INTRODUCTION

In the mid-20th century, increasing concerns about global food scarcity and the constraints of conventional farming methods led to a substantial change in agricultural strategies. Scientists and agricultural specialists acknowledged the pressing necessity to create novel crop kinds that can provide higher yields and resist pests and illnesses (Fischer, 2009). This endeavor resulted in the implementation of high-yield crop types, combined with the extensive acceptance of contemporary farming methods and the use of chemical inputs such as fertilizers and pesticides. This marked the beginning of what later became known as the Green Revolution. It had a significant global influence, especially in areas that were struggling with persistent food poverty (Patel, 2013). Nations such as Mexico, the Philippines and Pakistan witnessed substantial enhancements in agricultural productivity, resulting in surpluses in food production and effectively mitigating hunger for millions of individuals (Conway, 2012). This has significantly influenced global trade dynamics eventually by utilizing agricultural surpluses from Green Revolution countries to stabilize food prices and mitigate the risk of starvation in vulnerable regions.

The emergence of the Green Revolution signifies a pivotal moment in the course of human history, signaling a significant shift in worldwide agricultural methods and altering the path of food manufacturing. This revolutionary movement brought forth numerous advancements, such as high-yield crop varieties, advanced irrigation systems and chemical inputs (De Schutter & Vanloqueren, 2011). Nevertheless, its primary objective was to combat the prevalent issues of food insecurity and famine. During the same time, India saw significant transformations due to the Green Revolution, which led to substantial changes in its socio-economic structure and the attainment of exceptional growth in agricultural production.

During the late 1960s and early 1970s, India experienced significant progress in the Green Revolution, driven by the innovative work of agricultural pioneers like M.S. Swaminathan and supported by key officials in the union government. This era saw a substantial increase in the use of high-yielding varieties of wheat and rice, the development of irrigation systems and the adoption of modern agricultural methods (Alauddin & Quiggin, 2008). These initiatives marked a major advancement in India's efforts to improve food security and agricultural sustainability, leading to remarkable growth in the agricultural sector. As a result, agricultural yields increased significantly, leading to a substantial rise in food production. This achievement enabled India to attain self-sufficiency in essential crops like wheat and rice (Swaminathan, 2013). Consequently, India, once reliant on food assistance and imports to meet its nutritional needs, transformed into a net exporter of food grains, thereby strengthening its food security and enhancing its international reputation.

The Green Revolution had significant socio-economic consequences for India, especially in rural regions where agriculture was the only source of income for millions of individuals. The implementation of high-yielding crop varieties and sophisticated farming practices resulted in enhanced financial gains for farmers, elevated living conditions and the establishment of a thriving agricultural industry (Swaminathan, 2013). Nevertheless, the advantages of the Green Revolution were not uniformly allocated and discrepancies in the availability of resources and technologies persisted, intensifying socio-economic inequality in rural India.

Although the Green Revolution achieved substantial improvements in agricultural productivity and food security, it encountered various hurdles and received criticism. Environmental deterioration, soil erosion and water pollution have become significant concerns due to the extensive utilization of chemical inputs like fertilizers and insecticides (Fischer, 2009). In addition, the intensive monoculture farming methods linked to the Green Revolution resulted in a decline in biodiversity and the degradation of traditional agricultural knowledge (Aktar et al., 2009).

Demonstrating the effectiveness of science and technology in addressing pressing global challenges, the Green Revolution stands as a testament to human ingenuity and innovation. In India, it brought about a significant transformation in the agricultural sector, resulting in the upliftment of a large number of individuals from poverty and guaranteeing food security for a rapidly expanding population (Swaminathan, 2013). Nevertheless, the impact of the Green Revolution is intricate, as it has resulted in both accomplishments and shortcomings that have influenced the trajectory of agriculture in India and other regions.

The employment of pesticides during the Green Revolution represented a pivotal moment in agricultural methodologies, resulting in a notable surge in the yield of key crops since the 1960s (Patel, 2013). Pesticides have proven to be highly beneficial for agriculture as they provide essential defense against weeds, pests and diseases (Fischer, 2009). These factors are responsible for causing almost 40 percent of the world's potential crop loss each year. The presence of around thirty thousand species of weeds, three thousand species of worms and ten thousand species of plant-eating insects that compete for food crops highlights the crucial importance of pesticides in protecting agricultural productivity (Aktar et al., 2009).

Moreover, pesticides play an important role in safeguarding crops during the process of shipping and storage, where they are vulnerable to harm caused by insects, fungi and rodents. Furthermore, pesticides serve a major task in maintaining food security and minimizing food waste by increasing the longevity of crops and limiting losses after harvesting (Fischer, 2009).

In addition to protecting crops, pesticides also have a vital function in reducing food contamination caused by hazardous microbes and naturally occurring poisons. Applying pesticides reduces the likelihood of foodborne illnesses and guarantees the safety of food for consumers (Onyeaka et al., 2024). However, it is crucial to acknowledge that although pesticides are deadly to specific pests, they can also have unintended consequences on non-target species, including humans. The continuous dilemma of overuse and misuse of pesticides is primarily driven by the need for rapid and significant benefits in agricultural productivity (Damalas & Eleftherohorinos, 2011).

