Utility of cockroach as a model organism in the assessment of toxicological impacts of environmental pollutants

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Abstract

Environmental pollution is a global concern because of its associated risks to human health and ecosystem. The bio-monitoring of environmental health has attracted much attention in recent years and efforts to minimize environmental contamination as well as to delineate toxicological mechanisms related to toxic exposure are essential to improve the health conditions of both humans and animals. This review aims to substantiate the need and advantages in utilizing cockroaches as a complementary, non-mammalian model to further understand the noxious impact of environmental contaminants on humans and animals. We discuss recent advances in neurotoxicology, immunotoxicology, reproductive and developmental toxicology, environmental forensic entomotoxicology, and environmental toxicology that corroborate the utility of the cockroach (\textit{Periplaneta americana}, \textit{Blaptica dubia}, \textit{Blattella germanica} and \textit{Nauphoeta cinerea}) in addressing toxicological mechanisms as well as a sensor of environmental pollution. Indeed, recent improvements in behavioural assessment and the detection of potential biomarkers allow for the recognition of phenotypic alterations in cockroaches following exposure to toxic chemicals namely saxitoxin, methylmercury, polychlorinated biphenyls, electromagnetic fields, pharmaceuticals, polycyclic aromatic hydrocarbon, chemical warfare agents and nanoparticles.

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Conflict of Interest

The authors declare that there is no conflict of interest.

Credit author statement

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The review provides a state-of-the-art update on the current utility of cockroach models in various aspects of toxicology as well as discusses the potential limitations and future perspectives.

Keywords
Cockroach; Model organism; Toxicology; Environment health; Bio-monitoring

1. Introduction

The widespread incidence of environmental pollutants due to human activities related to improper discharge of industrial wastes, agrochemicals, wastewater treatment plants, sewage sludge and untreated landfills pose serious risks to the ecosystem and the human health globally (Khan et al., 2021, Sharma et al., 2021). Particularly concerning are the health risks associated with elevated levels of heavy metals, fertilizer-based chemicals, pesticides, pharmaceuticals, nanoparticles and microplastics which have been classified to be neurotoxic, hepatotoxic, teratogenic, endocrine disruptors, and immune modulators based on their mechanisms of toxicity (Luo et al., 2020). Exposure of organisms to several types of xenobiotics can occur at any stage of lifecycle (gestation, infancy, toddlerhood, childhood, adolescence, adulthood, middle age, and the aged), with some stages being more sensitive than others. Moreover, the degree of toxicity also varies with the organism’s position within its food web because bioaccumulation due to organism’s storage of toxicants in fatty tissues may ultimately create a trophic cascade and the biomagnification of the toxicants (Adeel et al., 2021). Therefore, with the growing rate of environmental problems, there is an urgent need for detection of environmental contamination levels and the delineation of toxicological mechanisms associated with their exposure in order to provide immediate health solutions for both humans and animals.

The significant contributions of different animal models to toxicology research have been demonstrated in unravelling the toxicological mechanisms of drugs, biological therapeutics, chemicals and environmental pollutants in humans and animals. The usefulness of such animal models has been related to their availability, convenience and ability to parallel human disorders. The use of vertebrate models in toxicology research was confined to rodents, cats, dogs, rabbits and pigs for years (Prior et al., 2021, Khabib et al., 2022). Notwithstanding the effectiveness of these mammalian models, their usage is associated with some drawbacks namely ethical problems, larger size, expensiveness, and longer reproduction cycles (Prior et al., 2021, Khabib et al., 22). However, the use of non-mammalian species as substitutes in toxicological testing in recent decades have proven the zebrafish (Danio rerio), fruit flies (Drosophila melanogaster), and nematode (Caenorhabditis elegans) to be excellent alternative models in toxicology studies. Despite the great advances in toxicology, comprehensive understanding of the impact of contaminants on the cockroach has yet to be appreciated.

The cockroach is a valuable insect model in biomedical research and environmental entomotoxicology- which deals with the use of insects as biosensors of environmental pollution (Hodecek, 2020). However, to the best of our understanding, there is no systematic
review reporting the use of cockroach as an invaluable model organism in toxicology, as well as the potential advantages and limitations of this species over other experimental model systems. Cockroaches are social insects belonging to the Blattodea order. They are cosmopolitan insects due to their resilience to tolerate and survive in different weather conditions, including the arctic cold and tropical heat climates. Approximately 4,500 cockroach species exist in the globe with only 30 species living around human territories in building crevices, bark of trees and under decomposing leaves, garbage, and floating materials at river banks (Gondhalekar et al., 2021). Cockroaches consume various substances such as human foods, organic wastes and sewage. Due to their soiled feeding and breeding habits, cockroaches are established agents that transfer pathogenic microorganisms through their cuticles, mouthparts, regurgitation and fecal deposition (Turner et al., 2021). Cockroaches pose a serious risk of mechanical transmission of foodborne poisoning, nosocomial infections and diseases (Nasirian, 2019) as well as cause allergic immune reactions in humans (Patel and Meher, 2016). Consequently, cockroaches are often considered as pest and a major threat to human health (Wang et al., 2019).

However, the significance of cockroach as an insect model in biology and toxicology has long been documented (Fisk and Mengle, 1956; Llewellyn et al., 1976; Shambaugh, 1969). The four major cockroach species widely used as model organisms in toxicological studies are described herein. *Periplaneta americana* (Linnaeus, 1758) is the predominant cockroach species often regarded as a pest. It originated from Africa and the Middle East. The adult cockroach can grow up to 4 cm long, and its wings enable it to fly (Bell and Adiyodi, 1981). *Blattella germanica* (Linnaeus, 1767), also known as German cockroach, is native to Southeast Asia. It is a small-sized species of about 1.1 to 1.6 cm in length. The colour varies from tan to nearly black with two, roughly parallel, lines which run from the head to the end of the wings. It has wings, but is unable to fly (Eaton et al., 2007).

*Nauphoeta cinerea* (Olivier, 1789), also known as speckled or lobster cockroach, originated from north-eastern Africa countries namely Egypt, Eritrea, Libya, and Sudan. It breeds easily in captivity and the adult cockroach can grow up to 3.0 cm in length with wings, but is unable to fly. *Blaptica dubia* (Serville, 1838), also known as Argentinian wood cockroach, is a medium-sized species of about 4-4.5 cm long. It is native to Central and South America. The adult insect is dark brown or black with slightly lighter orange streak. The adult male has completely developed wings but rarely flies (Wu et al., 2013). Furthermore, resembling humans, the cockroach live in environments with numerous harmful substances namely microbial toxins, pesticides, pharmaceuticals and other xenobiotics which consequently, predispose it as a non-target insect to their adverse effects via ingestion of contaminated food particles (Adedara et al., 2020).

