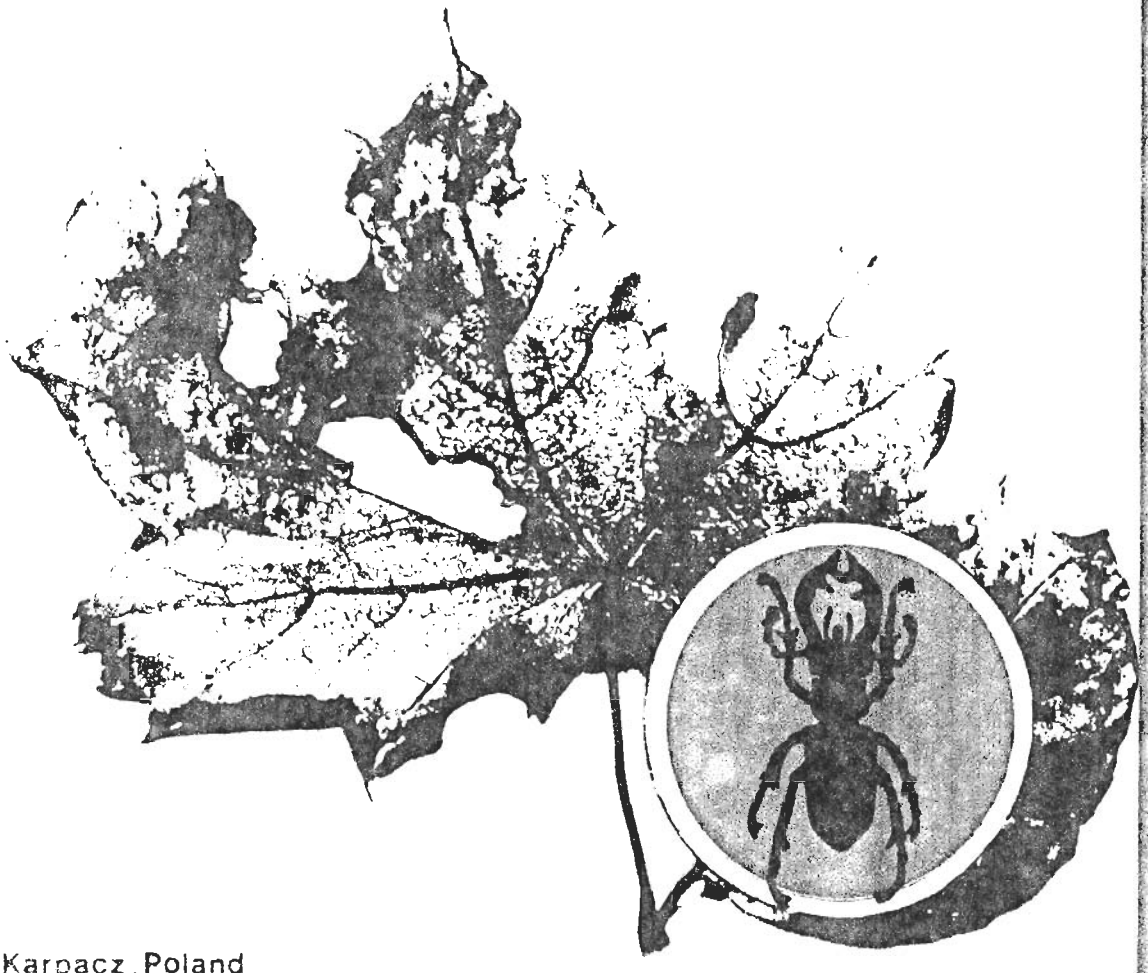
Regulation of Insect Development and Behaviour

International Conference

Part II



Karpacz, Poland
June 23–28, 1980

Wrocław 1981

Scientific Papers of the Institute of Organic and Physical Chemistry of Wroclaw Technical University, No 22, Conferences 7

METABOLISM AND ENVIRONMENTAL FATE OF JUVENOIDS: A DECADE'S RESEARCH IN PERSPECTIVE AND AN ASSESSMENT OF THE FUTURE

Bruce D. Hammock

Division of Toxicology and Physiology, Department of Entomology
University of California, Riverside, California
USA, 92521

ABSTRACT

Aspects of juvenoid metabolism and environmental degradation are reviewed with an emphasis on pathways of possible significance to insect endocrinology and to xenobiotic metabolism in general. The future of juvenoids as pest control agents is examined in light of their environmental stability.