Pesticides, crucial instruments in contemporary agriculture, can be categorized into different groups according to their intended purpose, toxicity, method of entrance, method of operation, chemistry and formulations. Pesticides are classified based on the pests they target and are designed to effectively combat specific types of pests.

Herbicides are specifically designed to manage the growth of undesired plants. They focus on weeds that compete with crops for essential resources like sunlight, water and nutrients. By getting rid of weeds, herbicides improve crop yields and increase agricultural output (Das, 2013).

Rodenticides, however, are specifically formulated to eradicate rodents, such as rats and mice, that present risks to stored grains and agricultural crops. Rodents not only devour and contaminate food sources but also harm crops and infrastructure (Das, 2013). As a result, rodenticides are crucial for controlling pests in both agricultural and urban environments.

Bactericides specifically combat bacterial infections, offering defense against diseases produced by harmful bacteria that can severely damage crops and undermine productivity (Das, 2013). Bactericides play a crucial role in managing bacterial infections, which in turn helps to preserve the health of crops and guarantee the production of high-quality food for consumption and commerce.

Fungicides are designed to control fungal infestations, which can negatively impact crops by impeding their growth, development and productivity (Das, 2013). Fungal diseases, including powdery mildew, rust and blight, pose significant risks to a wide range of crops, especially in areas characterized by elevated levels of humidity and precipitation.

Larvicides, a distinct class of pesticides, especially aim to eradicate insect larvae, hence impeding their growth into fully developed pests capable of inflicting harm on crops (Das, 2013). By disrupting insect pests' life cycles, larvicides help reduce pest populations and mitigate crop losses.

In India, where the tropical climate heightens vulnerability to insect infestations and other pest pressures, insecticide utilization is particularly high compared to other types of pesticides. Insecticides are essential for safeguarding crops against various insect pests, such as aphids, caterpillars, beetles and mites. If these pests are not controlled, they can substantially reduce agricultural productivity (Subedi et al., 2023).

The wide range of pesticides accessible to farmers allows them to efficiently control insect populations and protect the health of their crops, thereby promoting sustainable agriculture and ensuring food security. Nevertheless, it is crucial to employ pesticides prudently and in accordance with prescribed guidelines to limit detrimental effects on human health, the environment and non-target creatures (Tudi et al., 2021).

Diamides, a recent addition to the insecticide market, signifies a notable progression in pest control technology. These chemicals demonstrate potent and selective activity at specific target sites, allowing them to effectively manage a broad spectrum of pests with accuracy and effectiveness. Diamides offer a significant benefit due to their favorable toxicity profile, making them safer alternatives to conventional insecticides (Troczka et al., 2017). Diamides are a class of artificial compounds known as ryanoids. This group includes chlorantraniliprole, cyantraniliprole and flubendiamide. These compounds are specifically developed to imitate the way the natural pesticide Ryanodine, found in the *Ryania speciosa* plant, works. Gaining a comprehensive understanding of how diamides work is crucial for determining their efficacy and their uses in controlling pests (Troczka et al., 2017).

At the molecular level, diamides exert their insecticidal activity by targeting the ryanodine receptor, a calcium channel protein crucial for regulating calcium levels in insect cells. Ryanodine, a naturally occurring pesticide, attaches to the ryanodine receptor and inhibits the calcium channel to some extent, thus interfering with the movement of calcium within the cell (Figure 1.1). In contrast, diamides interact with the identical receptor, but they induce a persistent opening of the calcium channel (Troczka et al., 2017). The unregulated discharge of calcium reserves affects multiple cellular functions, resulting in physiological consequences such as fatigue, decreased appetite and, ultimately, mortality at the organismal scale. Mammalian tissues display three unique isoforms of the Ryanodine receptor (RyR): RyR1, predominantly found in skeletal muscles; RyR2, principally located in cardiac muscles; and RyR3, which is expressed in diverse tissues, including the brain, though at lower levels (Williams et al., 2001). Among the nonmammalian vertebrates, such as birds and fish, RyRα and RyRβ are the isoforms that are present (O'Brien et al., 1993).

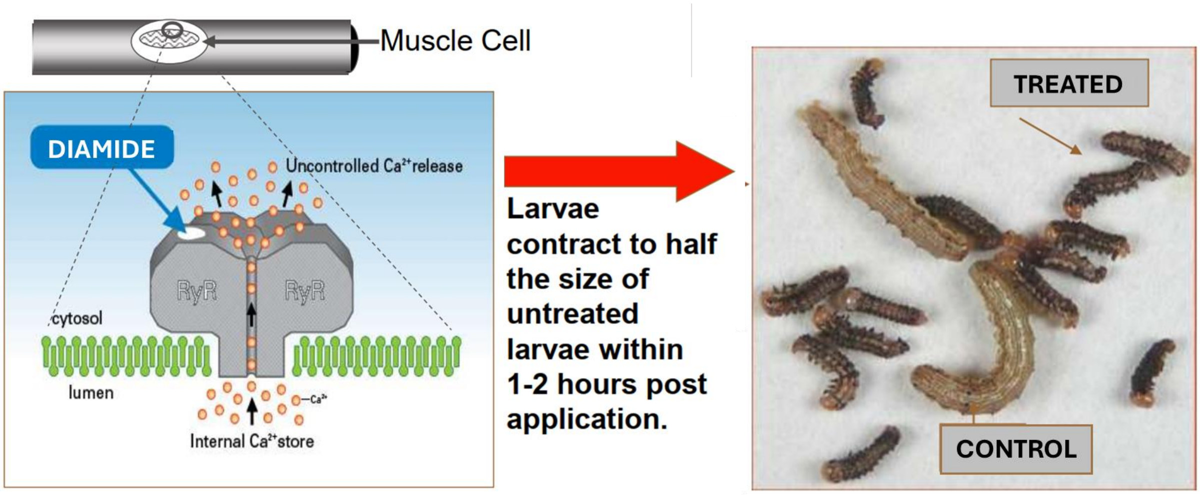
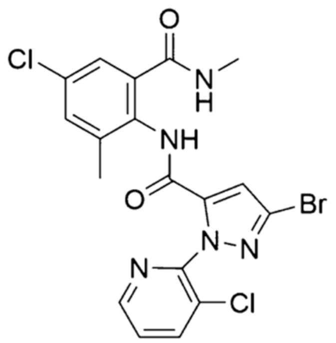