The objective of this review article is to discuss recent advances in the use of cockroaches as a suitable model organism in toxicology investigations. We discuss the basic biology, ecological significance, and the utility of *Periplaneta americana, Blaptica dubia, Blattella germanica* and *Nauphoeta cinerea* as an insect model in toxicology research. Specifically, the laboratory rearing and maintenance of cockroaches, routes of chemical exposure to experimental cockroaches and its application in the assessment of toxicological impacts of environmental pollutants as well as the limitations of cockroach model in toxicology and future perspectives are discussed. Cockroaches may be valuable systems to clarify
how different contaminants, which affect human health, modulate the physiology of living systems in an easy and high-throughput manner. Industrial products, such as potential insecticides could benefit from cockroach studies to improve their products as well.

2. Life cycle of cockroach

The cockroach undergoes gradual developmental process comprising of the egg, nymph, and adult stages (Fig. 1). It is commonly referred to as paurometabolous, hemimetabolous or incomplete metamorphosis due to absence of the pupa stage McGavin (2001). Cockroach species are generally grouped according to the three reproductive strategies, namely oviparous, ovoviviparous and viviparous. The great majority of cockroaches are oviparous because the young cockroaches grow in oothecae outside the mother’s body e.g Periplaneta americana and Blattella germanica (Ding et al., 1995, Fan et al., 2008) unlike ovoviviparous cockroaches where the oothecae grow inside the mother’s body, e.g. Nauphoeta cinerea and Blaptica dubia (Lanzrein et al., 1985, Brüning et al., 1985, Pick et al., 2010). Viviparous cockroaches grow their young ones in fluid within the mother’s uterus similar to mammals. The only species in this category is Diploptera punctata (Bell et al 2007, Mullins 2015). Both ovoviviparous and viviparous species give birth to live nymphs.

Usually, adult female cockroaches secrete sex pheromones (e.g., phenol, hexanoic acid and periplanone A and B), which appeal and draw the male cockroaches for mating during which spermatophores or sperm ampulla are released into the spermatheca of female cockroach (Nishino et al., 2011, Kou and Hsu 2013). Once fertilization is accomplished, the oviparous female cockroach deposits oothecae which contain several eggs tightly enclosed by strong protective protein in a safe place hidden from predators. The eggs attain full maturity and hatch to produce the nymphs after 28 to 60 days, depending on the species, in warm environments (Koehler et al., 1990, Mallery 2017). The nymphal stage involves about 7 to 8 moltings during which they shed their exoskeleton to grow bigger (Eggleston and Arruda 2001). The nymphs are characterized by lack of wings and fast movement. The nymphs attain full adult stage and coloration after the last molt, which takes 103 to 360 days, depending on the species and environmental conditions including temperature (Koehler et al., 1990, Robinson 2005). The lifespan of cockroaches varies from species to species, and it depends on moisture and temperature during each of the three stages. However, cockroaches generally live 6 to 18 months (Eggleston and Arruda 2001, Robinson 2005, Adedara et al., 2021b). The duration of stages and life spans of different cockroach species are illustrated in Table 1. Based on the cockroach life cycle, the insect represents a good multiple model organism because the eggs, nymphs and adults are valuable models in toxicology research. The eggs and the nymphs are models frequently used in developmental toxicity studies, whereas the nymph and adult cockroaches are useful for neurobehavioural experiments. For instance, topical application of 10 μg/μL of fenoxycarb to the ventral abdomen of newly molted first-, second-, third-, fourth-, and fifth- (last) stage nymphs of Blattella germanica caused mortality of the nymphs except the last instars (King and Bennett, 1988). However, the same concentration significantly delayed the final molting in Blattella germanica. These investigators concluded that the vulnerability of the last-instar cockroaches to the molt-inhibitory effects of fenoxycarb is associated with its interference with the period of ecdysone production by the prothoracic gland (King and Bennett, 1991).
Exposure of adults, developing nymphs and eggs of *Blattella germanica* and *Periplaneta americana* to 10-70 mg/L of ethyl formate in a 12 L desiccator and 0.65 m³ exposure chamber significantly inhibited the hatching rate in a dose-dependent manner and adversely affected all developmental stages and the adult insects (Kim et al., 2021). These observations suggest the versatility in the utility of cockroaches in toxicology. However, no study has demonstrated the possibility of using adult cockroaches to model degenerative diseases.

2.1. Ecological relevance of cockroaches

The interrelationships between organisms and their environment afford several benefits which are essential for the survival and well-being of both animals and humans. Although cockroaches may constitute nuisances in some circumstances, they are global lever pullers because their contribution to the ecosystem is indispensable (Bell et al., 2007; Adedara et al., 2020). Cockroaches are detritivores and proficient recyclers of nutrient in the ecosystem. They efficiently digest lignocellulose in plant materials by the cellulases in their gut. Cellulase activities in cockroaches enable them to have access to the cytoplasm as well as convert the cell wall into digestible, nutritive oligomers and monomers (Tamaki et al., 2014, Shelomi et al., 2020). The hindgut of the wood-feeding cockroaches houses bacteria that produce acetate and butyrate from ingested cellulose (Ottesen and Leadbetter, 2010).

Although symbiotic microbes are known to play a pivotal role in cell wall digestion (Gijzen et al., 1994), their communal activities with the insect digestive enzymes to completely digest cellulose into simple sugars have been established (Genta et al., 2003). Thus, the earlier dogma that symbionts are largely central in the fibrolytic digestive system of insects like cockroaches has been modified (Vera-Ponce de León et al., 2020). Previous studies demonstrated microbe-independent cellulolytic digestive activity in wood-eating insects including cockroaches (Genta et al., 2003, Tamaki et al., 2014, Arakawa et al., 2016), thus corroborating that cellulolytic enzymes originate from both the cockroach and the gut microbiota. Moreover, cockroaches feed on decomposing organic matter and release abundant nitrogen stuck in them into the soil through their faeces. The discharged nutrients as a result of cockroach activity are subsequently utilized by growing plants (Adedara et al., 2020). Further, cockroaches are good source of nutrition for several insectivores specifically birds, arthropods, reptiles and mammals therefore, constituting an essential component of the food chain in ecosystem and consequently, preserve the biosphere in a self-sustaining cycle (Adedara et al., 2021a). Some species like Australian burrowing cockroaches are ground-dwelling critters help to enhance soil quality and aeration through deep burrowing.

