PESTICIDE CHRONOTOXICITY TO INSECTS AND MITES: AN OVERVIEW

"The sun can no way catch up the moon, nor can the night outstrip the day: Each (just) swims along in (its own) orbit."

-The Holy Qur-an,

(An English translation of the meaning.)

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SUMMARY: Chronotoxicity may be defined as the changes in an organism's sensitivity to toxicants in relation to time. A review of research papers reveals numerous examples of a sensitive period to insecticides and miticides during a 24-hour cycle, or a circadian rhythm of sensitivity. In rare instances bimodal or tri-modal rhythms of susceptibility have been determined. The circadian rhythms of insect sensitivity to toxicants have been related to rhythmic biochemical processes. Thus, behavioral and physiological rhythms might be considered as marker rhythms for the rhythmic patterns of sensitivity to toxicants. However, the circadian peaks of sensitivity may not always be correlated with activity and metabolism. The practical application of the circadian sensitivity to toxins has had limited study and perhaps deserves greater attention.

Key Words: Pesticide chronotoxicity.

INTRODUCTION

Ever since living organisms have existed on earth they have been subjected to rhythmically alternating cycles of light and darkness associated with the movements of the earth around its axis and around the sun, and a host of other phenomena in the ecologic habitat niche and the universe as a whole. Consequently, they have developed rhythmic activity patterns synchronized with particular periods of the day to counteract or exploit as much as possible the environment in which they live. Many of the inputs are cyclic, with different frequencies, including some differing from the geophysical and social day and year; they provide variability to the data to the point that a they rhythms can not be detected, which does not meant that it does not occur.

Some of the rhythms of biological processes, whether physiological or behavioral, are direct responses to photoperiodic stimuli, while many are overt manifestations of internal oscillators which are in continuous motion, regardless of the external temporal clues (Zeitgeber = time giver) (2).

Of particular interest are those endogenous oscillations which have evolved with a periodicity approximating 24 hours (circadian rhythms). The periodicity is used by animals and plants to 'time' daily events thus allowing the organism to perform functions at the 'appropriate time of the day'.

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In view of the circadian behavioral, metabolic and physiological rhythms that have been described in insects (3,23) the probability would appear to be good that insects and mites should display time-related changes in their sensitivity to pesticides and other pharmacological or toxicological agents.

Basic concepts and terminology

The term chronotoxicity, as we may define, refers to the changes in an organisms sensitivity to toxicants in relation to time.

Time concepts are involved in the subject of chronotoxicity. The terms exogenous and endogenous were first introduced to differentiate between rhythmic patterns induced by external factors and those taking place even in the absence of temporal cues (20). An endogenous rhythm thus would continue to function in the absence of photoperiodic stimuli (Zeitgeber = time giver) and is said to be free-running.

The term photoperiod has been used ambiguously in much of the literature in that it is sometimes used to denote only the lighted portion of the cycle and also to denote the entire cycle of illumination and darkness (LD). The daylight portion of the photoperiod is referred to as photophase; the dark portion is termed scotophase (3).

Environmental periodicity is frequently matched by an appropriate endogenous rhythmicity which is a constituent and characteristic physiological feature of living components such as cells, tissues, etc. Organisms may possess circadian (ca. 24 hours), circatidal (ca. 12.4 hours), circaseptan (ca. 7 days), circasyzygic or semi-lunar (ca. 14.7 days), circalunar (ca. 29.4 days), or circannual (ca. 1 year) periodicities. Others have also been determined (14).

The period of a rhythm (τ) is the time elapsing between successive identical states (phases). When entrained under a photoperiodic regime of light and dark (DD or LL), however, the period of the rhythm is subjected to small daily adjustments to keep in synchrony with the environmental photoperiod, and it approximates 24 hours (22 to 28 hours). In such a case the rhythm is defined as an endogenous circadian rhythm. The term 'circadian' introduced by Halberg (16), was derived from the latin 'circa' (= about) 'dies' (= day) to describe all daily rhythms of approximately 24 hours, longer or shorter by a few minutes or hours.

