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Parameters for Grading the Toxic Severity of Test Chemicals: A Review Article in the Advancement of Unknown Drugs into the Clinic

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ABSTRACT

Grading of toxic severity of a test chemical is a routine procedure in experimental toxicology to support regulatory policy in categorisation and harmful labeling decisions. Harmful labeling decision is usually made on the basis of lethal dose administered to study animal. However, the toxic property of a chemical compound is diverse in which the undesired biological mechanism manifested on treated study animal may not cause death within the period of the experiment. The lengths of time at which undesired biological effects manifest on study animal depend on the amount of administered dose. The adverse effect of higher dose usually manifests within a shorter period of time than the lower doses.

The scientific basis of drug action is the interaction of a drug with its receptor or biological target by which undesired biological changes manifested at the cellular, organs or organismal level depending on the amount of administered test chemical. Drug metabolism is an important biological process to be considered in the study of pharmacological activity of test chemical compounds. It is one of the primary mechanisms by which a chemical compound is inactivated or activated depending on the metabolic enzyme systems involved in biotransformation. Biotransformation of test chemical perhaps leads to the production of reactive metabolites that could be more toxic, mutagenic or carcinogenic than parent chemical compound which could elicit different biological responses such as loss of appetite and suppressed immune response among others. The metabolic and immune systems are highly interrelated in which the proper function of one is highly dependent on the other. Metabolic dysfunction for instance leads to deteriorated immune system which can be evaluated by clinical parameters such as differential blood cell counts (i.e., T-lymphocytes and B-lymphocytes) and immunoglobulin concentration in blood serum.

In conclusion, grading of toxic severity of test chemicals should take into account not only the estimate of hazards on the basis of lethal dose administered into the biological system but also the different biological responses (immunological and physiological) and the length of time at which biological responses manifested in the course of metabolism. Quantitative analysis of undesired biological responses as toxic severity and toxic reaction rate is paramount for grading the toxic severity of test chemical compounds on study subject which allow better identification of real risks to public health safety.

Keywords: Grading, Toxic severity, Test chemical, Regulatory policy

INTRODUCTION

All chemical compounds are poisons with different intensity depending on the chemical nature of its component and amount of dose administered into the natural process of an organism. As a result, grading of the toxic severity of every test chemical is a routine procedure in the development of unknown drugs into pharmaceutical products [1,2]. It is usually determined on the basis of a dose of test chemical which caused death to treated study subject regardless of the length of time at which lethal effect manifested on treated study laboratory animal [1,2]. A test chemical at 5 mg/kg body weight, for instance, may cause death to study animal at 5 h after dosing and another test chemical at the same dose may cause death to the same study animal at 24 h after dosing. Even though the two test chemicals caused death to study animal at the same amount of dose, both test chemicals could not have the same grade of toxic severity. The toxic severity of test chemical which caused death at 5 h after dosing is more than the toxic severity of test chemical which caused death at 24 h after dosing.

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This review article focuses on brief explanation of physical and biological parameters that could help in grading the toxic severity of test chemicals administered into study laboratory animal. It illustrates the link between the dose, its toxicity and the length of time at which its adverse effect manifests on treated study subject and the interrelationship between metabolic and immune systems by which quantitative, undesired biological responses as toxic severity and toxic reaction rate could be determined within the biological system.

Acute toxicological studies conducted by Belay in 2011 and 2019 [17,18] showed that the dose had never limited the toxicity but the magnitude of adverse effect and length of time at which adverse effect manifested on treated Balb c mice. Even if the adverse effect was manifested within a short period of time when large amount administered into lab Balb c mice orally, it also remained after a long period of time when small amounts administered in the same route. Before illustrating the parameters for leveling the toxic severity of test chemicals, it would be first helpful highlighting the mechanisms by which the adverse effect of test chemicals is manifested within the biological system.

Test chemicals are administered into study subject by a route which is convenient and safe as possible during the period of the study. Each of the administered test chemicals is subjected for an investigation to determine dose-biological response relationship [3]. The scientific basis of drug action is the interaction of administered drug with its cellular receptor or other biological target and the biological changes that it has elicited in cells, organs and the whole organism depending on the amount of the administered test chemical [4]. This kind of drug action (what the drug does to the body) are known as pharmacodynamics [4].