Cockroaches, like other organisms, live in environments that are contaminated with numerous noxious substances, namely microbial toxins, pesticides, pharmaceuticals, heavy metals and other xenobiotics, which bio-accumulates in their tissues (Habes et al., 2001, Zhang et al., 2007, Small et al., 2016, Adedara et al., 2016). Therefore, the aforementioned attributes make cockroach an ecologically relevant animal model to investigate the impact of these contaminants on both target and non-target organisms (Fig. 2).

2.2. Cockroach as an alternative model organism in toxicology research

National and international organizations encourage scientifically-valid research to reduce, refine, or replace mammalian models in toxicological analysis and risk assessment with complementary and alternative methodologies and non-mammalian models (Peterson et al.,
Indeed, some cockroach species have increasingly gained the attention of scientists as excellent non-mammalian alternative and complementary models to assess chemical toxicity and the biological activities of pharmacological substances. Moreover, cockroaches possess hearts, brains, muscles, digestive tracts and reproductive organs which perform more or less equivalent functions to mammals (Harrison and Duell 2016). Cockroaches and mammals exhibit similarity in the biophysical principles of nervous system function, because they both possess analogous neurotransmitters, though their distribution differs (Huber et al., 1990; Stankiewicz et al., 2012). The head of a cockroach contains a tiny brain which consists of three pairs of lobes, namely the protocerebrum, the deutocerebrum, and the tritocerebrum (Granholm et al., 1995, Maestro et al., 1998). The protocerebrum consists of mushroom bodies which contain densely packed neurons (Kenyon cells) and constitutes a major portion of the brain. The deutocerebrum innervates the antennae whereas the tritocerebrum is associated with the labrum and integrates sensory information from the protocerebrum and deutocerebrum. The mushroom body is similar to the mammalian hippocampus, which is responsible for learning and memory formation.

The brain of cockroaches consists of major neurotransmitters found in humans, such as glutamate and gamma-aminobutyric acid (GABA) and neuromodulators namely acetylcholine, serotonin, dopamine, octopamine and norepinephrine (Troppmann et al., 2014, Hamanaka et al., 2016, Giese et al., 2018). Indeed, the cockroach has been shown to be a good model organism for neurobiological and physiological investigations (Walz et al., 2006; Blankenburg et al., 2015). Cockroach high sensitivity to noxious substances and its utility in toxicology research has delineated the behavioural responses and mechanisms of toxicity in treasured insects namely honey bees and other pollinators whose populations have reportedly diminished over the last several decade (Adedara et al., 2016a, Brettell et al., 2017, Cameron and Sadd 2020). In addition, cockroaches have numerous advantages over mammalian models in several bioassays as they are cheaper to maintain, smaller to handle and easier to breed in larger numbers due to their prolific reproductive capability (Harris and Moore, 2005).

The typical physiological and internal structures in the cockroach model are analogous to the mammalian counterpart, although with some specificities. Nutrient acquisition occurs via food processing with mouth parts and proventriculus. Analogous to the mammalian intestine, the cockroach midgut is a functional structure for food digestion, absorption and biochemical processing (Drake et al., 2010, Spence et al., 2011, Malone and Shah, 2021). The Malpighian tubules in cockroach are considered analogous to the mammalian kidney because they both perform equivalent function of excretion and osmoregulation. The cockroach Malpighian tubules are responsible for the filtration of water, solutes and metabolic wastes from the hemolymph (Nocelli et al., 2016). Energy storage in form of glycogen and triglycerides in cockroaches is similar to the mammalian counterpart. In contrast to the solid structure of the mammalian liver where glycogen is made and stored, the cockroach fat body is a loose tissue containing the adipocytes for energy storage. Moreover, the fat body is analogous to the mammalian liver owing to its multifunctional roles as an endocrine, lipid storage and detoxification organ (Kerkut and Gilbert, 1985). The cockroach hemocytes, which are immune effector cells involve in cellular response
to microbial infections, are analogous to the phagocytes in the mammalian innate immune system (Browne et al., 2013).

The reproductive systems of insects and mammals are similar in structure and function. The male reproductive system of cockroach comprises of a pair of testes located laterally in the 4th and 6th abdominal segments, a pair of vas deferens, ejaculatory duct and a pair of seminal vesicles (Simmons 2001, Resh and Carde, 2009). However, unlike in mammals where sperm produced by the testes is stored in the epididymis, the sperm produced by cockroach testes is stored in the seminal vesicles (Bunning et al., 2015). The female reproductive system of cockroach consists of two ovaries located in the 2nd and 6th segment of the abdomen (Resh and Carde, 2009). The cockroach ovaries are analogous to the mammalian ovaries in the production of eggs (Gullan and Cranston, 2005). The hemolymph, a fluid analogous to the mammalian blood, runs in the circulatory system of cockroaches and is responsible for the movement of solute molecules. However, in contrast to the blood, the hemolymph does not contain red blood cells (Kanost, 2009). Also, the mammalian respiratory system involves the lungs in which the airflow is bidirectional (in and out) whereas the cockroach respiratory system consists of a unidirectional airflow tracheal system for respiration (Szumlás 2002, Webster et al., 2015). Thus, based on the similarities in the physiological and internal structures, the feasibility of using the cockroach as an alternative model organism in toxicological studies, prior to the conventional vertebrate testing, is possible (Adedara et al., 2015).

2.3. Laboratory rearing and maintenance of cockroaches

Laboratory cockroaches are generally housed in mass colonies in plastic boxes or glass boxes. Usually, the insects are reared in mixed-age and mixed-sex colonies under standard conditions of a regulated temperature of 25 ± 2 °C and 70% relative humidity with a 12 h: 12 h light-to-dark photoperiod cycles. Each housing box is provided with materials like egg crates and corn cob bedding for nesting and reproduction, and a smaller container with cellulose sponge or cotton soaked with water. Cockroaches are commonly allowed to freely access water and food. The common feed for laboratory cockroaches are dog pellets, rodent feed, ground biscuits and fresh fruits which are all commercially available (Rodrigues et al., 2013, Zhou et al., 2014, Small et al., 2016, Afolabia et al., 2018, Mrdaković et al., 2019). However, specific cockroach feed formulated in our laboratory is not only very nutritious but very suitable for dietary exposure of chemicals to cockroaches (Adedara et al., 2015). A kilogram of this formulated diet contains 500 g of corn meal, 350 g of wheat flour, 100 g of sugar, 25 g of casein, 20 g of powdered cow milk and 5 g of commercial salt (NaCl supplemented with iodine, 20 μg of iodine per gram). A thin layer of grease is commonly applied to the upper part close to the lid of the housing box to prevent cockroaches from escaping. Alternatively, it may be painted with Fluon AD-1 to make the surface slippery for the insects (Ruebhart et al., 2011). Cleaning of cages is carried out periodically with care to avoid accumulation of dirt and prevent infections.