Description of rhythmic parameters

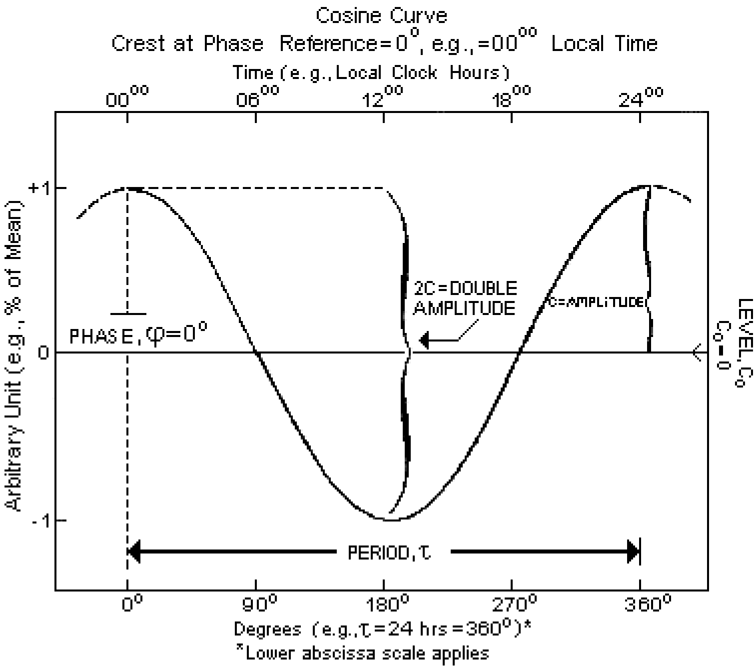
Many investigators have the convention of representing rhythmic functions in terms of curves that oscillate in a sine wave (cosine curve) (8). On such graphical representation, values on the abscissa corresponding to any arbitrary point (Φ) on the curve is called the phase angle (Ψ), and is expressed in degrees or in time units, where 15 phase angle is equivalent to one hour in a 24-hour cycle. The phase angle (Ψ) is referred to as the 'acrophase' when the arbitrary point (Φ) marks the time of the peak value of the rhythmically oscillating variable. The time elapsing between two successive acrophases is the period of the rhythm. The mesor is the average value or mean value of the mathematical function used to describe the rhythm (τ). The amplitude is the difference between the maximal and the mesor value of a sinusoidal function describing a rhythm. All these rhythmic parameters are visualized (Figure 1) (27).

Daily and circadian rhythms in insect sensitivity to toxicants

Circadian rhythms of mammalian, sensitivity to drugs were first recorded by Halberg and his associates (14,15). At different times of the 24-hour cycle, mice were found to react differently to equivalent doses of ethanol. Highest susceptibility was recorded (in LD 12:12) towards the end of the light period and lowest susceptibility close to midnight. A circadian susceptibility-resistance cycle to ethanol persisted in the mouse kept in continuous darkness for several days.

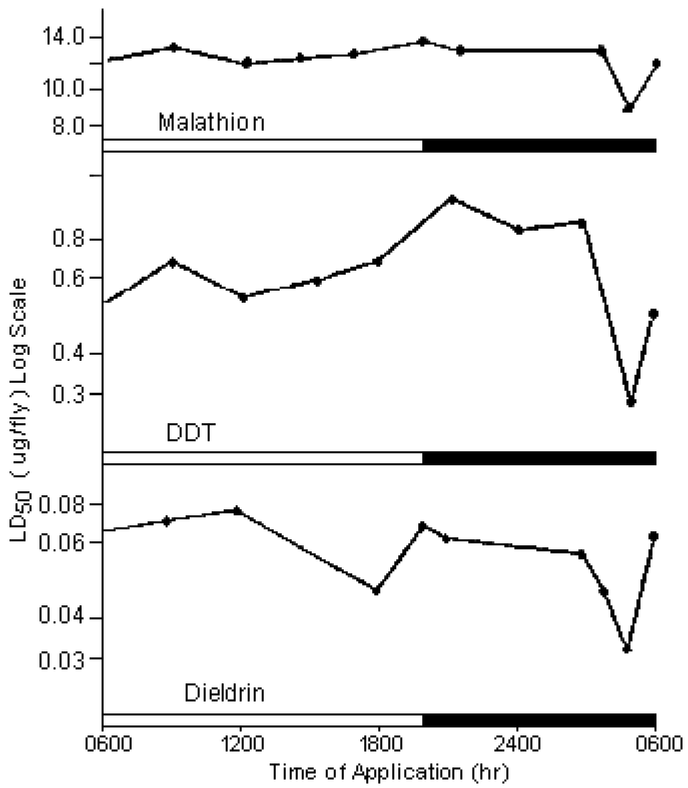
Daily rhythmic patterns of insect sensitivity to insecticides were first reported in the German cockroach, *Blattella germanica* maintained under a 12L:12D photoperiod (3). Potassium cyanide was least toxic at the beginning of the subjective day, but increased in toxic-