INTRODUCTION

The last decade witnessed numerous attempts to commercially develop several juvenile hormone mimics or juvenoids. Thus, it may now prove useful to reflect over what has been learned regarding the environmental fate and pathways of degradation of such compounds from both basic and applied perspectives. Such studies on the stability of juvenoids under a variety of conditions not only provide a scientific framework upon which to build a registration package leading to commercial use, but they also have applied and basic significance to several fields. In this chapter, some aspects of the evolution of juvenoid structure will be examined along with an overview of the major routes of degradation of several compounds. The investigation of these routes of degradation was interesting in itself because the juvenoids represented new structural types whose metabolism was not well studied in either insects or mammals. This work also has broad significance because juvenoids are models for

the natural insect hormones as well as being models for terpenoid compounds ubiquitous in our environment. Since terpenes are natural constituents of foods and fragrances as well as artificial additives to cosmetics and a host of other products, it is surprising that their metabolism has been so superficially studied.

This chapter is not designed to be an exhaustive review; rather, it will serve to highlight some of the interesting discoveries of juvenoid metabolism as they apply to broader fields. This information will then be interpreted in light of an assessment of the future for juvenile hormone mimics. For more exhaustive literature reviews, the reader should refer to Hammock and Quistad (1976, 1980) and Schooley and Quistad (1979).

EVOLUTION OF JUVENOID STRUCTURES AND PATHWAYS OF THEIR DEGRADATION

Even before epoxide hydration (1) and ester hydrolysis (2) were established as the two major pathways of juvenile hormone (JH) degradation, work was under way to develop structures which were more active and stable in the insect and less expensive to manufacture. Several such compounds are illustrated in Figure 1.

In methoprene, the *O*-methyl substituent is much more resistant to hydrolysis than the epoxide of juvenile hormone. The dienoate ester is more stable to base than the natural enoate ester of JH while the ethyl ester of hydroprene and the isopropyl ester of methoprene are more sterically hindered than the methyl ester of JH (Henrick *et al.*, 1973). These compounds have also proven to be refractory to hydrolysis in insects.

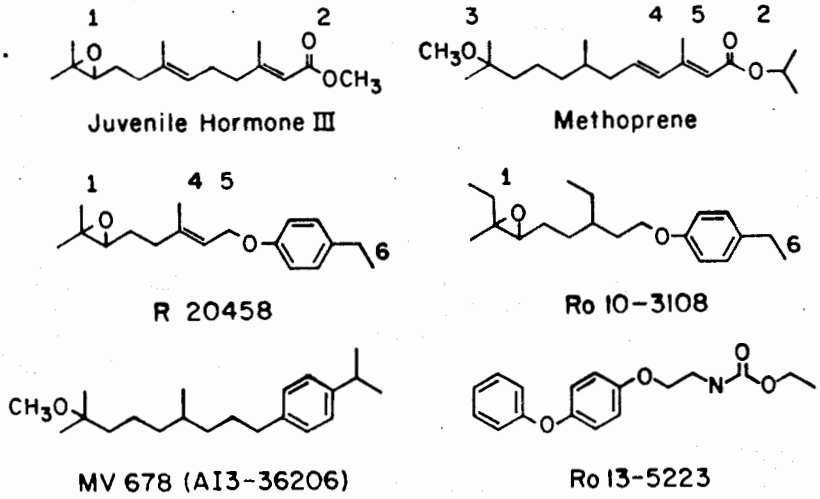


Fig. 1. Structures of representative juvenoids illustrating major sites susceptible to environmental degradation and metabolism.

The major pathways of degradation of methoprene include oxidative *O*-demethylation (3) (Fig. 1); oxidation, peroxidation, reduction, and isomerization of the dienoate olefins (4,5); ester hydrolysis (2); and degradation of the backbone of the molecule (Quistad *et al.*, 1974a, 1976; Schooley and Quistad, 1979, and included references). The instability of the dienoate moiety to ultraviolet radiation limits the usefulness of these compounds under conditions where they will be exposed to intense sunlight (Quistad *et al.*, 1975a; Henrick *et al.*, 1975). For most usages of methoprene, however, such exposure is not a problem. The *O*-methyl moiety is very susceptible to metabolism in some insect strains resistant to pesticides (Hammock *et al.*, 1977). Such potential resistance problems have not yet limited the use of methoprene.