2.4. Routes of chemical exposure to experimental cockroaches

Experimental cockroaches are commonly exposed to test compounds via different routes, depending on the study type, the nature of the test compound, and developmental stage.
of the cockroach. The three major routes of exposure of test compounds are via oral, injection and inhalation, similar to modes of treatment in mammalian models (Fig. 3). In oral exposure, the non-volatile test compounds are added to the diet or drinking water for both acute and chronic studies (Rodrigues et al., 2013, Adedara et al., 2015). In situations where cockroaches avoid food-contaminated diets due to taste, the best practice is starvation before exposure. If the test compound is volatile, the most suitable route of exposure is via vapour inhalation (Worek et al., 2016). Basically, the test compound is placed inside small glass beaker or flask without cover for spontaneous volatilization within the treatment boxes carefully positioned in a fume cupboard (Worek et al., 2016, Waczuk et al., 2019). Another important route of administration of test compounds is via microinjection. This technique is most suitable for acute study especially of substances such as venoms and pathogens. Generally, an anesthetized cockroach is positioned between the thumb and fingertips of one hand, ventral side up while the posterior side faces the analyst. The second hand is then used to freely operate the syringe (Ruebhart et al., 2011, Rossato et al., 2019). Cockroaches can be injected via an inter-segmental membrane between the third and the fourth abdominal segments or below the leg in the second thoracic segment near the ventral and dorsal cuticles junction (Abdel-Rahman et al., 2010, Ruebhart et al., 2011, Rossato et al., 2019). Direct injection into the hemocoel facilitates rapid diffusion into the tissues of the insects. It is advisable that pilot studies using varying volumes are tested to verify the final injection volume is well tolerated by the insects. It is important to mention that while injection route does not necessarily mimic human exposures, it allows for shortened experimentation, better control of the absorbed dose and understanding of mechanisms that are associated with toxic outcomes.

3. Cockroach as an insect model in the assessment of toxicological impacts of environmental pollutants

Recent studies focusing on the utility of different cockroach species in toxicology research specifically in neurotoxicology, immunotoxicology, reproductive and developmental toxicology, environmental forensic entomotoxicology and environmental toxicology are reviewed herein (Fig. 4).

3.1. Neurotoxicology

The cockroach is a valuable model in neurotoxicology. Both nymphs and adult cockroaches have been used in either acute or chronic studies to elucidate the neurotoxic effects of several substances including venoms, inorganic mercury, methylmercury and saxitoxin (Abdel-Rahman et al., 2010, Adedara et al., 2015). Laboratory reared adult female cockroach Periplaneta americana injected with scorpion (Scorpio maurus palmatus) neurotoxins exhibits permanent disability within 180 minutes post venom administration. The neurotoxic (paralytic) effect of this toxin is associated with inhibition of acetylcholinesterase (AChE), superoxide dismutase (SOD) and catalase (CAT) activities, and induction of oxidative damage evidenced by elevated protein carbonyl content levels in the treated insects (Abdel-Rahman et al., 2010). This evidence supports the notion that altered redox status and negative modulation of the cholinergic signaling play a role in the deleterious effects of this toxin. Saxitoxin, an alkaloid toxin produced by cyanobacteria,
contaminates water sources and bioaccumulates in the marine food chain. Human exposure to saxitoxin induces paralytic shellfish poisoning via inhibition of the voltage-gated sodium channels (Deeds et al. 2008; Durán-Riveroll and Cembella 2017). *Nauphoeta cinerea* injected with 30 μL of saxitoxin exhibits paralysis of all the limbs with an extension rather than a contraction with peak sensitivity of 60 min post-injection. The “knock down” (KD50) was 31.2 ng/g body weight at which dose the cockroaches were unable to right themselves from an inverted position. These data establish that this cockroach bioassay affords a quick, efficient, economical and simple to perform test for paralytic shellfish poisoning toxicity (Ruebhart et al., 2011). *Nauphoeta cinerea* has been demonstrated to be a good model to study the toxicity of organophosphate, namely, chlorpyrifos. Acute injection of chlorpyrifos caused behavioural (tremors, seizures, etc) and biochemical (AChE inhibition) changes similar to those observed in vertebrates. In addition, pralidoxime injection 10 minutes after chlorpyrifos reversed the biochemical and behavioural toxicity of chlorpyrifos (da Silva et al. 2018). The ability of the cockroach to exhibit similar therapeutic response to pralidoxime, a clinical drug well reported to be efficacious against organophosphorus poisoning in humans, evidently demonstrated that the cockroach can be used as an initial and valuable insect model to investigate the potential therapeutic effects of new agents against organophosphorus poisoning in vivo.

Organic and inorganic forms of mercury are widely acknowledged neurotoxic environmental contaminants. Exposure of lobster cockroach *Nauphoeta cinerea* to inorganic mercury chloride (HgCl₂) at 10, 20 and 40 mg/L in drinking water for 7 days fails to alter AChE activity, but causes a dose-dependent reduction in survival rate, peroxidase and thioredoxin reductase activities with concomitant increase in hydroperoxide and thiobarbituric acid reactive substances (TBARS) levels (Rodrigues et al., 2013). *Nauphoeta cinerea* nymphs exposed to methylmercury at 0, 0.03125, 0.0625, 0.125, 0.25 and 0.5 mg/g feed for 35 consecutive days show behavioural impairment, characterized by decreased locomotion and impaired motor patterns, leading to a marked decrease in the exploratory profile and homebase formation (Adedara et al., 2015). These neurobehavioral deficits were associated with reduced AChE and glutathione S-transferase (GST) activities, total thiol level, and elevated reactive oxygen and nitrogen species (RONs) and TBARS production in the exposed cockroaches, thus highlighting its utility as an alternative and complementary model in neurotoxicology research (Adedara et al., 2015, Adedara et al., 2016b, Afolabi et al., 2020). Neurotoxicity induced by chronic exposure to high level of methylmercury (2.5, 25, and 100 μg/g of diet) for 10 and 30 days was also observed in *Nauphoeta cinerea* after 60 days withdrawal period (Piccoli et al., 2020), supporting persistent effects even in the absence of the contaminant. These data on the sensitivity of the cockroach to neurotoxic chemicals like mercury is particularly exciting given the economic impact of exposure to these neurotoxic agents on human globally. These findings suggest that the cockroach may be a valuable complementary insect model to be developed for further research on neurodegenerative diseases.