Figure 1: Cosine curve (after Sullivan *et. al.*, 1970).



ity to reach a maximum in the middle of the subjective night. Dimetilan, a carbamate insecticide, was also more toxic during the middle of the subjective night, but the minimum sensitivity of the cockroach occurred during the latter half of the subjective day. Since then many reports on daily and circadian rhythms in insect sensitivity to insecticides have been given. A study was carried out on the boll weevil, Anthonomus grandis maintained under three different photoperiods. At different times of the day, the weevils were treated with a standard dose of methyl parathion. Regardless of the photoperiodic regime itself, a distinct peak of resistance occurred at the beginning of the scotophase and recurred at 6-hour intervals, alternating with peaks of high sensitivity (5). The house cricket, Gryllus domesticus, showed a daily rhythm of narcotic sensitivity to ether, chloroform and carbon tetrachloride (19). The house fly, Musca domestica vicina was found to display a daily rhythm of sensitivity when treated with trichlorofon, an organophosphate insecticide. The rhythm was maintained when the insects were kept under different light/dark regimes (10). House flies and Madeira cockroaches, Leucophaea maderae, reared under alternating cycles of light and darkness, exhibited on daily sensitivity rhythm to pyrethrum. Both species were most susceptible to insecticides during the last quarter of the daily light span, about mid-afternoon (27). The sensitivity rhythm of house flies was tested using malathion, DDT and dieldrin (25). The insects were most susceptible to the three insecticides at the end of the scotophase, one hour before light-on (Figure 2). Furthermore, the endogenous nature of the rhythm of susceptibility to DDT was tested by rearing the insects in complete darkness from the pupal stage till the time of insecticide application. Under such conditions the insects were found to display the same pattern of susceptibility as that recorded under the LD regime. Adults of the yellow mealworm, Tenebrio molitor reared under 12:12 LD regime, showed bimodal susceptibility rhythm when treated with methyl parathion. Peaks of maximum sensitivity occurred at the onset of both light and dark phases (12). Males and females of Pectinophors

Figure 2: Effect of time of application on LD50 values (ug/fly) of three insecticides for the house fly, Musca domestica, under LD 14:10 (Adapted from Shipp and Otton,[25]).



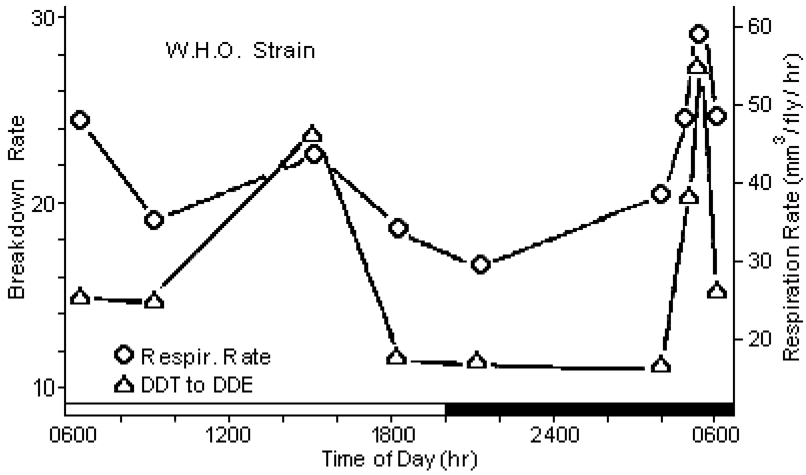
gossypiella showed rhythmic susceptibility to azinphos-methyl. The peak of maximum sensitivity occurred one hour after lights-on. In addition, males possessed a second peak of susceptibility that took place 2.5 hours after the onset of darkness (29). The larvae of the mosquito, Aedes aegypti, were most susceptible to chlorpyriphos (Dursban) one hour after lights-off (22). Circadian changes of insecticide sensitivity in three strains of A. aegypti were reported in both larvae and adults (1).

