2nd draft

ANALYSIS OF TOXICOLOGY STUDIES WITH LBAM AND RELATED LEPIDOPTERAN PHEROMONES

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Preamble

The light brown apple moth (LBAM) is an invasive pest with the capacity to damage an extremely wide range of important agricultural crops. Its control is therefore critical for economic reasons and to protect the food supply. One way to do this is to employ pheromones (airborne hormones) and their synthetic counterparts (semiochemicals) that act as sexual attractants to male moths. Some of these are quite specific for a single Lepidopteran (moths and butterflies) species and others work for several, closely related ones. Chemically they are classified as tetradecenyl (14 carbon-chain) acetates. By flooding an area with these pheromones it becomes impossible for the males to home in on females and complete the breeding cycle. The hoped-for result is a drastic reduction in the moth population. These agents are attractive due to their very low acute toxicity by comparison with conventional insecticides and their short biological half-life, the result of photo-oxidation and enzyme destruction. The mode of application, however, varies widely. There is little concern with the use of slow release baits placed in numerous locations within the target area, but the use of aerial spraying in urban centers raises legitimate concerns over the safety of humans within the area as well as concerns regarding the environmental impact.

Two commercial products are available for use. Check®Mate OLR-F is a pheromone attractant for a family of leaf-roller moths and to LBAM. Check®Mate LBAM is specific to the LBAM. The USDA Environmental Assessment document (2007) states that the latter will be used when available.

Analysis of available data

One of the major documents provided to me in relation to the aerial spraying of these pheromones is the U.S. Department of Agriculture report entitled "Treatment of Light Brown Apple Moth in the Seaside Area of California: Environmental Assessment, July, 2007." Three references were provided in this document purporting to provide toxicity data relevant to human exposures. Each of these will be examined in turn.

DOCUMENT A. OECD 2002. OECD Series on Pesticides. #12. Guidance for registration requirements for pheromones and other semiochemicals* used for arthropod pest control. 25pp (Environment Directorate, Organization for Economic Cooperation and Development.)

*N.B. Semiochemicals are synthetic pheromones chemically indistinguishable from their natural counterparts.

This document contains some interesting statements. In discussing the safety of these agents relative to non-target organisms, their volatility is cited as a factor contributing to their safety.

Page 12, bullet 4 states that "Individually placed dispensers generally give season-long control, while broadcast formulations are usually applied at lower rates more than once in a season."

Comment: This clearly indicates significant advantages to the bait/dispenser approach.

Page 13 bullet 1 states that "Semiochemicals are **generally assumed** to dissipate rapidly in the environment…"

Comment: This hardly seems a ringing testimony to their short biological life.

References to toxicity data appear first on Page 14. It begins with the statement (bullet 1) that "The US EPA, Canada's PMRA and the European Union's regulatory authorities have received no reports of adverse effects to human health or the environment associated with semiochemicals registered for use in mating disruption of arthropods and other applications. Most are SCLPs". (SCLP = semiochemical lepidopteran pheromone.)

Comment: No mention is made of the method of application used in these situations. It seems very unlikely that aerial spraying was used, else much more would have been made of the lack of adverse reactions in humans.

Page 14 bullet 2 reviews the acute mammalian toxicity studies and concludes that acute studies indicate low toxicity by the oral, dermal or inhalation routes, and no evidence of mutagenicity in the Ames Salmonella test. There was mild skin and eye irritation.

Comment: These conclusions are correct but acute toxicity studies conducted over a very short time span (hours or days) have little relevance to the potential for adverse reactions when repeated exposure take place over several months or years.

Page 14, bullet 4- Results of two sub-chronic studies in rats are cited. In one[1], rats were fed a high dose (up to 1 gm/kg) of "a commercial blend of branched acetates with an aliphatic chain length of between C_{10} to C_{14} for 90 days." The Other study[2] was a developmental study in which pregnant rats were exposed by inhalation to unbranched primary alcohols with chain length C_8 to C_{10}. No developmental defects were observed in the fetuses. The OCED document states, regarding the oral study[1], that "The results indicated that no significant signs of toxicity other than those expected with longer-term exposures to high dose of a hydrocarbon, namely, histopathologic evidence of nephropathy in males and increased liver and kidney weights in both sexes."