3.2. Immunotoxicology

Environmental pollutants modify immune responses, which often elicit immunotoxicity. The development of novel approach to investigate immune system function in non-mammalian
organisms is essential for reducing vertebrates use as well as to work with simple alternative models which can be instrumental to understanding basic mechanisms associated with immunotoxicology (Rossato et al., 2019). *Nauphoeta cinerea* nymphs injected with varied concentrations ($2 \times 10^5 - 2 \times 10^{10}$ CFU) of human pathogenic bacteria *Staphylococcus aureus* show marked alterations in the cellular metabolism and immunization of nymphs, as evidenced by decreased hemolymph metabolites (glucose, amino acids, total proteins, and cholesterol), permanent morphological damage, reduced food intake, increase in isolation, and increased colony-forming unit (CFU) until death of the cockroaches (Rossato et al., 2019). Moreover, adipokinetic hormones, which play pivotal role in insect immunity, enhance entomopathogenic fungus *Isaria fumosorosea* mediated immunotoxicity in the *Periplaneta americana* (Gautam et al., 2020). Cockroaches injected with 5 μL of sample containing 500,000 fungus blastospores exhibited metabolic stimulation, diminished haemolymph nutrients (carbohydrates and lipids) and catalase activity in the cockroach’s gut, whereas co-treatment with 2 μL (40 pmol) of adipokinetic hormones restores lipid level, but intensifies the increase in carbohydrate level and catalase activity. The exacerbation in toxicological response due to the growth and development of this fungus has been related to nutritional abundance (Gautam et al., 2020). The aforementioned studies demonstrate that the cockroach can serve as a suitable and promising model to investigate mechanisms associated with host-pathogen interaction during bacterial and fungal infections possibly resulting from exposure to environmental contaminants.

### 3.3. Reproductive and developmental toxicology

Exposure to environmental pollutants is well known to be associated with reproductive health risks. The utility of cockroach as a prospective model insect for studying the reproductive and developmental toxicity has been reported elsewhere (Small et al., 2016, Lambiase et al., 2005). The effects of gold nanoparticles on the reproductive system of exposed cockroaches corroborate findings obtained from a mammalian model, showing that gold nanoparticles exposure poses a serious reproductive and developmental toxicity risk to developing organisms. Specifically, newly emerged females cockroach *Blattella germanica* orally exposed to gold nanoparticles at 87.44 μg/g diet to adulthood evidenced bioaccumulation of gold nanoparticles without affecting the time for the formation and eclosion of ootheca. However, it markedly decreased ootheca viability, the number of nymphs hatched and the postembryonic developmental parameters namely the nymphal survival and life span (Small et al., 2016), thus indicating the adverse reproductive effect of gold nanoparticles on non-target organism. Unfortunately, while these data on gold nanoparticles-mediated changes in the life-traits and population dynamics of cockroaches are interesting, the knowledge gap on main organ accumulation of gold nanoparticles and the regulatory mechanisms for reproductive toxicity in the cockroaches have yet to be investigated.

Polychlorinated biphenyls are pervasive environmental pollutants originating from widely industrial usage and inefficient disposal, thus posing a serious health risk to both vertebrates and invertebrates (Li et al., 2010, Adeogun et al., 2016). *Blattella germanica* injected with to 0.13, 0.26, 0.78, 1.3 μg of polychlorinated biphenyls exhibited a dose-dependent increase in pathological lesions in the digestive system, fat body and male gonads with
marked destruction of spermiogenesis at 15 and 20 days post-injection, thus indicating the
noxious impact of polychlorinated biphenyls exposure on tissues of non-target organism.
Indeed, the complete disappearance of spermatids and spermatocytes with the discontinuous
cellular boundaries of spermatocytes observed in cockroaches exposed to high doses of
polychlorinated biphenyls demonstrates the utility of the cockroach as an insect model
in reproductive toxicity study (Lambiase et al., 2005). However, the available studies on
reproductive toxicity are largely descriptive without detailed mechanistic approach. This
aspect of research can be improved with more data from both insect sexes and biochemical
endpoints related to reproductive function.

3.4. Environmental forensic entomotoxicology

Environmental forensic entomotoxicology is a new branch of entomotoxicology which
highlights the utility of insects as bio-detectors of environmental toxicants Hodecek (2020).
The use of toxic substances and chemical warfare agents by terrorist groups continues to
pose a threat to international communities. The effectiveness of conventional defensive
strategies against these deadly agents, which involve environmental decontamination,
physical protection and medical treatment strongly relies on early warning and detection
(Worek et al., 2016). Cockroach has been demonstrated to be an appropriate sentinel for
exposure to chemical warfare agents owing to their rapid response to a wide-ranging of
deadly agents, noticeably visible and unique reactions, ease of handling, broad availability
and low cost. Exposure of South American cockroaches Blaptica dubia to nerve agents
namely sarin, cyclosarin, tabun, soman or venomous agent X (VX) through inhalation
exhibits identical reaction sequence characterized by a smooth transition from hyperactivity
to loss of control, supine position and death (Worek et al., 2016). The signs of toxicity
induced by blister agent lewisite were largely similar to nerve agents, while sulfur mustard
exposed cockroaches exhibited rapid behaviour responses alongside fast alterations in the
neuronal electrical field potential, loss of control, reduced motility and a state of agony
related to DNA alkylation, disruption of biochemical processes and cellular dysfunctions
(Worek et al., 2016, Popp et al., 2018). The findings from these studies demonstrate the
relevance of cockroach in environmental forensic entomotoxicology to create awareness
of possible deadly agents and consequently contribute to the security of international
communities.

3.5. Environmental toxicology

The integrity of the ecosystems is increasingly threatened due to continuous discharge
of xenobiotics during anthropogenic activities. Bio-monitoring of environmental health
is of great interest globally, and has indeed attracted much attention in recent decades.
Organisms that assimilate environment pollutants and respond in an easily quantifiable
manner represent valuable biosensors to investigate the magnitude of contamination and its
consequences on the ecosystems. Earlier investigations evidently demonstrate cockroach to
be a suitable model insect for environmental risk assessment of xenobiotics in non-target
insect species (Maliszewska et al., 2018, Mrdaković et al., 2019, Adedara et al., 2020a).