The small workers of the ant, Acromyrmex octospinosus reared under LD 12:12 showed a distinct rhythm of susceptibility to parathion. The peak of sensitivity occurred at a time when the lights were switched on (17). Drosophilae melanogaster reared under LD 12:12, showed maximum sensitivity to parathion at the end of the scotophase and the beginning of the photophase (18). Male, non-virgin female and virgin female American cockroaches, Periplaneta Americana, were standardized on a regimen of light for 14 hours, alternating with darkness for 10 hours (LD 14:10) (9). They were tested for sensitivity to an organophosphorus insecticide, dichlorvos, on this regimen and on a regimen providing for one day's maintenance in continuous red light. Results indicated endogenous circadian changes in cockroach sensitivity in the three groups. Again, American cockroaches held under an LD 14:10 regimen demonstrated circadian rhythm of sensitivity to propoxur. Maximum sensitivity occurred 3 hours after the onset of the scotophase, whereas minimum sensitivity occurred somewhat in the middle of the photophase (7).

Non-rhythmic sensitivity patterns

A circadian pattern of sensitivity of insects to insecticides has not always been found, although critical sta-

Figure 3: Effect of time of treatment of adult Musca domestica with DDT on respirator, rate (open circles) and metabolism of DDT to DDE (open triangles) under LD 14:10 (Adapted from Shipp and Otton,[26]).



tistical treatment has been lacking. Little or no rhythmicity was detected in the sensitivity of the German cockroach. Blattella germanica to sodium fluoride, sodium azide and 2,4-dinitrophenol (3). However, no data is given to support the above statement. Likewise, house flies maintained under different photoperiodic regimes are said to lack daily sensitivity rhythms toward malathion (13). Analysis of their data by cosinor and least squares reveals a circadian rhythm in those subjected to 10:14 LD and 14:10 LD. Adults of 3 species of mosquitoes, Anopheles culcifacies, Anopheles annularis and Aedes aegypti did not display a rhythmic pattern in susceptibility to both DDT and malathion (6). The latter study was conducted with field collected blood-engorged female. No statistical treatment of the data was reported.

Daily rhythm in mite sensitivity to toxicants

Chronotoxicity studies on mites are limited. Daily rhythms have been shown using adults of the two-spotted spider mite, Tetranychus urticae using a number of insecticides and pharmacological agents. In LD 14:10 the maximum sensitivity to dichlorvos occurred about 2 hours into the photophase (after dawn).

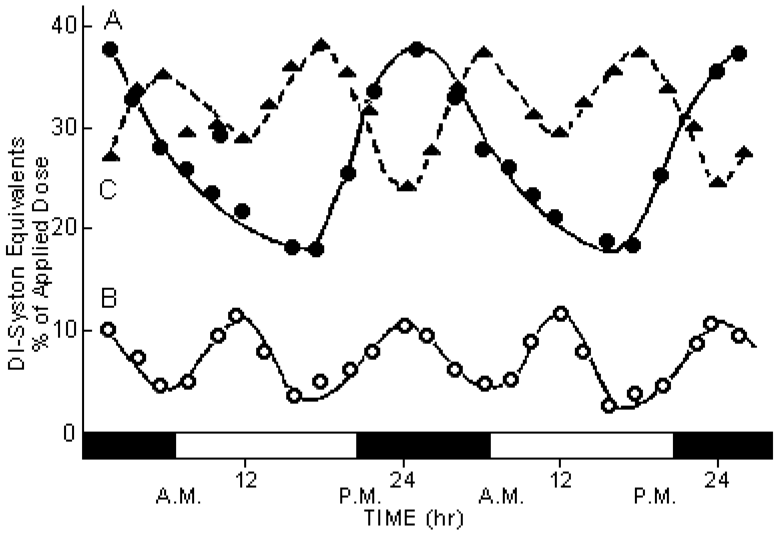
The mites were least sensitive to dichlorvos about two hours into the scotophase (21). Maximum mite sensitivity to ether, chloroform and carbon tetrachloride occurred about dusk (19). A bimodal rhythm of susceptibility to dicofol was detected in the two-spotted spider mite. The peaks of maximum sensitivity occurred at dawn and before sunset (11).