A second approach toward designing a stable, relatively inexpensive structure is illustrated by Stauffer's R 20458 (Pallos, 1971) which evolved from Bowers' (1969) terpenoid ethers. The epoxide moiety is retained, but the ester moiety is replaced by the much more hydrolytically stable phenoxyether linkage. Major routes of degradation involve hydration or rearrangement of the 6,7-epoxide functionality (1) (Fig. 1), epoxidation of the 2,3-olefin (4), allylic hydroxylation leading in some cases to cleavage of the ether linkage (5), and oxidation of the ethyl substituent primarily in the alpha but also in the terminal beta position (6) (Gill *et al.*, 1972, 1974; Hoffman *et al.*, 1973; Hammock *et al.*, 1974a; Ivie *et al.*, 1976).

Ro 10-3108, developed by Hoffman LaRoche, illustrates how minor modifications can substantially increase the stability of a molecule. The ethyl branch on the epoxide functionality (C7) sacrifices activity on Diptera for increased activity on Lepidoptera and several other insects (Zurflueh, 1976). Interestingly, the increase in hydrophobicity about the epoxide increases its stability to acid catalyzed hydrolysis (Hangartner *et al.*, 1976) and this concept was extended to several other compounds (Mumby and Hammock, 1979). Elimination of the 2,3-olefin leads to some loss in activity but also leads to a much more stable compound since the terpenoid chain is no longer susceptible to allylic hydroxylation. The major routes of metabolism still involve epoxide hydration (1) and oxidation on the phenoxyethyl side chain (6) as in R 20458 (Hangartner *et al.*, 1976; Dorn *et al.*, 1976). The phenoxyethyl side chain of both R 20458 and Ro 10-3108 is susceptible to rapid oxidation. The epoxide moiety is quite labile to hydrolysis under acidic conditions, but it is much more stable than has been widely assumed. Resistant strains of house flies have not been shown to have increased epoxide hydrolase activity (Hammock *et al.*, 1977; Yu and Terriere, 1978a), and epoxide hydration does not appear to be the major route of JH degradation in many insects. Thus, knowledge of the major mechanisms of hydration of the epoxide moiety

in model ecosystems and in target insects may allow the structure to be stabilized while retaining insecticidal activity. In general, these terpenoid phenoxy ethers are more uv stable than the dienoate juvenoids, but they are unstable under conditions where epoxide hydration can occur.

A continuation of this evolution in structure is illustrated by MV 678 (Fig. 1) where the terpenoid phenoxy ether linkage is eliminated (Schwarz *et al.*, 1974). This modification might lead to enhanced stability since oxidation alpha to a heteroatom is common in biological systems; however, it also opens a second site for benzylic oxidation. In contrast, the isopropyl substituent on the phenyl moiety may sterically hinder benzylic oxidation, in some cases.

The decisions to develop methoprene, hydroprene, R 20458 and Ro 10-3108 were all made at a time when society seemed to desire very nonpersistent compounds. Although each of the above compounds is more stable than the parent JH's, they are very unstable when compared with many classical pesticides. Since juvenoids act only at certain times during insect development, their biostability must be consistent with their mechanism of action. Thus, the above compounds were only suitable for use under fairly restrictive sets of circumstances. Apparently, the disadvantages of juvenoids including their inability to rapidly kill the larval stages of many destructive insects outweighed the advantages offered by the above compounds for many applications.

The concept has recently evolved that persistence need not be necessarily equated with biostability and the resulting biomagnification and accumulation in a food chain. Juvenoids are needed which are persistent enough under a variety of field conditions to effectively control pest insects. They need to be refractory to rapid metabolism in certain pest insect species that have developed very high capacities to degrade foreign compounds, while still having several sites susceptible to metabolism in a variety of organisms. These and other constraints on future juvenoid development will be discussed later in this chapter and by Menn and others elsewhere in this volume.

CONTRIBUTIONS OF JUVENOID RESEARCH TO THE SCIENCE OF XENOBIOTIC METABOLISM

The study of xenobiotic metabolism certainly has applied significance; mainly in gaining approval to market a compound and subsequently in gleaning information which might lead to the production of more useful derivatives by increasing or decreasing their biostability, changing their distribution in various pools within an organism or in an ecosystem, or even designing intrinsically more active compounds. Yet, xenobiotic metabolism is also a basic science. An understanding of how living creatures cope with foreign as well as intrinsic compounds is fundamental to an understanding of how

they exist in ecosystems filled with both natural and man-made poisons.