Comment: The only longer-than-acute inhalation study referred to in the OECD document is the one by Nelson *et al*[2]. It looks at the behavioral and developmental toxicity of a series of <u>industrial alcohols.</u> It was necessary to access the original paper to obtain more details of this report. These alcohols were administered by inhalation for 7hr/day on days 1-19 of gestation. This

short exposure period could hardly be taken as evidence of the safety to humans of multi-month/year exposures to aerosols. Moreover the extrapolation of results from a group of industrial alcohols, including methanol and ethanol, to insect pheromones involves a breathtaking leap of logic and self-deception. Further, the tridecenyl acetates used in the Daughtrey study[1] are not the tetradecenyl acetates of the pheromones in question.

THIS STUDY BY NELSON *ET AL.* APPEARS TO BE THE ONLY PURPORTED EVIDENCE OF THE SAFETY OF EXPOSURE TO THE PHEROMONE AEROSOL CAPSULES LISTED IN THIS OECD DOCUMENT. NEITHER THE CHEMICALS USED NOR THEIR PHYSICAL STATE (VAPORS) IS RELEVANT TO THE QUESTION OF LONG TERM, REPEATED EXPOSURES OF HUMANS TO PHEROMONES OR SEMIOCHEMICALS IN MICROCAPSULE FORM SPRAYED FROM AIRCRAFT. IT IS INTERESTING TO NOTE THAT THE TITLE OF THE PAPER (SEE 2 BELOW) WAS OMITTED FROM THE CITATION IN THE REFERENCES IN THE OECD DOCUMENT. THIS COULD HAVE BEEN AN OVERSIGHT, OR IT COULD INDICATE THAT THE AUTHORS OF THE OECD DOCUMENT DID NOT READ THE ORIGINAL ARTICLE, OR IT COULD HAVE BEEN LEFT OUT TO OBSCURE ITS LACK OF RELEVANCE.

1. Daughtrey WC, Smith JH, Hinz JP, Biles RW. Subchronic toxicity evaluation of tridecenyl acetate in rats. Fundam Appl Toxicol 14(1): 104-112, 1990.

2. Nelson, BK, Brightwell WS, Krieg EF Jr. Developmental toxicity of industrial alcohols: a summary of 13 alcohols administered by inhalation to rats. Toxicol Indust Health 6(3-4): 373-387, 1990.

DOCUMENT B. U.S. EPA Office of Prevention: Pesticides and Toxic Substances. Reregistration Eligibility Decision, Tridecenyl Acetates. EPA 738-R-96-021. 1996

The toxicity data referred to in this document relate entirely to the use of the sexual attractant pheromones tridecenyl (not tetradecenyl) acetates to disrupt the mating behavior of tomato pinworms. It refers to "technical grade active ingredients or TGAIs" (the pheromones) that may be encapsulated in beads, embedded in polymeric fibers or contained in solid polymeric matrix dispensers. It notes that the encapsulated beads or fiber embedded TGAIs can be applied as sprays although no mention of aerial spray application is made. Mention is made to the low acute human toxicity and short half-life but no references or specific data are provided.

Comment: None of this information is especially relevant to the aerial application used in the Monterey situation. Under the Environmental Hazard paragraph however (page 4), the following statement appears.

"The following environmental hazard statement must appear on the labeling of all products containing tridecenyl acetates: 'Do not apply directly to water, or to areas where surface water is present or to intertidal areas below the high water mark. Do not contaminate water when disposing of equipment wash water or rinsate'. This statement no doubt relates to the demonstrated toxicity to aquatic and marine species of these agents (see below).

Given the geographic nature of the Monterey Peninsula the aerial application would appear to constitute a significant environmental risk.

DOCUMENT C: Weatherston I, Stewart R. Regulatory issues in the commercial development of pheromones and other semiochemicals . Use of pheromones and other semiochemicals in integrated production. IOBS* wprs Bulletin 25: 1-10, 2002.

*This should be IOBC, the International Organization for Biological and Integrated Control of Noxious Animals and Plants. West palaearctic regional section.

This document reviews a number of factors relating to biological pest control agents. In comparison to the United States, the European Community has approved far fewer agents and Finland, Sweden, Denmark, Ireland, Belgium and the United Kingdom have not approved any pheromone/attractants (as of 2002). The authors attribute the much lower approval rate to "…the alarming trend of almost universal adoption of the Precautionary Principle in Europe in regard to the use of any new technology, process, chemical or any new technology whatsoever." (page 9, para 3).

Note that the authors do not define "area-wide", which could refer to either aerial or ground spraying. The authors review acute toxicity data for some avian species and fish (bluegills, rainbow trout) as well as the common water flea *Daphnia magna*. They note the susceptibility of aquatic species.