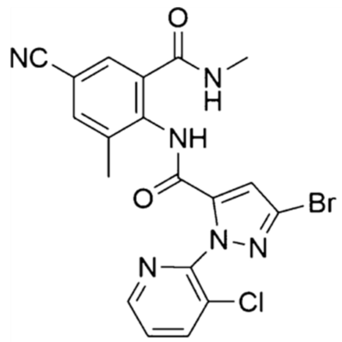
Figure 1.1: Diamide mode of action
(https://irac-online.org/documents/dsiamide-moa-rotation/)

Based on their chemical properties, diamides can be categorized into two groups: Anthranilic Diamides and Phthalic Diamides. Chlorantraniliprole and cyantraniliprole are classified as anthranilic diamides, but flubendiamide is categorized as a phthalic diamide (Figure 1.2). Chlorantraniliprole and cyantraniliprole are distinguished by the inclusion of chlorine and cyanide groups in their respective chemical structures. Flubendiamide, however, falls within the group of phthalic diamides and has a distinct chemical structure that incorporates fluoride atoms within its chemical composition. These components function as the core structure that is accountable for their insecticidal properties (Teixeira, 2013).

Anthranilic Diamides

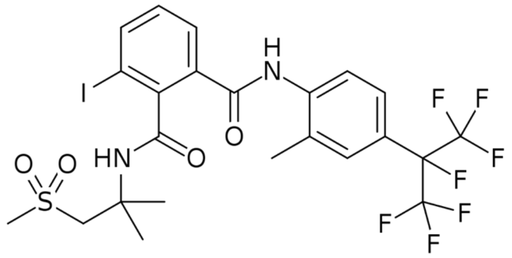


Chlorantraniliprole



Cyantraniliprole

Phthalic Diamide



Flubendiamide

Figure 1.2: Structural classification of diamides

Flubendiamide has become widely recognized and adopted in pest management because of its excellent selectivity and minimal harm to non-target organisms. It is widely employed to safeguard crops such as rice, cotton, corn, grapes and assorted fruits and vegetables from lepidopteran pests (Das et al., 2017). Its specific targeting of harmful pests and minimal harm to other creatures make it a desirable choice for integrated pest management systems. Moreover, flubendiamide has surfaced as a possible substitute for organophosphate and organochlorine insecticides, which present substantial environmental and health hazards.

The incorporation of diamide insecticides such as flubendiamide signifies a noteworthy advancement in the implementation of sustainable pest control methods. Flubendiamide effectively controls targeted pests while limiting the negative impact on beneficial insects, thus promoting ecological equilibrium and reducing reliance on broad-spectrum pesticides (Teixeira, 2013). Moreover, its minimal harm to animals and aquatic organisms reduces the likelihood of adverse effects on human health and the environment.

Flubendiamide and other diamides offer a promising solution to the challenges faced in agricultural pest management. Due to their precise targeting of specific sites, low toxicity to non-target organisms and minimal environmental impact, they are highly valuable in integrated pest management systems (Das et al., 2017). As the agricultural industry evolves, diamides are expected to play an increasingly important role in sustainable pest management strategies. This will contribute to the long-term sustainability of agricultural fields and environmental conservation.

A recent experiment has highlighted the harmful effects of diamides on developing avian embryos, revealing that even at low concentrations, diamides can be detrimental. The treated groups exhibited a wide range of qualitative abnormalities compared to their untreated counterparts, as described by Abbas et al. (2018). These abnormalities included microcephaly, hydrocephaly, edematous swelling, hematoma formation, abnormal body pigmentation, microphthalmia, distorted beak, agnathia, micromelia, amelia, omphalocele and ectopia cordis. The findings indicate that even low-level exposure to diamides can cause severe disruptions in the normal development of bird embryos.

Research conducted by Sarkar et al. (2017) has shown that flubendiamide significantly reduces fertility in non-target dipteran insects like *Drosophila melanogaster*. By diminishing reproductive ability, flubendiamide has the potential to impact insect populations that it was not intended to target. Another study by Sarkar et al. (2018) found that flubendiamide can alter the visual and locomotory activities of *Drosophila melanogaster*, suggesting that the pesticide affects the behavior and physiology of these insects.