The first studies on the metabolism and environmental fate of juvenoids came at a time when the techniques of metabolism chemistry were rapidly evolving. The use of radiotracers, functionality selective derivatives, and thin-layer chromatography was supplemented by high performance liquid chromatography and mass-spectrophotometry interfaced with gas liquid chromatography. These techniques allowed the separation of metabolites of very similar chemical properties and facilitated structural assignments using very small amounts of samples. The studies on JH and juvenoid metabolism in insect organs held *in vitro* and in isolated hepatocytes represent attempts to bridge the gap between metabolism studies performed in well defined cell-free systems and the more complex *in vivo* studies. It is safe to predict that such techniques will continue to evolve and find increasing application in metabolic studies involving a variety of xenobiotics.

The study of juvenoid metabolism has unearthed several interesting metabolic pathways in both target and nontarget organisms. In some cases, the study of juvenoid metabolism has provided information on the metabolism of the natural hormone in insects. Even before the metabolism of the natural JH's was investigated, the high biological activity of certain juvenoids indicated that epoxide hydrolases and esterases were probably involved in JH metabolism. The report that the diol was a major metabolite of the juvenoid R 20458 in a locust was coincident with a report that JH diol was an important JH metabolite in several insects (Gill *et al.*, 1972; Slade and Zibitt, 1972). An unexpected metabolic pathway of methoprene in the house fly involved isomerization of the active 2*E* isomer to the much less active 2*Z* isomer (Quistad *et al.*, 1975b). The involvement of a thiol group in this reaction is expected since thiophenol is used to isomerize dienoate acids during their manufacture by a reversible Michael-like addition (Siddall, 1976). The reaction was verified *in vivo* in resistant and nonresistant house fly strains and glutathione was shown to be capable of catalyzing both the forward and the reverse reaction as well as capable of very slowly conjugating with methoprene and JH, but an adequate *in vitro* model was not demonstrated (Hammock *et al.*, 1977, unpublished). This observation certainly deserves further exploration, especially as it might apply to the metabolism of the natural hormones.

An important point in the investigation of the metabolism of biodegradable pesticides was raised by Quistad *et al.* (1974b). When a pesticide or other xenobiotic was administered to a nontarget organism, rapid and quantitative excretion of the radiolabel was considered an indication of biodegradability. The opposite situation was usually taken as an indication of undesirable persistence. In mammals, methoprene was found to be rapidly excreted as a variety of metabolites, including CO_2, indicating that it was rapidly metabolized, but a surprisingly large

proportion of the dose remained, apparently tissue bound. It was then demonstrated that methoprene was metabolized to ^{14}C acetate which was then incorporated into natural metabolites. Thus, the lack of total excretion of methoprene indicated neither persistence nor the formation of reactive metabolites but, in contrast, extreme biodegradability. As pest control agents are patterned more closely after natural products, this phenomenon is likely to be commonly observed.

Studies on the metabolism of R 20458 led to several interesting problems. Metabolism by cell-free liver homogenates from several species qualitatively demonstrated the major routes of metabolism observed *in vivo* with one major exception. Phenols were major *in vivo* metabolites while significant ether cleavage was not detected *in vitro* (Hoffman *et al.*, 1973; Gill *et al.*, 1972, 1974; Hammock *et al.*, 1974a). Thus, an *in vitro* model for the major route of metabolism of these compounds has not been established.

Hoffman *et al.* (1973) reported metabolites of R 20458 in which the ethyl substituent on the phenyl ring was replaced by a *p*-hydroxy substituent. These workers speculated that the metabolites might be produced from a Baeyer-Villiger-like oxidation of the acetophenoxy moiety. Once again, this interesting reaction was not duplicated *in vitro*. These two observations are certainly deserving of further work since they might illustrate new pathways of metabolism which could be applicable to a wide range of xenobiotics.