Blaptica dubia nymphs exposed to fluoranthene, a widespread polycyclic aromatic
hydrocarbon, at environmentally relevant concentrations of 0.2 and 18 ng/g dry weight
of diet for 30 days show significant effect on midgut antioxidant and detoxification enzyme activities evidenced by increased SOD, CAT, and GST activities, but decreased glutathione level, thus suggesting the use of cockroach as biomarkers of polycyclic aromatic hydrocarbon pollution (Mrdaković et al., 2019). Several volatile chemicals frequently used as solvents and for industrial processes are known environmental pollutants with immunological, neurological and reproductive dysfunctions consequences in animals and humans. For example, exposure of Nauphoeta cinerea nymphs to vinylcyclohexene via inhalation at concentration range of 3.41 to 7.03 nmol/μL for 35 and 70 days does not affect water consumption but significantly decreases the survival rate and body weight gain. Vinylcyclohexene inhalation increases GST activity and reactive species in the head, fat body and reproductive organs without affecting brain AChE activity, thus suggesting the deleterious impact of vinylcyclohexene inhalation and the utility of Nauphoeta cinerea as an insect model to study environmental toxicants (Waczuk et al., 2019).

Electromagnetic field is associated with the environment because it is produced by both natural and man-made activities. There are mounting concerns about the potential health risks associated with exposure to electromagnetic fields in both humans and animals (Hardell and Sage 2008, Engels et al., 2014, Kaplan et al., 2016). Exposure to electromagnetic fields in rodent model promotes behavioral and physiological responses associated with disruption of neuronal, molecular and neurochemical systems (Akdag et al., 2010, Ciejka et al., 2011, Sekeroğlu et al., 2012). Similarly, Periplaneta americana entirely exposed to electric fields exhibits notable changes in normal locomotion, characterized by hypolocomotion and increased turning angle (Jackson et al., 2011). Indeed, the influence of electromagnetic field on the response to noxious heat was studied in cockroach. Periplaneta americana exposed to electromagnetic field (50 Hz, 7 mT) for 24 h, 72 h and 7 days exhibits significant increases in the escape latency from noxious heat. Exposure to electromagnetic field diminishes glutathione level but increases the malondialdehyde level in the whole-body homogenate after 24 h of exposure, which persists for 7 days after exposure. Thus, exposure to electromagnetic field induces oxidative stress and modifies heat perception in cockroach (Maliszewska et al., 2018), emphasizing the relevance of cockroach as a biosensor of electric fields. The movement of animals, including cockroaches, is aided by magnetoreception, a sense to perceive the earth’s magnetic field required for orientation and navigation (Vácha 2006, Vácha et al., 2009). This magnetic sensitivity in cockroaches has been associated with the rapid transduction of a chemical magnetoreceptor called flavoprotein Cryptochrome (Slaby et al., 2018). Earlier investigation demonstrated that Periplaneta americana exposed to radio frequency magnetic fields exhibited marked disruption of magnetoreception (Vácha et al., 2009). However, whether Cryptochrome was affected along with the disrupted magnetoreception in the exposed cockroaches remains unknown. This finding establishes the susceptibility of cockroaches to the potential adverse effect of radio frequency magnetic fields.

Comparative assessment of the influence of chronic exposure to a static magnetic field and an extremely low frequency magnetic field in Blaptica dubia nymphs revealed that both exposures significantly decreased the body mass and glycogen content in the fat body, but increased locomotion. Chronic static magnetic field increased total lipid content in the fat body whereas a decreased is verified in chronic extremely low frequency magnetic field.
Moreover, increased SOD and CAT activities with concomitant decreased glutathione reductase and GST activities were found in gut masses of cockroaches exposed to magnetic field, thus indicating magnetic fields as potential stressor influencing gut mass and antioxidant defense system in cockroach (Todorović et al., 2019).

Environmental contamination by human and veterinary pharmaceuticals is globally acknowledged to be a serious health risk to the ecosystems (Rasheed et al., 2020, Chaturvedi et al., 2021). *Nauphoeta cinerea* has been demonstrated to be a valuable biosensor for monitoring ecotoxicological hazard of pharmaceuticals. *Nauphoeta cinerea* nymphs exposed to diclofenac-contaminated food at 0, 0.5, 1.0 and 2.0 μg/kg feed for 42 consecutive days exhibited significant decrease in maximum speed, mobility time and episodes, distance travelled, turn angle, body rotation, and path efficiency, but increased freezing duration. Paralleling the behavioral data, diclofenac reduced antioxidant enzymes and AChE activities, but elevated the levels of nitric oxide, RONS and lipid peroxidation in the head, midgut, and hemolymph of the exposed cockroaches, thus suggesting induction of neurotoxicity and oxidative inflammatory stress in exposed insects (Adedara et al., 2020a).

Moreover, *Nauphoeta cinerea* exposed to ciprofloxacin (0.5 and 0.25 μg/g feed) alone or jointly with atrazine (1.0 and 0.5 μg/g feed) for 63 consecutive days showed that the combined exposure caused greater locomotor and exploratory deficits than individual exposures. The decrease in AChE and antioxidant enzyme activities with elevated levels of biomarkers of oxidative stress were markedly intensified in the hemolymph, midgut and head of cockroaches co-exposed to ciprofloxacin and atrazine (Adedara et al., 2021a). These data underscore the usefulness of cockroach-based models in assessing impact of co-exposure to environmental contaminants related to pharmaceuticals and herbicides.

