryonic development (Chen *et al.* 2024). Overall, the results demonstrate that butylcaptax and Dropp act as potent inducers of oxidative stress, leading to significant membrane damage in hepatic organelles, with microsomal membranes showing particularly high sensitivity. From a mechanistic perspective, the elevated MDA levels reflect not only enhanced free radical oxidation of polyunsaturated phospholipids but also secondary membrane remodeling processes involving phospholipase activation and lysophospholipid accumulation. These processes likely underlie the structural and functional disturbances associated with pesticide-induced hepatotoxicity (Chen *et al.* 2025; Kanner *et al.* 2025; Burkhanov *et al.* 2025). Thus, the oxidation products of butylcaptax and Dropp stimulate lipid peroxidation reactions in the mitochondrial and microsomal membranes of the maternal and embryonic liver, leading to the structural and functional impairment of these organelles.

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