When flubendiamide was tested on organisms that were not the intended targets, there were concerns expressed regarding the wider ecological impact of the chemical. The effects of flubendiamide on the Chinese tiger frog (*Hoplobatrachus chinensis*) were investigated by Li et al. (2014) and their findings suggest that flubendiamide may have negatively impacted amphibian populations. As an additional point of interest, the discovery of flubendiamide residues in human milk (Liu et al., 2022) has brought forth substantial public health concerns, particularly regarding the health of mothers and infants.

Considering these findings, it is of the utmost importance to carry out exhaustive safety evaluations of flubendiamide to determine the potential developmental poisoning it may cause. This includes conducting in-depth research on its impact on organisms that are its goal, as well as organisms that are not its target and how it may affect human health. It is clear from the evidence that stringent regulatory measures and additional research are required in order to reduce the dangers that are linked with the use of flubendiamide in agricultural management methods.

Chick as a model

The chick embryo has become a crucial model in developmental biology due to its intriguing characteristics. It shares significant molecular, cellular and anatomical similarities with the human embryo, making it highly valuable for research. Its rapid development, combined with ease of observation and experimental manipulation, enhance its utility. The relatively large size and planar form of the embryo during its early stages facilitate in-depth research. Live optical imaging of the chick embryo is easily achieved by creating a small window in the eggshell. This window, along with various cell-marking techniques, provides a reliable tool for real-time monitoring of cell movements and destinies (Vergara & Canto-Soler, 2012).

An advantage of the chick embryo model over mammalian models is that it enables precise interventions to be performed at particular phases of embryonic development. Additionally, the chick embryo model allows for the potential of continuing development by simply shutting the window and re-incubating the egg (Burt, 2005). It has been discovered through the sequencing of the chicken genome that the chicken has a gene count that is comparable to that of humans, a high level of sequence conservation and a significantly more compact diploid genome. In the field of comparative genetics, as well as in the study of gene regulation and evolution (Stern, 2018), these qualities are quite desired.

The chick embryo model is advantageous both economically and practically. Eggs and their housing are affordable, enabling a wide range of laboratories to conduct extensive research due to their accessibility. Eggs are consistently available year-round in predictable quantities, facilitating easier planning and execution of research projects. Additionally, embryonic chicks possess the unique capability to regenerate their retinas at specific developmental stages, making them suitable for studying regenerative eye biology (Vergara & Canto-Soler, 2012).

Subsequently, taking into consideration India's agricultural economy and the widespread practice of poultry farming in rural regions, it is common for chicks in these surroundings to be subjected to pesticides, both directly and indirectly, through the consumption of food grains

(Figure 1.3). In light of this, the chick embryo is an excellent model for the investigation of the effects of pesticide exposure, which is significant to the contexts of both agronomic and public health.

Figure 1.3: Rhode Island Red hens eating food grains from the ground (https://www.thehappychickencoop.com/rhode-island-red/)

Origin of problem

The chick embryo model has been used to investigate the developmental toxicity of diamide pesticides, particularly flubendiamide. This research revealed significant embryonic malformations, suggesting a potential threat to embryonic development. Initial laboratory studies identified various developmental anomalies in chick embryos exposed to flubendiamide, including insufficient angiogenesis, eye and limb deformities and incomplete ventral body wall closure. These findings indicate that flubendiamide, a pesticide commonly used in agriculture for insect control, may disrupt essential developmental processes.

The observed disruptions in angiogenesis and morphogenesis highlight the need for a more comprehensive examination of the mechanisms by which flubendiamide induces these teratogenic effects. Understanding these mechanisms is crucial for assessing the broader implications of flubendiamide exposure on vertebrate development and for framing regulatory policies on pesticide use.

Objectives of this study

The main aim of the investigation is to elucidate the underlying mechanisms behind the structural and functional abnormalities triggered by flubendiamide during the developmental stages of chick embryos.

This will be achieved through three parallel studies listed below, which can be treated as specific objectives.

1. To examine the efficacy of flubendiamide on the morphology and hematology of developing chick.
2. To decipher the function of flubendiamide in the process of chorioallantoic membrane (CAM) angiogenesis in chick embryogenesis.
3. To unearth the role of flubendiamide in the domestic chick's eye development and patterning.
4. To elucidate the impact of flubendiamide on the liver of newborn chicks.

Morphology and hematology

Wilson (1973) defines developmental toxicity as the set of abnormalities that influence an organism's growth, such as malformations, functional deficits, developmental delays and mortality. These issues can be the result of a variety of chemical or physical factors and typically manifest across multiple organ systems prior to birth, suggesting early teratogenic effects. It is imperative to conduct a comprehensive investigation and comprehend the toxicity of pesticides in light of their potential developmental interference and the increasing introduction of these products into the market.

Hematological parameters are sensitive indicators of systemic toxicity, organ dysfunction and immune system activation, providing valuable insights into the overall health of the organism (Witeska et al., 2023). It is imperative to comprehend the hematological profile in order to evaluate the health status and physiological responses of organisms that have been exposed to environmental stressors, such as pesticides. The importance of hematological profiling in ecotoxicological studies has been underscored by recent research, which emphasizes its role in assessing the sublethal effects of environmental contaminants on wildlife populations (Pérez-Cadahía et al., 2020).