Drug metabolism is an important biological process to be considered in the study of pharmacological property of a chemical compound [5]. The pharmacological activity of a chemical compound perhaps reduced or enhanced by metabolic enzyme systems [5]. This means that between administration and excretion of a drug from the body, it undergoes biotransformation to either highly or moderately reactive intermediates which ultimately elicits multiple biological signals [6,7]. Ingestion of methanol for instance does not result in significant illness and mortality by itself but it is rapidly metabolized by alcohol dehydrogenase to formaldehyde which is subsequently converted to highly toxic formic acid by aldehyde dehydrogenase [8]. Drug metabolism is therefore one of the primary mechanisms by which a chemical compound is inactivated or activated depending on the metabolic enzyme systems involved in biotransformation [9]. The metabolic enzyme systems do not create new toxic metabolites but produced it from the chemical compound in which it has been already incorporated by means of chemical reaction. The composition of a chemical compound could be either highly toxic or moderately toxic or both chemical elements combined together by means of chemical reaction which might be reversibly produced in the metabolic system known as catabolism (phase I metabolism) [10,11] or it may be transformed into new chemical species in another metabolic system known as anabolism (phase II metabolism) [10]. During metabolic reaction, for instance, glucose is converted into carbon dioxide and water which is known as catabolism [11]. Carbon dioxide is a toxic metabolic by-product of glucose molecule which has to be excreted through the respiratory system. And again glucose and oxygen are produced when carbon dioxide and water react together within the natural process of a plant in the presence of light and chlorophyll. The glucose molecule is again transformed into starch, fats and oils for storage which is known as anabolism [11].

A chemical compound has always different chemical characteristics from the chemical elements from which it has been formed by means of chemical reaction. The extent of toxic metabolic products of a chemical compound also varies depending on the enzyme involved in the metabolic system of a cell [11]. The toxic property of a chemical substance could be manifested either at specific phase or multiple phases of metabolism depending on the nature of its chemical component which perhaps entails the manifestation of its adverse effect within the biological process of living organism at different length of time as metabolic phases are often happening sequentially [4]. There are different stages of cellular metabolism where different biotransformation processes have taken place from food to waste products [12]. These series of metabolic reactions produces not only metabolites with different biological and pharmacological properties but also ATP which is used to provide power for biosynthetic reactions and other energy requiring processes such as transport of nutrients across biological membranes within an organism [13]. The first stage of cellular metabolism usually involves degradation of large molecules which mostly occurs outside the structure of a cell although special organelles called lysosomes could also degrade them within the cell [13]. The second stage of cellular metabolism occurs mainly in the cytosol except some i.e. the final step of conversion of pyruvate to acetyl CoA that occurs in the mitochondria in which the third stage of cellular metabolism (oxidative phosphorylation) takes place **(Figure 1)** [13].

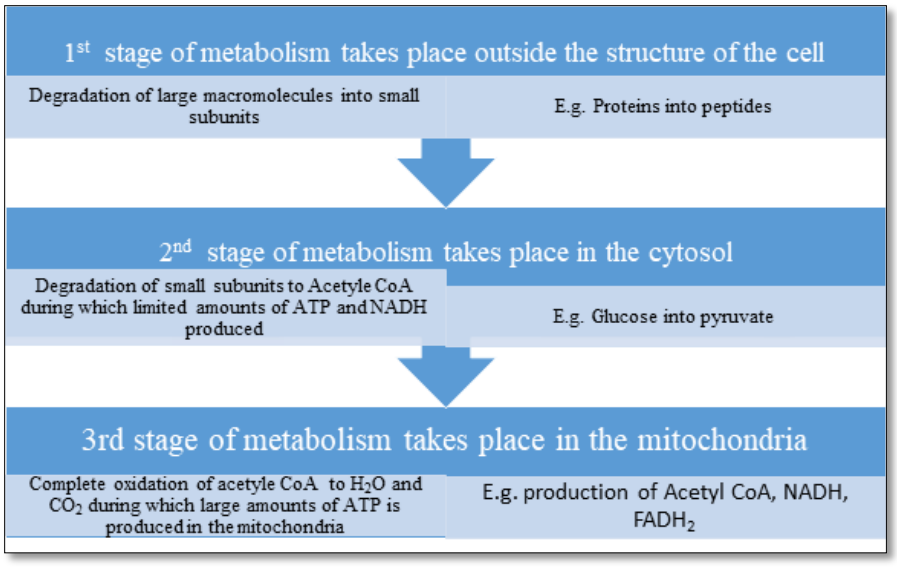

Figure 1. Stages of cellular metabolism.