Several interesting pathways of environmental degradation involve either the 6,7-epoxide or an epoxide formed in the 2,3-position. Hydration of the 6,7-epoxide (A) (Fig. 2) (10,11 epoxide of JH) to the corresponding diol (B) was shown quite early to occur under a variety of conditions. It was logical to assume that epoxidation and subsequent hydration of the 2,3-olefin (C) would lead to a 2,3,6,7-tetraol (D) (Fig. 2) (Gill *et al.*, 1972; Ajami and Riddiford, 1973). Instead, it was found that hydrolysis of the diepoxide (C) catalyzed by either enzymes or acid or oxidation of the 2,3-olefin of the 6,7-diol led to a diol-epoxide (E) intermediate which rapidly cyclized at or below physiological pH to a variety of products. Two tetrahydrofuran diols (F) were found to be the major products, and under some circumstances, the only products (Hammock *et al.*, 1974a,b; Gill *et al.*, 1974). These cyclized products represent minor *in vivo* and *in vitro* metabolites of R 20458. Analogous reactions have been demonstrated with JH (Hammock *et al.*, 1975) and there is good evidence that the resulting products are JH metabolites in the house fly (Hammock *et al.*, 1977; Yu and Terriere, 1978b). Isoprenoid compounds commonly exhibit such 1,5-unsaturation, and it is likely that many of these compounds are metabolized to analogous cyclic products. The intermediate diol epoxide is quite reactive, but with R 20458 and related compounds it, fortunately,

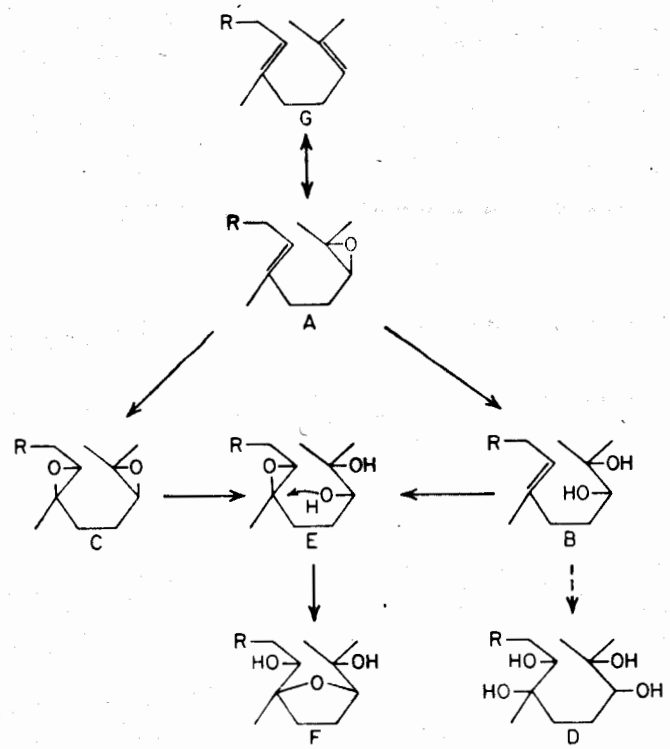


Fig. 2. Metabolism of the epoxide and olefinic functionalities in terpenoid molecules.

appears to be a poor alkylating agent.

Another very interesting observation regarding the 6,7-epoxide moiety of R 20458 was also made by Hoffman *et al.* (1973). When R 20458 was orally administered to rats, several metabolites with the 6,7-olefin were isolated implying epoxide reduction. Since Gill *et al.* (1972, 1974) failed to find such compounds following intraperitoneal administration of R 20458 to rats and mice, Hoffman *et al.* (1973) speculated that such metabolites might have arisen from olefinic impurities in the R 20458 administered to the rats. This dilemma was explained when the fate of R 20458 in steers was examined (Ivie *et al.*, 1976; Ivie, 1976). These workers found that under anaerobic conditions the rumen contents were capable of effectively reducing R 20458 and several other epoxides to their corresponding olefins (F) (Fig. 2). Such reactions would be anticipated to be much more important following oral administration than following intraperitoneal administration. Analogous reactions have been found to be catalyzed by the gut contents of a variety of mammals, including man. Since the 6,7-olefin of R 20458 (and the analogous olefins of squalene and a variety of terpenes) is readily epoxidized, the reaction should effectively be reversible *in vivo*. The metabolism

of epoxidized compounds is being extensively studied in relation to mutagenicity and carcinogenicity, so it is surprising that this apparent epoxide reduction has not been further investigated.