CAM Angiogenesis

Angiogenesis, the process of creating new blood vessels, is a fundamental step in embryonic development. It plays a crucial role in building an intricate network of blood vessels that is essential for supporting life. Throughout this complex procedure, newly forming blood vessels act as channels for transporting vital oxygen and nutrients to developing tissues, guaranteeing their appropriate growth and functionality (Felmeden et al., 2003; Wittig & Münsterberg, 2016). This network not only promotes the increase in size but also plays a role in maintaining the stability and balance of tissues, enabling tasks such as healing and the exchange of nutrients (Felmeden et al., 2003). A notable example highlighting the importance of angiogenesis is the CAM, a crucial extraembryonic tissue responsible for gas exchange, nutrient transport and waste elimination in the developing chick embryo (Ahmed et al., 2022).

During the development of the chick embryo, CAM angiogenesis occurs in a series of steps, starting with the production of blood islands on day 2 and ending with the full development of the vascular system by day 21. At first, basic blood vessels appear and then develop into a complex network through several processes, such as the creation of the bilayered CAM and the following stages of growth and branching (Schmidt et al., 2019). Significantly, this ongoing process reaches its peak by days 15 to 18, resulting in increased vascular density. This enhances the exchange of gases and transfer of nutrients, hence facilitating the growth of the embryo (Schmidt et al., 2019). By day 21, the completion of CAM angiogenesis indicates the formation of a strong vascular system that is essential for supporting the growth of the developing embryo.

During embryonic development, blood arteries play a dual role. They not only transport nutrients but also contribute to the regulation of cellular balance and support metabolic functions. Vascular endothelial growth factor (VEGF) plays a crucial role in the process of angiogenesis, which is the creation of blood vessels. VEGF acts as a critical regulator and triggers a series of important molecular events (Hiratsuka et al., 2005). Research has shown that VEGF plays multiple roles in angiogenesis. It activates bone morphogenetic proteins (BMP2 and BMP6) and triggers downstream pathways such as the PI3K-Akt/mTORC2 pathway. These pathways are crucial for the proliferation of endothelial cells and the formation of new blood vessels (Karar & Maity, 2011; Guo et al., 2018; Pulkkinen et al., 2021).

In addition, the interaction between VEGF and its receptor KDR (VEGFR-2) with proliferating cell nuclear antigen (PCNA) highlights their crucial function in stimulating the proliferation of endothelial cells and the construction of blood vessels (Hiratsuka et al., 2005; Strzalka & Ziemienowicz, 2011) (Figure 1.4). The sonic hedgehog (SHH) signaling pathway has been identified as an important regulator that affects the dynamics of endothelial cell cytoskeleton through the PI3K/AKT pathway, thereby playing a role in angiogenesis. This has been demonstrated in studies by Zavala et al. (2017), Salybekov et al. (2018) and Lei et al. (2020).

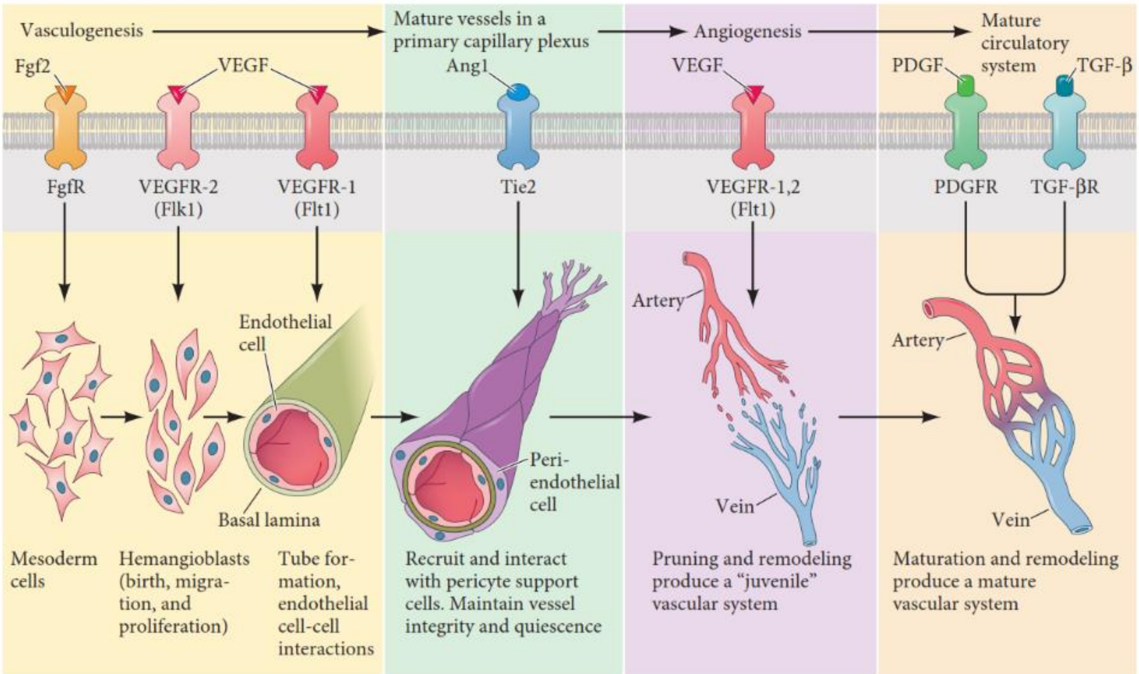


Figure 1.4: Vasculogenesis and Angiogenesis (Gilber 11th Edition)

Within the framework of CAM vascularization, the process of blood vessel sprouting is regulated by signaling molecules such as WNT7A and BMPs. This process is crucial for the formation of a fully developed network of capillaries, known as a capillary network plexus (Ahmed et al., 2022). BMP2 and BMP6 are important in maintaining the flexibility of endothelial cells during sprouting. They work together with SHH and WNT7A to preserve the properties of endothelial cells (Benn et al., 2017; Olsen et al., 2017). Therefore, the coordinated activity of several signaling pathways and molecules guarantees the strength and flexibility of the CAM vascular system, which is crucial for facilitating embryonic development.