The impact of psychoactive drugs which coexist in the environment has also been investigated in cockroaches. Exposure of *Nauphoeta cinerea* nymphs to carbamazepine (1.5 and 3.0 μg/kg diet) and diazepam (0.5 and 1.0 μg/kg diet) for 42 consecutive days showed that diazepam *per se* elicited no adverse effect on the behavioral and antioxidant responses. Carbamazepine alone and the combination of both drugs significantly diminished locomotor and exploratory activities. Activities of AChE and antioxidant enzymes decreased markedly, whereas inflammatory and oxidative stress indices increased significantly in the midgut, hemolymph, and head of cockroaches exposed to carbamazepine alone and the mixtures, thus suggesting induction of neurotoxicity associated with inflammatory and oxidative stress. Collectively, exposure of cockroaches to a wide range of contaminants at environmentally relevant concentrations significantly interfered with the neurobehavioral and cellular responses, thus reinforcing the utility of cockroach-based models to environmental toxicology (Adedara et al., 2021b). The various tissue samples and elucidated mechanisms of actions of environmental pollutants in experimental cockroach models are summarized in Table 2. Microplastics are ubiquitous group of synthetic materials widely recognized as contaminants of emerging concern which are increasingly gaining attention due to their noxious additives impact on the living organisms (Conti et al., 2021; Ya et al., 2021). Although some limitations in the detection and quantification of microplastics level in water and sewage samples were overcome using a standardized and improved analytical spectroscopy and software technology (Strungaru et al., 2019), there...
is no study reporting the impact of microplastics on the cockroach. Thus, future studies in cockroaches as a biosensor should be carried out to explore microplastics toxicity.

4. Limitations of cockroach model in toxicology

Despite the increasing availability of scientific information on the utility of cockroach as a promising model organism in toxicology, there are some limitations that are associated with this biosensor. Limited amount of genetic data is available which definitely hinders gene identification, analyses of gene expression, genetic manipulation and consequently, insight into its molecular biology function. Recent advances in transcriptome studies have indeed been encouraging in the utility of cockroach model. Transcriptome analysis of the *Periplaneta americana* midgut identified 82,905 unigenes of which 64 genes putatively participate in detoxification (37 genes), digestion (11 genes) and oxidative stress response (16 genes) all of which are associated with the adaptation mechanism of this specie (Zhang et al., 2016) whereas the foregut consists of cytochrome P450s (70 genes), glutathione S-transferases (12 genes), carboxylesterases (7 genes) and adenosine triphosphate-binding cassette (7 genes) transporters largely involved in xenobiotic detoxification (Zhang et al., 2018). Data on the maturing oothecae, nymphs, adult females and males of *Blattella germanica* identified genes putatively coding for detoxification enzyme systems (263 genes), digestion (244 genes), immunity (133 genes), insecticide targets (28 genes) and chemoreception (33 genes) pathways (Zhou et al., 2014). Transcriptome analysis of fat body and head of *Nauphoeta cinerea* identified 62,121 transcripts of which 367 genes are related to stress and detoxification include cytochrome P450s (85 genes), glycosyl transferases (37 genes), acetylcholine and carboxyl esterases (28 genes) and heat shock proteins (29 genes) (Segatto et al., 2018). The aforementioned transcriptome data, though limited, are essential to facilitate the genetic insight into the specific biological processes of the insect model in future investigations. Moreover, we recommend investigation on the potential use of strategies to perform genetic modulation in cockroach during toxicological studies. For instance, to perform functional genomics studies using RNAi mechanism, the cockroaches are treated with specific dsRNA which decreases the protein it encodes, and resulting in phenotypes that reveal the functional role of the knocked down protein (Bellés, 2010). Other limitations of this model in comparison with mammalian models mainly include its less complexity in adaptive immune system, brain and other major organs as well as the specificity of some pathogenetic factors.

5. Concluding remarks and future perspectives

There is considerable evidence implicating environmental pollutants in serious health risks in both humans and animals, thus the imperativeness for the detection of contamination levels as well as delimiting the toxicological mechanisms involve in such toxic exposure with the purpose of improving the health conditions of both humans and animals. This review article emphasizes the need to complement the mammalian models with non-mammalian species to further understand the noxious impact of contaminants on humans and animals. Specifically, the four major cockroach species namely *Periplaneta americana, Blaptica dubia, Blattella germanica* and *Nauphoeta cinerea* which have been shown to be valuable insect model in toxicology were review herein. The use of complementary
non-mammalian models is necessary because there is no animal species that fully mimics human situations. The cockroach represents a valuable model organism to investigate basic research questions related to toxicological responses because of the similarities in some important physiological and internal structures with mammals in addition to sharing the same environment and chemical exposure with human. The utility of the cockroach model in neurotoxicology, immunotoxicology, reproductive and developmental toxicology, environmental forensic entomotoxicology and environmental toxicology with cellular mechanisms similar to mammals substantiates its validity as an optimal model organisms for toxicology investigations. However, the limited genetic data which is a major drawback in the utility of this important model is highly expected because it is an emerging model organism in toxicology which requires concerted multi-disciplinary approach to fully unravel its genome which currently is unknown.

The cockroach system shows a promise to extend far into the future and positively contribute to the understandings of toxicological mechanisms associated with toxicant exposure. Overall, the utility of cockroach models as interesting strategies to study adverse effects of xenobiotics on the ecosystem has been reviewed herein. However, there are some open questions still requiring further scrutiny (Table 3) to fully validate this promising model organism as a complementary system in environmental toxicology research. We are quite aware that these highlighted questions can be expanded with specific experimental design by researchers to further substantiate the utility of cockroaches in understanding toxicological mechanisms.

Acknowledgments

I. A. Adedara is indebted to TWAS-CNPq for financial support in form of TWAS-CNPq 2013 Postdoctoral Fellowship (FR number: 3240274252). The financial support of CAPES for the award of the “Jovem Talento com Experiência no Exterior” (CAPES Print Program, File No. 88887.568833/2020-00) to I.A. Adedara is gratefully acknowledged.

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Fig. 1.
The life cycle of cockroach comprising of the developmental phases namely the egg, nymph and the adult stage.
Fig. 2.
Schematic overview of the ecological relevance of cockroach as an animal model to investigate the impact of environmental pollutants. Cockroaches as decomposers help in proficient recycling of nutrients for plants’ growth. Heavy metals, microbial toxins, pesticides and pharmaceuticals via anthropogenic activities can reach the terrestrial environments and cause harmful effects on the organisms. Cockroaches are suitable bioassays to elucidate how contaminants (separately or jointly) affect biochemical and neurobehavioral responses in both ecotoxicological and translational perspectives. Cockroaches are indispensable in the self-sustenance of the food chain where they serve as source of nutrition to several insectivores in the ecosystem.
Fig. 3.
Schematic overview of laboratory cockroach species, experimental protocols and basic anatomy to investigate toxicological responses. (A) Cockroach species commonly used as model organisms in toxicological studies. (B) The major routes of exposure of experimental cockroaches to test compounds. (C) The typical internal structures in the cockroach model.
Fig. 4.
The utility of cockroach-based models to assess toxicological impacts of a wide range of environmental pollutants.
Table 1
The duration of stages and life spans of different laboratory cockroach species