The toxic property of a chemical compound could also manifest at different phases of biosynthesis reactions that could ultimately manifest on the immune system of an organism which is described as follow: Metabolic reactions are divided into three major categories all of which takes place within the cell simultaneously **(Figure 2)** [12]. The three major metabolic reactions are [12]:

1. Degradation of nutrients (fuelling reaction), i.e., Glucose → $CO_2 + H_2O$
2. Biosynthesis of small molecules (biosynthesis reaction), i.e., fatty acids and nucleotides.
3. Biosynthesis of large molecules (polymerisation reaction), i.e., Glucose → glycogen

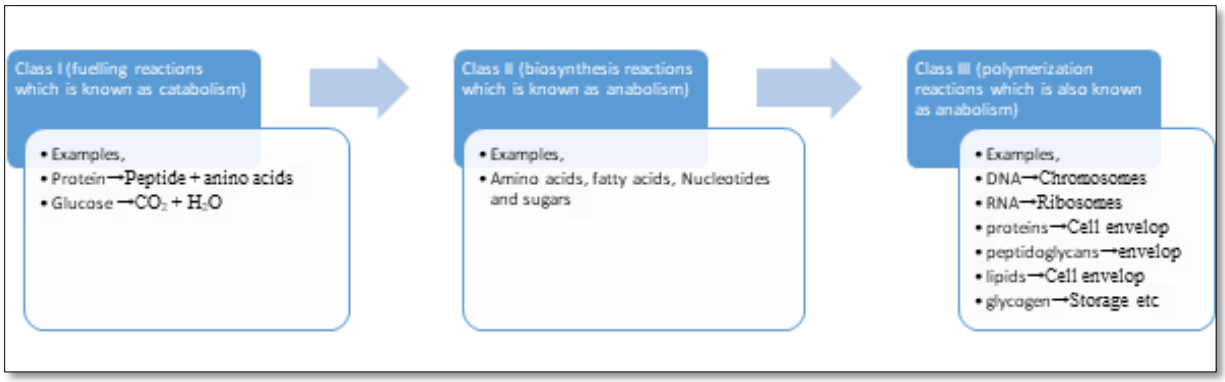

Figure 2. The three major categories of metabolic reactions within a cell.

The first metabolic reaction produces energy in the form of adenosine triphosphate (ATP) whereas the second and third metabolic reactions require energy which involves in making of chemical bonds in the molecules found in the biological system of an organism [12]. This means that the energy which was produced in the first metabolic reaction (catabolism) would be used in the second and third metabolic reactions (anabolism) which makes harmonious progression of life of a living thing. The insufficiency in the first metabolic reaction would lead to limited biosynthesis activity of the cell which ultimately causes undesired biological phenomenon at the organismal level, i.e., loss of weight (wasting syndrome), ageing, growth retardation and infertility.

Metabolism of nitrogenous compounds has an oxidation level between carbohydrates and lipids in which they could be used as nitrogen, carbon and energy sources [14]. Proteins are hydrolysed into peptides and further to amino acids by proteases [14]. Amino acids are first converted to organic acids by deamination reaction (removal of amino group) which may be oxidative or reductive depending on the metabolic enzyme system involved in the biotransformation [14]. Ammonia released from deamination is used for protein and nucleic acid synthesis as a nitrogen source and organic acids could be further oxidized

for energy production (ATP) whereas the amino groups is exchanged for the keto group of α-keto acid [14].

Metabolism of hydrocarbons on the other hand requires oxygen in which only few organisms could metabolise it [14]. The first step in metabolising hydrocarbons is oxygenation by oxygenase enzymes in which hydrocarbon molecules are oxidized into alcohol, aldehyde, organic acid and acetyl CoA metabolic products subsequently which finally enters to Krebs cycle/Citric acid cycle in the mitochondria [14].

The biotransformation mechanism of a chemical compound within the biological system could be aerobic, anaerobic or autotrophic metabolism. Anaerobic metabolism is the production of energy in the absence of oxygen which uses the same pathways as in aerobic metabolism but differ in the use of an alternative electron acceptor, e.g. Nitrate, NO^{3-} [14,15]. Many organisms grow without using electron transport chain (ETC) which generates the energy called fermentation [14,16]. Since there is no electron transport used in this metabolic process, the organic substrate must undergo a balanced series of oxidative and reductive reactions [14,16].