Eye Formation

The development of eyes in chick embryos is a highly organized process controlled by an intricate interaction of signaling molecules. The complex procedure initiates by designating the head ectoderm to receive signals from brain bulges, resulting in the formation of a bias that leads to the construction of a lens (Saha et al., 1989). Following this, the OTX2 protein specifies the anterior neural tube, whereas the activation of PAX6 inhibits this process. This leads to a series of transcription factors that are essential for defining the eye field, ultimately resulting in the creation of a single eye field (Zuber et al., 2003; Zuber, 2010) (Figure 1.5). The process of dividing the single eye field into two side fields is directed by the expression of SHH, which inhibits PAX6 in the middle region (Macdonald et al., 1995).

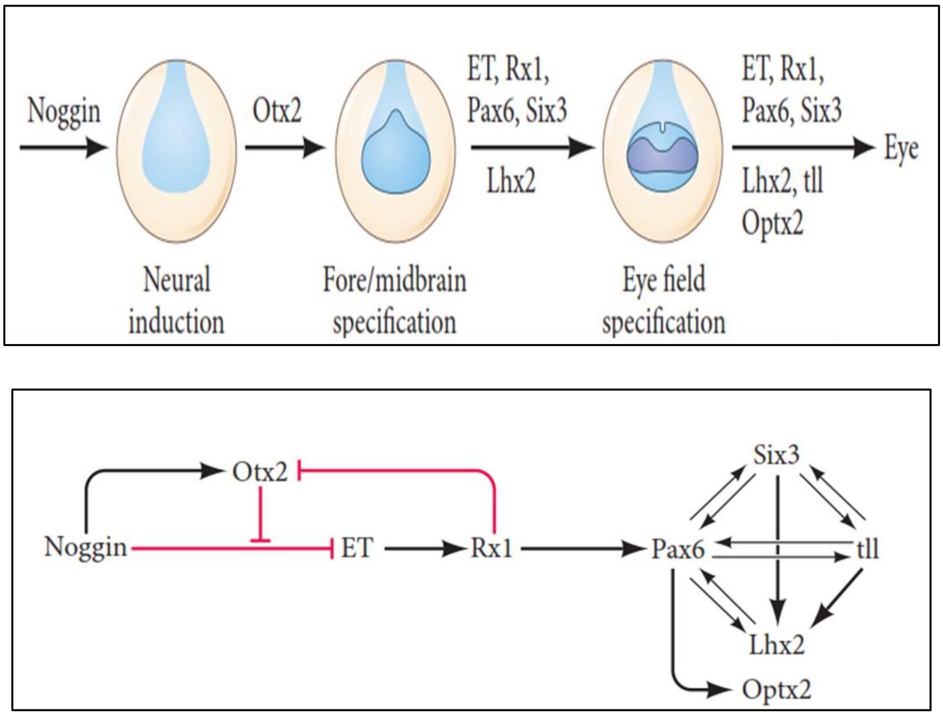


Figure 1.5: Oculogenesis- Eye field formation (Gilber 11th Edition)

During chick embryo development, the formation of the eye progresses through distinct stages. It begins on day 2 with the emergence of the optic stalk and optic cup. Interactions between the outer layer of cells and developing eye structures initiate a process where the outer layer cells transform into the lens placode. This transformation culminates in the formation of the lens pit through invagination (Hilfer, 1983). By the third day, the lens vesicle forms from the lens pit, followed by the subsequent development of the retina and cornea. Pigmentation occurs on the fourth day (Hamburger and Hamilton, 1951; Khan and Ackerman, 2023).

During the development of the eye, several chemicals have important functions in coordinating activities such as cell growth, motility, organization and programmed cell death (Khan and Ackerman, 2023). It is worth mentioning that noggin causes the activation of OTX2 in the front part of the neural tube, whereas PAX6 plays a crucial role in determining the eye field and starting a series of transcription factors (Zuber et al., 2003; Zuber, 2010).

SHH, a crucial signaling molecule, regulates the organization and splitting of the single eye field into two by inhibiting the expression of PAX6 in the midline (Cavodeassi et al., 2019). The dysregulation of SHH-PAX6 signaling disrupts optic cup growth, lens cell apoptosis and overall eye development (Yamamoto et al., 2004).

In addition, genes like WNT11 and adhesion molecules like CDH1 and CDH2 are essential for the creation of the eye field and cell migration. WNT11 enhances the unity of eye field cells; however, excessive expression of WNT11 can hinder the movement and arrangement of cells (Cavodeassi et al., 2005). On the other hand, reduced levels of CDH1 and CDH2 impair the necessary movement of cells for the creation of the eye field (Niessen et al., 2011; László and Lele, 2022).