| Cockroach species       | Incubation (days) | Nymph (days) | Life span (days) | References                        |
|-------------------------|-------------------|--------------|------------------|-----------------------------------|
| *Blattella germanica*   | 28–30             | 60–100       | 120–200          | Eggleston and Arruda 2001, Robinson 2005 |
| *Blaptica dubia*        | 28–30             | 180–380      | 250–530          | Mallery 2017, Koehler et al., 1990 |
| *Nauphoeta cinerea*     | 30–36             | 65–78        | 344–365          | Adedara et al., 2021b             |
| *Periplaneta americana* | 30–60             | 150–360      | 100–700          | Robinson 2005                    |
| Experiment                                                                 | Biochemical assay                          | Specie of cockroach | Tissues                      | Main results                                                                 | References          |
|---------------------------------------------------------------------------|--------------------------------------------|---------------------|------------------------------|-------------------------------------------------------------------------------|---------------------|
| Exposure to mercury chloride (10-40 mg/L in drinking water) for 7 days    | AChE, total thiol, GST, GPx, GR, CAT, TrxR, RONS, TBARS | *Nauphoeta cinerea* | Head                         | Induction of oxidative stress without affecting AChE activity.                | Rodrigues et al., 2013 |
| Dietary exposure to MeHg (0.125-0.625 mg/kg) for 35 days                 | AChE, total thiol, GST, RONS, TBARS        | *Nauphoeta cinerea* | Head                         | Increased oxidative stress and reduced AChE activity.                          | Adedara et al., 2015 |
| Incubation with fungi (4.2 × 10^9 spores/mL) for 48 hr                   | CAT, H2O2, LDH, MDA                         | *Periplaneta americana* | Fat body, midgut, whole body, hemolymph | Induction of oxidative stress                                                 | Chaurasia et al., 2016 |
| Electromagnetic field (50 Hz, 7 mT) for 24 h, 72 h and 7 days            | MDA, GSH                                   | *Periplaneta americana* | Whole-body                    | Induction of oxidative stress                                                  | Maliszewska et al., 2018 |
| Injection of *S. aureus* (2 × 10^5 –2 × 10^10 CFU) for 30 days           | Glucose, amino acids, proteins, and cholesterol | *Nauphoeta cinerea* | Hemolymph                    | Reduced metabolites of glucose, amino acids, total proteins, and cholesterol  | Rossato et al., 2019 |
| Inhalation of vinylcyclohexene (3.41-7.03 nmol/L) for 35 and 70 days     | GST, AChE, RONS                             | *Nauphoeta cinerea* | Head, Fat body, Reproductive organ | Induction GST activity and reactive species generation.                      | Waczuk et al., 2019  |
| Dietary exposure to fluoranthene (0.2 and 18 ng/g diet) for 30 days       | GSH, GST, SOD, CAT                          | *Blaptica dubia*     | Midguts                      | Increased antioxidant enzymes and reduced GSH level                           | Mrdaković et al., 2019 |
| Dietary exposure to diclofenac (0.5-2.0 μg/kg feed) for 42 days          | AChE, NO, GSH, GST, SOD, CAT, RONS, MDA     | *Nauphoeta cinerea* | Head and fat body            | Induction of neurotoxicity, inflammatory and oxidative stress                | Adedara et al., 2020 |
| Injection of streptozotocin (74 and 740 nmol)                            | Triglyceride, glycogen, TBARS, AChE         | *Nauphoeta cinerea* | Head and fat body            | Glucose dyshomeostasis and oxidative stress                                  | Olagoke et al., 2021 |
| Dietary exposure to ciprofloxacin (0.5 and 0.25 μg/g feed) and atrazine (0.5-2.0 μg/g feed) for 63 days | AChE, NO, GSH, GST, SOD, CAT, RONS, MDA | *Nauphoeta cinerea* | Head, midgut and hemolymph | Induction of neurotoxicity, inflammatory and oxidative stress                | Adedara et al., 2021a |
| Dietary exposure to carbamazepine (1.5 and 3.0 μg/kg diet) and diazepam (0.5 and 1.0 μg/kg diet) for 42 days | AChE, NO, GSH, GST, SOD, CAT, RONS, MDA | *Nauphoeta cinerea* | Head, midgut and hemolymph | No adverse effect with diazepam alone. Exacerbation of neurotoxicity, inflammatory and oxidative stress upon co-exposure | Adedara et al., 2021b |

Acetylcholinesterase (AChE), glutathione S-transferase (GST), Glutathione peroxidase (GPx), Glutathione reductase (GR), Superoxide dismutase (SOD), Catalase (CAT), Thioredoxin reductases (TrxR), Reactive oxygen and nitrogen species (RONS), thiobarbituric acid reactive substance (TBARS), Nitric oxide (NO) and Malondialdehyde (MDA)
Table 3

Selected open questions associated with the utility of cockroach in toxicology research.

| Main aspects                          | Open questions needing further research                                                                 |
|--------------------------------------|----------------------------------------------------------------------------------------------------------|
| Sex and specie                       | Are there sex- and specie-specific differences in the toxicological responses in cockroach models? Does hormone differences between male and female affect toxicity in cockroaches? |
| Age                                  | What is the influence of age or stages of metamorphosis on the toxicological responses in cockroach model? |
| Population                           | Are there population-specific differences between cockroaches reared in the laboratory environment and those in the natural habitat? How does domestication influence toxicological response of cockroach model? |
| Transgeneration                      | Can toxicological responses produce multigenerational effect in cockroach model?                         |
| Metabolic factors and food           | Do metabolic factors and food (e.g., hungry versus fed cockroaches) influence toxicological responses in cockroach model? |
| Neurodegenerative disorders          | Can the cockroach be used to model neurodegenerative disorders following exposure to multiple environmental stressors? |
| Organ and tissue specificity         | Are there organ- and tissue-specific differences in the toxicological responses in cockroach models? Do reproductive toxicants differentially affect testes and ovaries in cockroaches? |
| Routes of exposure                   | Are there toxicological responses differences in the routes (e.g., oral, injection and inhalation) of toxicant exposure in cockroaches? |
| Testing conditions                   | Can behavioral and toxicological responses in cockroaches depend on testing conditions (e.g., individual vs. group-raised animals)? |
| Behavioral and biochemical endpoints| How do changes in biochemical endpoints correlate with specific behavioral phenotypes in cockroach-based models? |