The toxic property of a chemical compound is diverse in which suppressed appetite is one of commonly manifested biological responses as its toxic effect starts from the cellular level where there is nutrient sensing system [4]. Grading of toxic severity should take into account not only the estimate of hazards on the basis of the lethal effect of doses administered into the biological system but also clinical signs and symptoms and the length of time at which signs and symptoms manifested in the course of metabolism. Quantitative analysis of undesired biological responses as toxic severity and toxic reaction rate need to be computed for leveling the poisoning severity of test chemical compounds on study subject which allow better identification of real risks to public health safety [17-19].

An integrated biological approach (physiological, immunological and histopathological investigations) in toxicological evaluation is an essential scientific approach due to the fact that it forms the basis for modern clinical medicine in which data for qualitative and quantitative biological responses could be collected using fewer laboratory animals than lethal end point acute toxicity evaluation. It has economic importance because it reduces time and wastage of resources by conducting comprehensive pharmacological analyses in which all stages of toxicological assessments in the advancement of unknown test chemical into clinical trial could be evaluated with a single laboratory setting. It maximises public health safety with having scientifically and adequately validated toxicological data that would be used in drug discovery and development.

The degree of toxic severity and toxic reaction rate of test chemical compounds in the biological process of a living thing is generally dependent not only on the amount of administered dose and chemical nature of its component but also on the biological property (e.g. immune strength and sensitivity of other biological systems) of an organism and metabolic enzyme systems involved within the cell [11,19]. Biotransformation of a chemical substance perhaps leads to bio-activation which involves the production of reactive metabolites that could be more toxic, mutagenic or carcinogenic than parent chemical compound which could elicit multiple biological responses within the biological system of an organism [20]. Of the biological responses, loss of appetite and suppressed immune response are among other responses that might be manifested as a result of noxious chemical compound administered into the natural process of study animal. The ability to resisting the harmful effect of test chemicals against the biology of an organism needs strong immune response during exposure which in turn needs biological organisation with viable metabolic system that is highly capable of processing and storing energy [4]. The activity of the immune system and metabolic regulation within the biological process of higher animals are highly interrelated in which the proper function of one is highly dependent on the other [4]. Metabolic dysfunction for instance leads to deteriorated immune system which could be evaluated by either differential blood cell counts, i.e., T-lymphocytes and B-lymphocytes or concentration of immunoglobulins in blood serum **(Table 1)** [21]. The mechanism of toxicity of test chemicals is divers which perhaps first affect the metabolic system of a cell by which it is bio-activated and ultimately manifested on the immune system. The immune system detects any aberrant biological phenomenon happening within the biological system of an organism that could be manifested as different clinical signs and symptoms. The adverse effect of test chemicals could be manifested on threated laboratory animal either by blocking major catabolic or anabolic or both signaling pathways both of which are different metabolic systems which often takes place sequentially [4]. The long term disturbance of metabolic system by noxious test chemical leads to abnormal immune responses. A direct relationship and coordinated regulation of metabolic and immune responses have therefore potential benefits in the determination of poisoning severity of test chemicals administered into the biological system of study animal.

Table 1. Changes in concentration of serum immunoglobulins at 4 h after treatment of Balb-c mice with different doses of test chemicals [18,19].

Test chemicals	Tested doses	Quantitative immunoassay before treatment as reference test		Quantitative immunoassay at four hour after treatment for comparison		*ΔIg* serum conc.
		IgG	IgM	IgG	IgM	*Δ Ig*
Dichlorvos	10 mg/kg	<1100 mg/L	70 mg/L	<1100 mg/L	90 mg/L	+20 mg/L
	50 mg/kg	<1100 mg/L	70 mg/L	<1100 mg/L	80 mg/L	+10 mg/L
	90 mg/kg	X	X	X	X	X
Chlorpyrifos	10 mg/kg	<1100 mg/L	90 mg/L	<1100 mg/L	120 mg/L	+30 mg/L
	50 mg/kg	<1100 mg/L	50 mg/L	<1100 mg/L	70 mg/L	+20 mg/L
	90 mg/kg	<1100 mg/L	90 mg/L	<1100 mg/L	80 mg/L	-10 mg/L
Cypermethrin	10 mg/kg	<1100 mg/L	70 mg/L	<1100 mg/L	90 mg/L	+20 mg/L
	50 mg/kg	<1100 mg/L	80 mg/L	<1100 mg/L	70 mg/L	-10 mg/L
	90 mg/kg	<1100 mg/L	80 mg/L	<1100 mg/L	50 mg/L	-30 mg/L