Epoxide hydration to the diol (B) (Fig. 2) accounts for a major proportion of both *in vivo* and *in vitro* metabolites in mammals; however, when the reaction was studied in more detail, it was found to be largely catalyzed by a soluble enzyme apparently located in both the cytosolic and mitochondrial subcellular fractions of mouse liver homogenates (Gill *et al.*, 1972, 1974). This observation was surprising because it had been widely assumed that epoxide hydrolases were exclusively membrane bound (Oesch, 1973). Subsequently, a soluble epoxide hydrolase has been found to occur in the cytosol and mitochondrial lumen in a variety of tissues from numerous vertebrates (Gill and Hammock, unpublished). In contrast to earlier assumptions, this enzyme appears to hydrate a variety of structurally dissimilar epoxides including known mutagens and suspect carcinogens (Ota and Hammock, 1980; Hammock *et al.*, 1980a,b). This discovery and the observations mentioned above aptly illustrate how the investigation of the metabolic fate of juvenoids has expanded our understanding of xenobiotic metabolism in general.

FUTURE OF JUVENOIDS: CONSTRAINTS ON DEVELOPMENT

The hope and the reality. Williams (1967) predicted a bright future for third generation pesticides due to their many advantages over the more classical pesticides. The effort spent in developing juvenoids as the primary example of the third generation pesticides has certainly not been wasted since the information gained has greatly expanded our knowledge in a variety of fields. Very important to pest management is the evolution in thought leading to a broader definition of the third generation pesticide concept. Initially, a third generation pesticide was defined in terms of a final product acting to disrupt chemical mediation in insects. One can conceive of a third generation pesticide in broader terms as a compound designed to have many of the attributes of an ideal pest management agent whose development has been based on a knowledge of the comparative physiology and biochemistry of target and nontarget organisms. Thus, the third generation concept becomes a dynamic term describing the evolution of a pest management agent rather than simply a mechanism of action (Sparks and Hammock, 1980).

On the other hand, the practical use of juvenoids has been very limited. Only the dienoate juvenoids have been registered, and of these, methoprene accounts for most use, largely in the control of dipterous pests. Juvenoids have failed to have a significant impact in controlling insects on field, row or orchard crops. From an insect pest management standpoint, juvenoids have thus failed to have an important

impact on the patterns of pesticide usage since most pesticides are used for crop protection. Such large volume markets are obviously the most profitable, and juvenoids have failed to acquire a share of these markets. The capital investment leading to the development of juvenoids has been socially important in expanding basic knowledge and in providing an effective control agent for some medically important insects. However, the small return on this investment makes it of questionable value from an industrial standpoint.

Conditions for future development. Certain social and economic conditions may again make the development of juvenoids and related innovative compounds attractive to future investment. These constraints concern a void in the pesticide market under conditions which the importance of the many advantages of juvenoids will be maximized. This void may be provided by the development of resistance to phosphates, carbamates and/or pyrethroids in certain key pests (Georghiou and Saito, 1980). Although insects can clearly become resistant to juvenoids, it is reassuring that there is no good evidence for resistance involving the site of action. The juvenoids to be developed must not be susceptible to high levels of cross resistance in major pests selected with classical pesticides. Thus, including several resistant strains at some stage in a screening program would be wise (Sparks and Hammock, 1980).

The philosophy of insect pest management has attracted scientists for years, and recently there seems to be a growing acceptance of well-designed insect pest management programs. The selectivity of juvenoids among insect orders and even families is much greater than that commonly observed with classical pesticides. This advantage of juvenoids may make them more economically attractive as pest management programs become increasingly common.

During the 1960's and 1970's, there was a trend toward developing compounds that were much less persistent than the organochlorine pesticides due to a concern over the accumulation of pesticides in food chains and the effect which trace levels of dietary pesticides might have on man. Although juvenoids are quite biodegradable, so are a number of classical pesticides. Thus, the persistent organocholorine pesticides generally with very low mammalian toxicity, were often replaced with less persistent organophosphate and carbamate pesticides often with high mammalian toxicity. Protection of the environment and the consumer from trace levels of pesticides was, thus, often obtained at the expense of agricultural workers having to use acutely toxic materials. During the last part of this decade there has been increasing concern for the health of the workers employed in the manufacture, distribution and use of pesticides as well as for those inadvertently exposed during cultivation or harvest or due to pesticide drift. If this trend continues, the extraordinarily low mammalian toxicity of juvenoids may provide an economic incentive

encouraging their development. A number of factors, certainly including resistance, can be anticipated to create potential markets for new pesticides. With increasing concern for compounds which are safe to use and which can be integrated into pest management programs, new juvenoids may find application.