Marker rhythms

A number of reports have suggested that circadian rhythms in insect sensitivity to toxicants might be expected to be reflections or rhythmic biochemical processes involved in the mode of action and metabolism of the toxicants. Therefore, one might consider behavioral and physiological rhythms to be marker rhythms for the rhythmic patterns of insect sensitivity to toxicants.

Maximum house fly sensitivity to DDT coincided with highest respiratory metabolism, using measurement of rates of oxygen uptake (Figure 3); conversion of DDT to DDE was also maximal at this time (26). The sensitivity of house crickets, Gryllus domestica, to the anesthetics ether, chloroform and carbon tetrachloride was also found to be at its daily maximum during the early scotophase, which corresponds with the time of greatest locomotor activity (19). An enzyme study uti-

Figure 4: Effect of photoperiod on the rates of metabolism of disulfoton by larvae of Heliothis zea. (A) oxidative metabolites in larval extracts; (B) hydrolytic metabolites in larval extracts; (C) hydrolytic in fecal extracts (modified from Bull and Lindquist, [4]).



lizing the specific activity of Mg2+ ATPase revealed a tri-modal (8-hour period) rhythm in larvae of the mosquito, Aedes aegypti; this enzyme was inhibited by the insecticide gamma chlordane in a parallel daily rhythm. Thus, the percentage inhibition was at its lowest in samples taken at the time the specific activity of the control samples was also at its lowest (30).

Metabolic measurements also show relationships with toxic effects. Studies of the metabolism of the insecticide disulfoton in the bollworm (4) showed that larvae reared on a meridic diet at constant temperature and under 14L:10D photoperiod exhibited two daily maximal of oxidative metabolites, considered to be the toxic components. The maximal occurred at 6 a.m. and 6 p.m. Extracts from feces did not reveal a rhythmic pattern. Hydrolytic products (non-toxic) followed a cyclic pattern of 24 hours. Larvae reared in continuous dim light did not exhibit rhythmicity in either oxidative or hydrolytic products (Figure 4). Experiments involved insecticide treatment by injection at 2 hour intervals and determinations were of radio phosphorus labeled disulfoton.

Metabolic relationships studied in the confused flour beetle, Tribolium confusum showed a significant relationship between oxygen consumption and sensitivity to the insecticide dichlorvos. Using a LD 12:12 regimen, the highest sensitivity to dichlorvos occurred shortly after midnight, while maximum oxygen consumption occurred about 2 hours later. Analysis included fitting cosine curves to the data to estimate rhythm characteristics (28).

Rhythms of circadian insecticide sensitivity and locomotion in American cockroaches showed a similarity of rhythmicity peaks within 3 hours after the onset of the dark span. Assuming sinusoidality, the times of highest sensitivity to dichlorvos led the acrophase of the locomotor activity rhythm by about 2 hours (8).

A positive relationship was also found between maximum locomotor activity in house crickets, Acheta domestica and sensitivity to anesthetics ether, chloroform and carbon tetrachloride. The positive relationship occurred during early scotophase (19).

The circadian rhythm of a critical enzyme acetylcholine esterase appeared to be synchronized with sensitivity to carbaryl and malathion in the rice grasshopper, Aiolopus thalassinus. Both insecticides are inhibitors of the enzyme. The acrophases of both rhythms occurred during the photophase at nearly the some time. Rhythms of AChE activity and sensitivity to carbaryl seem to be endogenous since they persisted under DD conditions. Continuous illumination had the same effect on both rhythms, causing them to damp out (24).

Exceptions to the generalization that insecticide sensitivity has circadian rhythmicity peaks at or near the time of circadian peaks of insect, Leucophaea maderia and the house fly, Musca domestica, a diurnal species. Similar rhythmicity peak were found in sensitivity to pyrethrum, whereas activity and metabolism would be distinctly different in a 24-hour period (27).

ACKNOWLEDGEMENTS

The senior author is grateful to Professors F. Halberg, E. Halberg and L. K. Cutkomp, University of Minnesota, USA for introducing me to the field of Chronotoxicology. Further, acknowledgement is made for the cosinor analysis of the data of Frudden and Wellso (1968) by Dr. Germaine Cornelissen in the laboratory of Dr. Franz Halberg.

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