[X] *represents treated mouse which died much earlier than the time for blood specimen collection*

ELECTRON TRANSPORT AND OXIDATIVE PHOSPHORYLATION SYSTEM OF A CELL

The life of animals and humans is dependent on oxygen molecule (O_2) for respiration, the process by which cells derive energy in the form of Adenosine triphosphate (ATP) from the controlled reaction of hydrogen with oxygen to form water [13]. In addition, molecular oxygen is incorporated into a variety of substrates by enzymes designated as oxygenases by which many drugs, pollutants, and chemical carcinogens are metabolized by enzymes of this class, known as the cytochrome P450 system [13,22]. Oxidative phosphorylation is the process in which ATP could be formed from electron transport chain (ETC) within mitochondria [13,23]. A number of drugs and poisons such as amobarbital and cyanide, carbon monoxide respectively, inhibit oxidative phosphorylation, usually with fatal consequences [13].

ATP synthesis is a reaction in which energy is required in order for it to occur [13,23]. This energy is derived from the oxidation of nicotinamide adenine dinucleotide (NADH) and Flavin adenine dinucleotide ($FADH_2$) by the four protein complexes of the electron transport chain (ETC) which are embedded in the inner mitochondrial membrane **(Figure 3)**. Nicotinamide adenine dinucleotide exists in an oxidized and reduced form, abbreviated as NAD+ and NADH, respectively [13]. In metabolism, nicotinamide adenine dinucleotide is a similar compound with $FADH_2$, which involves more actively in redox reactions, carrying electrons from one reaction to another [13]. Flavin adenine dinucleotide ($FADH_2$) is a redox cofactor which is created during the Krebs cycle and utilized during the last part of respiration, known as the electron transport chain within the mitochondria of the cell [13].

The ten NADH that enter the electron transport in the mitochondria originate from each of the earlier processes of respiration that has been taken place both in the cytosol and mitochondria of a cell, i.e., two NADH originate from glycolysis which takes place in the cytosol, two others from the transformation of pyruvate into acetyl-CoA and six others from the citric acid cycle both of which take place within the mitochondria of the cell [18]. The two $FADH_2$, however, originate in the citric acid cycle within the mitochondria of a cell [13,24].

Damage of cellular metabolism as a result of noxious chemicals therefore leads to the following [25,26]:

1. Inhibition of electron transport chain (NAD-NADH system and blocking of cytochrome oxidase which again causes cytotoxic anoxia [25,26].
2. Uncoupling of oxidative phosphorylation which leads to uncouple ATP production which again leads to depleted free energy storage within the body [25,26].
3. Inhibition of nucleic acid synthesis which leads to alteration in transcription and replication which again leads to depleted normal structural enzymes interferes with fat mobilization which again leads a lot of fat being accumulated within the cell [25,26].

Components of the respiratory chain are contained in four protein complexes embedded in the inner mitochondrial Membrane **(Figure 3)** [13]. These are:

The NADH-Q oxidoreductase complex (I)

The succinate Q reductase complex (II)

The Q-cytochrome c oxidoreductase complex (III)

The cytochrome c oxidase complex (IV)

The events of the electron transport chain involve NADH and FADH, which act as electron transporters as they flow through the inner mitochondrial membrane [22,27]. Electrons flow through the respiratory chain from NAD+ or NADH to $O_2/2H_2O$, passing through three protein complexes: NADH-Q oxidoreductase complex (I), where electrons are transferred from NADH to coenzyme Q also called ubiquinone; Q-cytochrome c oxidoreductase complex (III), which passes the electrons on to cytochrome c; and cytochrome c oxidase complex (IV), which completes the chain, passing the electrons to O_2 and causing it to be reduced to H_2O **(Figure 3)** [13]. Some substrates with more positive redox potentials than NAD+/NADH (e.g. succinate) pass electrons to ubiquinone via a fourth complex called succinate Q reductase complex (II), rather than complex I **(Figure 3)** [13].