BMP4 and BMP7 coordinate the process of optic vesicle development and lens induction, while FGF8 plays a crucial role in the initiation and differentiation of the neuronal retina and lens (Furuta and Hogan, 1998; Vogel-Höpker et al., 2000; Cagen and Reh, 2010). The synchronized expression and interactions of these molecules guarantee the prosperous development of the eye in chick embryos (Figure 1.6).

The existence of transcription factors such as OTX2 and PAX6 in the outer layer of cells in the head before the lens is formed, together with the activation of SOX2 in the area where the lens will develop by BMP4 released from the optic vesicle, indicates the beginning of lens specialization (Reza et al., 2007). Vimentin is essential for maintaining cellular integrity and stabilizing cytoskeleton connections, which are crucial for cellular attachment, migration and signaling (Arrindell and Desnues, 2023). The many chemical pathways work together to contribute to the precisely regulated process of eye development in chick embryos.

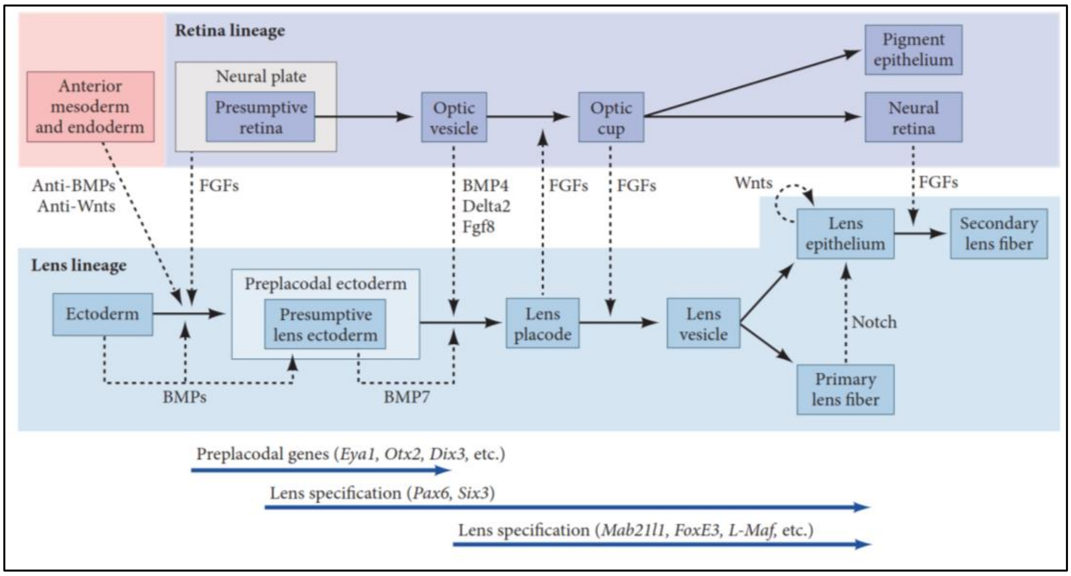


Figure 1.6: Oculogenesis- lens development (Gilber 11th Edition)

Abnormalities in the activity of these transcription factors during the initial stages of eye development can cause birth defects, as demonstrated in experiments with mice. Changes in the signaling of SHH and PAX6 led to serious conditions such as small eyes (microphthalmia) and the absence of eyes (anophthalmia) (Graw, 1996). The occurrence of congenital eye abnormalities highlights the significance of comprehending the fundamental causal causes (Guarnera et al., 2024).

Liver in xenobiotic biotransformation

The body's immune system responds to pollutants or xenobiotics by metabolizing and detoxifying these compounds, primarily through the liver in vertebrates. The liver plays a crucial role in converting lipid-soluble compounds into forms that are more soluble in water, which helps in their removal from the body (Grant, 1991) (Figure 1.7). The development of the liver in chick embryos is similar to that of mammals. The liver lobes begin to form between days 5 and 7 and become functional by day 14 (Wong & Cavey, 1992; Suksaweang et al., 2004). During embryonic development, the fetal liver plays a significant role as a hematopoietic organ. It contributes to the production of red blood cells and other blood cells, as well as the growth of hematopoietic stem cells (Nakamura-Ishizu et al., 2014).

When harmful compounds are encountered, the liver's protective mechanism initiates the activation of phase I and II enzymes, such as the cytochrome P450 system, to metabolize and eliminate these substances from the body (Zhao et al., 2021). Nevertheless, this procedure produces free radicals and reactive oxygen species (ROS), resulting in oxidative stress. Superoxide dismutases (SODs), catalases and glutathione reductases are antioxidant mechanisms that aid in the prevention of oxidative damage (Apel & Hirt, 2004; Schieber & Chandel, 2014; Ighodaro & Akinloye, 2018). Although organisms have developed defense systems, prolonged exposure to chemicals such as insecticides can still result in hepatic injury (Abdollahi et al., 2004; Cataudella et al., 2012).