Even assuming that the socioeconomic constraints are adequate for the development of a juvenoid, there are certain constraints on the compound itself. In addition to efficacy, these constraints are related to the selectivity, cost and persistence of the candidate insecticides. Since many of the advantages of juvenoids are also disadvantages, balances must be obtained. The selectivity of juvenoids is certainly an advantage, but high selectivity more so than other factors has probably limited their use in the field because the available markets are not large enough to bear the expense of registration. Only a very few individual insects, such as the boll weevil, could support the cost of developing a pesticide. On the other hand, a compound selective for the order Lepidoptera could represent an important pest management tool as well as a sound investment. The effectiveness of juvenoids indicates that they will have a high profit margin. Still, the development of compounds which are less expensive to produce than the earlier juvenoids is attractive.

In this chapter, aspects of the fate of juvenoids in the environment and in nontarget organisms have been examined. It is clear, for many reasons, that more stable juvenoids should be developed under conditions of use. The approach described, in part, by Hangartner *et al.* (1976) in which an estimate of environmental stability is incorporated quite early into efficacy studies, might be valuable in assessing new compounds. From the numerous studies on the degradation of juvenoids and other xenobiotics, certain functionalities are obviously too unstable to incorporate into a molecule designed for field use. A move toward making a compound stable enough to effect good insect control is not necessarily synonomous with the development of a persistent pesticide. For many applications, stability to light is a critical factor while hydrolytic stability is less important in determining efficacy under field conditions. The presence of one or more hydrolytically unstable moieties will lead to a compound that will not be overly persistent once it has weathered or entered a food chain. A fairly complex parameter to adjust is the stability in target vs nontarget organisms. Compounds highly refractory to metabolism are not socially acceptable due partly to their tendency to accumulate in a food chain, yet many target organisms are remarkably efficient at degrading xenobiotics. One approach is to develop compounds moderately resistant to rapid biological oxidation while still retaining some sites susceptible to hydrolysis. The juvenoid Ro 13-5223 discussed by GÜnthart and coworkers

in this volume may approach many of the attributes discussed here. The data presented in this volume clearly indicate the effectiveness of the compound, and it will be interesting to see if it will also prove to be economically profitable.

ACKNOWLEDGEMENTS

Dr. S. S. Gill critically reviewed the manuscript and J. J. Menn provided valuable suggestions. NIEHS Research Career Development Award K04 ES00046-02 provided salary support during the preparation of this chapter.

REFERENCES

1. A.M. Ajami and L.M. Riddiford, *J. Insect Physiol.* 19, 635-645 (1973).

2. W.S. Bowers, *Science* 164, 323-325 (1969).

3. S. Dorn, G. Oesterhelt, M. Suchý, K.H. Trautmann, and H.-K. Wipf, *J. Agr. Food Chem.* 24, 637-640 (1976).

4. G.P. Georghiou and T. Saito, Eds., *Pest Resistance to Pesticides: Challenges and Prospects*, (Plenum Press, New York, 1980), (in press).

5. S.S. Gill, B.D. Hammock, I. Yamamoto, and J.E. Casida, in *Insect Juvenile Hormones: Chemistry and Action*, J.J. Menn and M. Beroza, Eds. (Academic Press, New York, 1972), pp. 177-189.

6. S.S. Gill, B.D. Hammock, and J.E. Casida, *J. Agr. Food Chem.* 22, 386-395 (1974).

7. B.D. Hammock, S.S. Gill, and J.E. Casida, *Pestic. Biochem. Physiol.* 4, 393-406 (1974a).

8. B.D. Hammock, S.S. Gill, and J.E. Casida, *J. Agr. Food Chem.* 22, 379-385 (1974b).

9. B. Hammock, J. Nowock, W. Goodman, V. Stamoudis, and L.I. Gilbert, *Mol. Cell. Endocrinol.* 3, 167-184 (1975).

10. B.D. Hammock and G.B. Quistad, in *The Juvenile Hormones*, L.I. Gilbert, Ed. (Plenum Press, New York, 1976), pp. 374-393.

11. B.D. Hammock, S.M. Mumby, and P.W. Lee, *Pestic. Biochem. Physiol.* 7, 261-272 (1977).

12. B.D. Hammock and G.B. Quistad, in *Progress in Pesticide Biochemistry*, Vol. 1, D.H. Hutson and T.R. Roberts, Eds. (John Wiley & Sons, New York, 1980) (in press).