In complex I, electrons are passed from NADH to the electron transport chain, where they flow through the remaining complexes. NADH is oxidized into NAD+ during this initial process of the respiratory chain [27,28]. Complex II oxidizes FADH into FAD, garnering still more electrons for the respiratory chain [27,28]. At complex III, no additional electrons enter the respiratory chain, but electrons from complexes I and II flow through it [27,28]. When electrons arrive at complex IV, they are transferred to a molecule of oxygen [27]. Since the oxygen molecule gains electrons, it is reduced to water. While this oxidation and reduction reactions take place, another event occurs in the electron transport chain. The movement of electrons through complexes I-IV causes protons (hydrogen atoms) to be pumped out of the intermembrane space of mitochondria into the cell cytosol [27,28]. As a result, a net negative charge from the electrons builds up in the matrix space while a net positive charge from the proton pumping builds up in the intermembrane space. This differential electrical charge establishes an electrochemical gradient which drives ATP synthesis in oxidative phosphorylation [13].

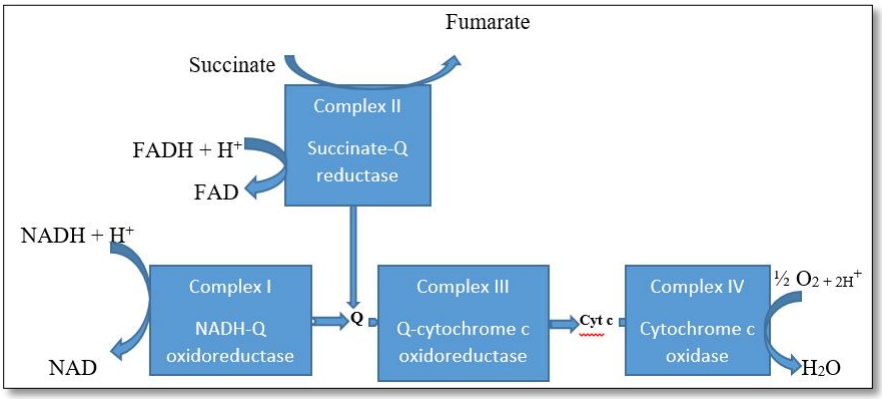

Figure 3. Diagram showing how electrons flow through the respiratory chain: Q is co-enzyme or ubiquinone: Cyt c is cytochrome c.

THE BASICS IN GRADING TOXIC SEVERITY

In the previous subtitles, the review has briefly demonstrated the relationship between the immune and metabolic systems and how this relationship affects the toxicity of test chemical compound administered into the biological system. The toxic property of a chemical compound is diverse in which multiple biological signals could be manifested in the biological system of living organism which would be detected as multiple signs and symptoms of toxicity. A test chemical compound administered to study subject in the oral route is subjected for first-pass metabolism in the intestinal mucosa and liver before reaching the circulatory system [29]. As a result, a fraction of administered dose of test chemical compound would reach the systemic circulation depending on the chemical nature of its metabolite, physiological state of the body and biological membranes. However, the drug concentration that reaches the vicinity of the biological receptor is usually unknown whether it has been administered intravenously or orally. An integrated biological approach (biological signals and length of time at which the signals manifested in the biological system) has been used in the previous two studies to roughly determine the toxic severity and concentration of tested chemicals that has been reached the biological receptors as it is briefly explained under this subtitle below. The length of time at which biological responses involving alterations in receptor function or the activation of undesired biological signals in the course of metabolism against to the amount of dose administered into the biological system could be, therefore, having more importance than anything else in the determination of toxic severity [18,19]. The undesired

biological effect of administered test chemical compounds would be manifested when its reactive dose has overwhelmingly antagonized the defense mechanism of exposed living organism. The strength of undesired biological signals caused by the harmful effect of test chemicals administered into study subject could be judged quantitatively as the toxic severity and toxic reaction rate within the biological system which is also briefly explained as follow:

The toxic severity (*s*) and toxic reaction rate (*r*) of administered test chemicals at different level of doses into lab Balb c mice have been analysed in the previous two studies [17,18]. An integrated biological approach (physiological and immunological analysis) was employed in the study to determine the biological responses as toxic severity and toxic reaction rate during the course of metabolism in treated Balb c mice **(Tables 2 and 3)** [17,18]. The toxic severity is the magnitude of poisoning caused by a dose of test chemical administered into the biological process of study animal in one of drug administration routes [19]. It has been computed using mathematical formulation *(s=r/d × 100)%/sec* where *s* is toxic severity, *r* is toxic reaction rate and d is administered dose [17,18]. The toxic severity of a dose at 90 mg/kg body weight of Balb c mice prepared from Chlorpyrifos and Cypermethrin pesticides was 11 and 33.3%/sec, respectively **(Table 2)** [18]. This means that the poisoning severity of Cypermethrin was greater than the poisoning severity of Chlorpyrifos by 22.3%/sec to treated Balb c mice. The toxic severity of tested chemicals determined the lifespan of treated Balb c mice. The higher the toxic severity of tested chemicals, the higher the administered dose and the shorter the lifespan of treated Balb c mice was after dosing **(Table 2)** [17,18].

Table 2. Toxic severity (*s*) of tested chemicals computed at four hour after dosing [18,19].

Test chemicals	Doses tested	Toxic severity (*s*) in %/s
Dichlorvos	10 mg/kg	-199.0
	50 mg/kg	-19.8
	90 mg/kg	X
Chlorpyrifos	10 mg/kg	-299.0
	50 mg/kg	-39.8
	90 mg/kg	11.1
Cypermethrin	10 mg/kg	-199.0
	50 mg/kg	20.0
	90 mg/kg	33.3

[X] *Represents lab Balb c mouse which died earlier than the time for toxic severity evaluation*

The toxic reaction rate on the other hand refers to the proportion of administered dose of test chemical that has been reached the vicinity of biological receptor and has been elicited adverse biological response on treated study animal [19]. It has also been computed using mathematical formulation ($r=d/t - \Delta Ig$) mg/s where *r* is toxic reaction rate, *d* is administered dose, *t* is elapsed time for adverse effect manifestation and ΔIg is change in immune response after dosing [17-19]. The toxic reaction rate of a dose at 90 mg/kg body weight prepared from Chlorpyrifos and Cypermethrin was 10 and 30 mg/s, respectively **(Table 3)**. This implies that the toxic reaction rate of Cypermethrin was greater than the toxic reaction rate of Chlorpyrifos by 20 mg/s in the biological process of treated Balb c mice. The study also showed that all the amount of administered doses prepared from each test chemicals has never been involved in the manifestation of adverse biological effect on treated Balb c mice. Of the administered doses at 90 mg/kg prepared from each of Chlorpyrifos and Cypermethrin, for instance, only 11.1% (r/d × 100=(10) / 90 × 100=11.1%) and 33.3% (r/d × 100=(30) / (90) × 100=33.3%) of the administered dose caused adverse biological effects on treated Balb c mice respectively [19]. The proportion of administered test chemical that has been manifested adverse biological effect was inversely related to the strength of immune response of treated Balb c mice. The higher the strength of the immune response, the less toxic severity and toxic reaction rate of test chemicals within the biological process of treated Balb c mice orally **(Tables 1-3)** [19]. The toxic reaction rate determined the safety margin of test chemicals administered into lab Balb c mice in the oral route. The laboratory Balb c mice which was treated with the dose that had computed toxic reaction rate less than zero survived from death whereas laboratory mice treated with the dose that had computed toxic reaction rate more than zero died at different length of time after dosing depending on the toxic severity of tested chemicals [18,19].

Both biological responses (toxic severity and toxic reaction rate) are dependent on the amount of administered dose,

length of time at which adverse effect manifested after dosing and strength of the defense mechanism of treated laboratory animal [18,19]. When the toxic severity and toxic reaction rate of test chemical compound is high, the adverse effect would be manifested on study subject within short period of time after dosing. However, the toxic severity and toxic reaction rate within the biological process of study animal would be low when there is high strength of defense mechanism which implies that the harmful biological responses of administered test chemical is inversely related to the strength of immune response of treated laboratory animal **(Figure 4)** [18,19]. The toxic severity would be high when the toxic reaction rate of test chemical is also high in the biological process of treated laboratory animal. In general, quantitative, undesired biological responses during the course of metabolic pathways could be computed as toxic severity and toxic reaction rate which could help to roughly determine the level of poisoning severity and concentration of test chemicals that has been reached the vicinity of biological receptors or other biological targets in the biology of study subject respectively **(Tables 2 and 3)**.