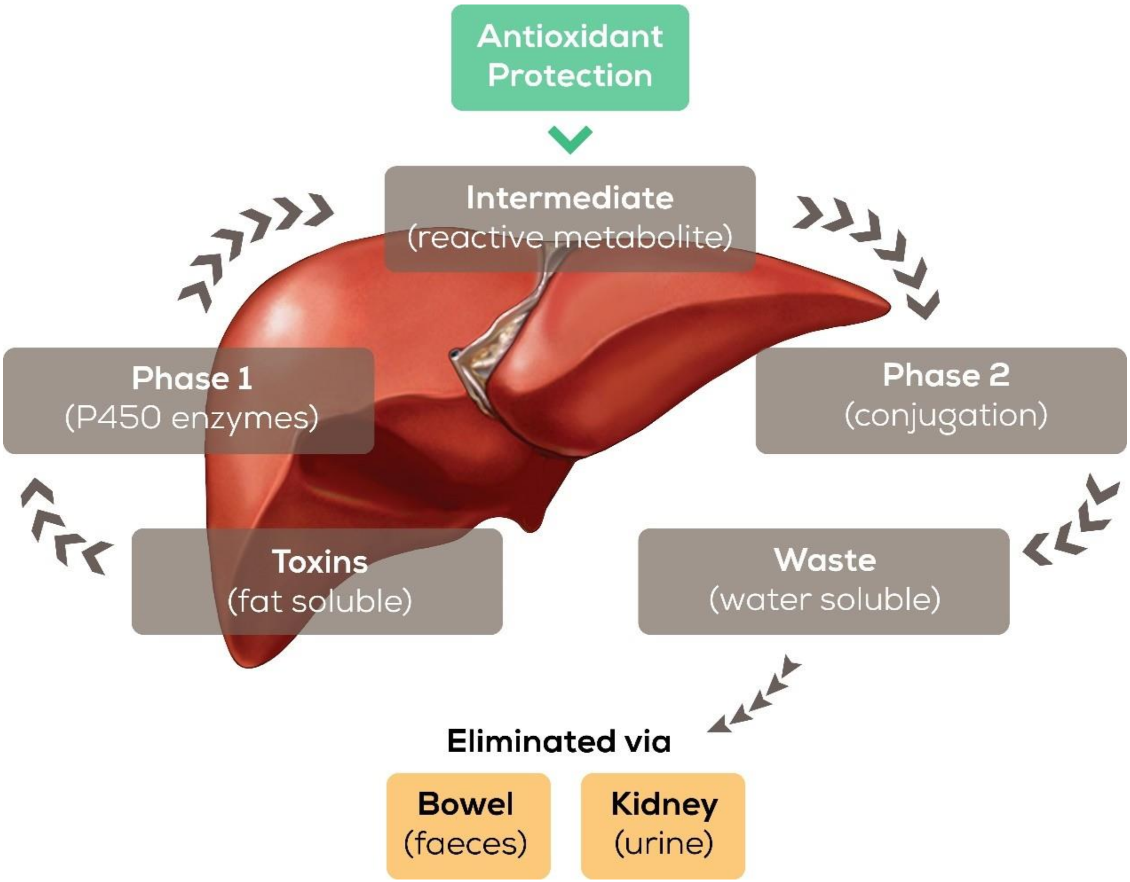


Figure 1.7: Mechanism of detoxification of drugs entering the system
(https://mommypotamus.com/liver-detox/)

Measuring specific enzymes like alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in blood serum is one method to assess liver damage caused by toxic substances (Gotaro et al., 1980; Kew, 2000). Elevated levels of these enzymes indicate severe liver injury, often associated with the release of reactive oxygen species during pesticide metabolism (Manna et al., 2004).

Furthermore, fetal liver dysfunction can affect the production of blood cells, particularly those in the erythro-myeloid and lymphoid lineages, since the liver is crucial for blood cell formation during development (Nakamura-Ishizu et al., 2014; Linklater & Higgs, 2016). Lymphocytes, which reflect the functional state of lymphoid organs, play vital roles in immune responses (Palis & Segel, 2010).

The liver synthesizes serum proteins like albumins and globulins, which regulate blood volume and osmotic pressure. Reduced levels of albumin and globulin, known as hypoproteinemia, can occur due to fetal liver dysfunction (de Brito Galvao et al., 2011), while progressive cirrhosis can lead to hypoalbuminemia (Bernardi et al., 2012).

Understanding the effects of pesticides on liver function and structure during embryonic development is crucial for elucidating toxicity mechanisms and identifying indicators of exposure or harmful outcomes, given the liver's essential role in detoxification.

In summary, this investigation aims to elucidate the underlying mechanisms behind the structural and functional abnormalities triggered by flubendiamide during the developmental stages of chick embryos. Through four parallel studies, we will examine the effects of flubendiamide on morphological, hematological, CAM angiogenesis, eye development and liver function. Studying hematology and morphology in pesticide-induced embryonic models provides insights into the developmental toxicity mechanisms of pesticides and helps evaluate their potential impacts on organism health and development. Angiogenesis is critical for providing necessary nutrients and oxygen to the developing embryo and disruptions in this process can have profound implications for embryonic viability. Eye formation, a highly orchestrated process regulated by various signaling molecules and transcription factors, can be significantly affected by alterations in these pathways, potentially leading to congenital defects. The liver, essential for xenobiotic metabolism and detoxification, plays a pivotal role in protecting the organism from harmful substances; its development and functionality during embryogenesis are crucial for overall health. Understanding how flubendiamide influences these processes will provide insights into its teratogenic potential and broader implications for developmental biology and toxicology. This research not only contributes to the fundamental knowledge of embryonic development but also has significant implications for environmental health and regulatory policies regarding pesticide use.