13. B.D. Hammock, S.S. Gill, S.M. Mumby, and K. Ota, in *Molecular Basis of Environmental Toxicity*, R.S. Bhatnagar, Ed. (Ann Arbor Science Publishers, Ann Arbor, MI, 1980a), pp. 229-272.

14. B.D. Hammock, M. El-Tantawy, S.S. Gill, L. Hasegawa, C.A. Mullin, and K. Ota, in *Microsomes, Drug Oxidations, and Chemical Carcinogenesis*, M.J. Coon, A.H. Conney, R.W. Estabrook, H.W. Gelboin, J.R. Gillette, and P.J. O'Brien, Eds. (Academic Press, New York, 1980b) (in press).

15. W.W. Hangartner, M. Suchý, H.-K. Wipf, and R.C. Zurflueh, *J. Agr. Food Chem.* 24, 169-175 (1976).

16. C.A. Henrick, G.B. Staal, and J.B. Siddall, *J. Agr. Food Chem.* 21, 354-359 (1973).

17. C.A. Henrick, W.E. Willy, D.R. McKean, E. Baggiolini, and J.B. Siddall, *J. Org. Chem.* 40, 8-14 (1975).

18. L.J. Hoffman, J.H. Ross, and J.J. Menn, *J. Agr. Food Chem.* 21, 156-163 (1973).

19. G.W. Ivie, *Science* 191, 959-961 (1976).

20. G.W. Ivie, J.E. Wright, and H.E. Smalley, *J. Agr. Food Chem.* 24, 222-227 (1976).

21. S.M. Mumby and B.D. Hammock, *J. Agr. Food Chem.* 27, 1223-1228 (1979).

22. F. Oesch, *Xenobiotica* 3, 305-340 (1973).

23. K. Ota and B.D. Hammock, *Science* 207, 1479-1481 (1980).

24. F.M. Pallos, J.J. Menn, P.E. Letchworth, and J.B. Miaullis, *Nature (London)* 232, 486 (1971).

25. G.B. Quistad, L.E. Staiger, and D.A. Schooley, *J. Agr. Food Chem.* 22, 582-589 (1974a).

26. G.B. Quistad, L.E. Staiger, and D.A. Schooley, *Life Sci.* 15, 1797-1804 (1974b).

27. G.B. Quistad, L.E. Staiger, and D.A. Schooley, *J. Agr. Food Chem.* 23, 299-303 (1975a).

28. G.B. Quistad, L.E. Staiger, and D.A. Schooley, *Pestic. Biochem. Physiol.* 5, 233-241 (1975b).

29. G.B. Quistad, L.E. Staiger, and D.A. Schooley, *J. Agr. Food Chem.* 24, 644-648 (1976).

30. D.A. Schooley and G.B. Quistad, in *Pesticide and Xenobiotic Metabolism in Aquatic Organisms*, M.A.Q. Khan, J.J. Lech, and J.J. Menn, Eds., *Amer. Chem. Soc.*, Washington DC, 1979, pp. 161-176.

31. M. Schwarz, R.W. Miller, J.E. Wright, W.F. Chamberlain, and D.E. Hopkins, *J. Econ. Entomol.* 67, 598-601 (1974).

32. J.B. Siddall, *Environ. Hlth. Perspec.* 14, 119-126 (1976).

33. M. Slade and C.H. Zibitt, in *Insect Juvenile Hormone: Chemistry and Action*, J.J. Menn and M. Beroza, Eds., (Academic Press, New York, 1972), pp. 155-176.

34. T.C. Sparks and B.D. Hammock, in *Pest Resistance to Pesticides: Challenges and Prospects*, G.P. Georghiou and T. Saito, Eds., (Plenum Press, New York, 1980) (in press).

35. C.M. Williams, *Sci. Am.* 217, 13-17 (1967).

36. S.J. Yu and L.C. Terriere, *Insect Biochem.* 8, 349-352 (1978a).

37. S.J. Yu and L.C. Terriere, *Pestic. Biochem. Physiol.* 9, 237-246 (1978b).

38. R.C. Zurflueh, in *The Juvenile Hormones*, L.I. Gilbert, Ed., (Plenum Press, New York, 1976), pp. 61-74.