Table 3. Toxic reaction rate (*r*) of tested chemicals computed at 4 h after dosing [18,19].

Test chemicals	Doses tested	Approximate length of time undesired effect significantly manifested	Toxic reaction rate (*r*) in mg/s
Dichlorvos	10 mg/kg	60 min	-19.9
	50 mg/kg	30 min	-9.9
	90 mg/kg	15 min	X
Chlorpyrifos	10 mg/kg	2:30 h	-29.9
	50 mg/kg	1:30 h	-19.9
	90 mg/kg	30 min	10.0
Cypermethrin	10 mg/kg	25 min	-19.9
	50 mg/kg	12 min	10.0
	90 mg/kg	9 min	30.0

[X] *represents lab Balb c mouse which died earlier than the time for blood specimen collection*

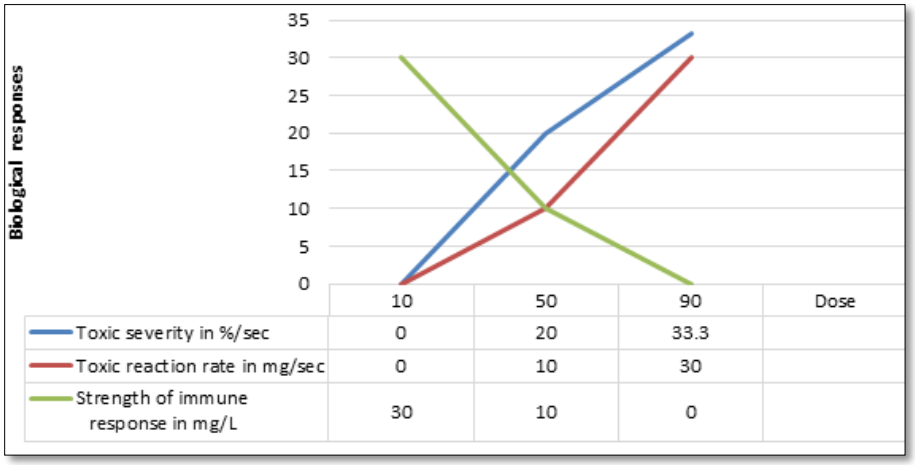

Figure 4. The toxic severity and toxic reaction rate of tested chemicals inversely realted to the strength of immune response of treated Balb C mice.

The catabolic and anabolic pathways are different metabolic systems in which the toxic property of test chemicals perhaps manifesting during both or one of the metabolic pathways. Each of the metabolic pathways has a first committed step which is regulated at different level of the biological system of higher animal even though it occurs in specific subcellular compartments [29,30]. Once a chemical compound is metabolised and its toxic metabolite is produced, it could not be possible to reverse its adverse biological effect within the biological system. An integrated biological approach is therefore preferable scientific approach in the determination of toxic severity of test

chemicals administered into study animal. The following parameters need to be considered in grading the poisoning severity of prepared test chemicals [13,14]:

a. The amount of dose administered into study subject.

b. The length of time at which undesired biological responses manifested after dosing.

c. Quantitative analysis of the immune response after every dosing at standard metabolic time interval need to be considered for adequate period of time.

d. The number of sampled laboratory animals in a cage should be as fewer as possible as high population will influence the biological state of each laboratory animals.

CONCLUSION

The adverse effect of test chemicals could be manifested either during the catabolic or anabolic or during both metabolic pathways both of which are different metabolic systems. The immune response and metabolic regulation are highly interrelated biological systems in which the proper function of one is highly dependent on the other. A direct relationship and coordinated regulation of both metabolic and immune systems have therefore potential benefit in the determination of undesired biological responses as toxic severity and toxic reaction rate within the biological process of a living thing. An integrated biological approach in experimental toxicology is crucial:

1. To determine the toxic severity (s) and toxic reaction rate (r) of test chemicals which is intended for advancement to the clinic.
2. To collect scientifically validated data for the categorisation and regulatory decision of the toxicity of test chemicals.
3. To collect adequate pharmacological data with fewer number of laboratory animals as compared to lethal end point acute toxicology.
4. To conduct comprehensive pharmacological property of test chemicals with a single laboratory setting which avoids wastage of time and resources.

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