Comment: Once again, this toxicity review seems to consist of hopeful statements not supported by any hard evidence. It contains statements such as "In the U.S. the regulatory agency believes that with the majority of pheromones there is no evidence of risk when the use does not exceed 150 gm of active ingredient per acre per year." And "while area-wide use of semiochemical products would generally lead to a greater exposure than use in traps, such exposure is likely to be insignificant." (page 5, last para). The authors further conclude **"the burden of demonstrating that use is safe or will not cause unreasonable effects on health and the environment should be**

significantly less than for conventional chemical insecticides."(page 6, last para)

Toxicity studies do not normally conclude that a substance is believed to be likely safe. I cannot imagine any pharmaceutical product being approved on such a basis, and it hardly seems a compelling reason to allow less rigorous safety testing. One wonders whether trained pharmacologists or toxicologists were involved in the toxicity testing.

Other Toxicology Studies Taken from the Scientific Literature

1. Beroza M, Inscoe MN, Schwartz PH Jr, Keplinger ML, Mastri CW. Acute toxicity studies with insect attractants. Toxicol Appl Pharmacol 31: 421-429, 1975.

This is one of the earliest toxicology studies in this field. The authors examined the acute toxicity of a number of insect attractants including one for the gypsy moth. The authors generally found low acute toxicity for the agents tested but noted the higher sensitivity of rainbow trout and bluegills.

2. Abdel-Hgani SB, Martinez-Lopez E, Perez-Perttejo Y, et al. Cytotoxicity and mutagenicity of four insect pheromones in CHO-K1 cells. Bull Environ Contam Toxicol 73: 963-970, 2004.

The authors studied the *in vitro* toxicity of four lepidopteran pheromones using several strains of *Salmonella typhimurium* (as in the Ames test) and the mammalian cell culture type CHO-K1. The authors found no evidence of mutagenicity in the Salmonella test but did find significant cytotoxicity in the cell culture test. They proposed that the safety of these agents was dependent in large part on their high protein-binding propensity. The authors refer to a previous study from their laboratory that reported similar cytotoxicity of other pheromones. The lack of mutagenicity has been reported by several laboratories.

3. Rosa E, Barata C, Damasio J, Bosch MP, Guerrero A. Aquatic ecotoxicity of a pheromonal antagonist in Daphnia magna and Desmodesmus subspicatus. Aquat Toxicol 79(3): 296-303, 2006.

Moderate toxicity was observed for this pheromone on these aquatic organisms and the authors express concern for their effects in the aquatic environment.

Comment: The cytotoxicity (cell poison) effect observed with several pheromones is of concern. If the authors are correct in surmising that protein binding is an important protective mechanism, given their absorbability through the skin, vulnerable populations with pre-existing

medical conditions could be at increased risk.

One additional document was obtained from the internet by this reviewer. This is the U.S. EPA document "Lepidopteran Pheromones: Tolerance Exemption". (Federal Register: Aug. 30, 1995, Vol. 60, # 168). This document "..establishes an exemption from the requirement of a food tolerance for residues of certain Lepidopteran pheromones resulting from the use of these substances independent of formulation, mode of application or physical form or shape with an annual application limit of 150 gm per acre for pest control in or on all raw agricultural commodities" (page 1). While this statement would appear to include aerial spraying in the exemption, a subsequent statement in the document contradicts this. On page 3, para 3, it is stated that "For pheromone products, especially those directly applied to food, one problem has been a lack of subchronic toxicity studies and an estimate of the actual pheromone residues occurring with use. Some pheromone uses in solid matrix dispensers have been registered based on the low probability of exposure justifying the waiver of the subacute toxicity studies, namely the 90 day-feeding, the developmental toxicity and immunotoxicity studies. **However, the Agency has held that sprayable formulations or other modes of application that may increase the likelihood of human exposure would still require the subchronic toxicology studies."**

This reviewer has found no evidence that such studies, appropriate to aerial spray application, have been conducted.

The waters are further muddied in the following paragraph (II. Human Health, Page 3). To quote, "Data has been submitted to date on compounds *similar in structure* (my italics) to the Lepidopteran pheromones and published in the peer reviewed, public literature. The information submitted covered compounds that were from six to sixteen carbon, unbranched alcohols acetates and aldehydes. Since the Agency is basing this tolerance exemption on chemical structure, it is relevant to consider the available subchronic toxicity for this group. The results given in these literature reports indicate that there is no significant acute toxicity associated with the primary alcohols, acetates or aldehydes mentioned."

Although no references are provided, this quotation would appear to refer to the published papers by Doughtrey *et al.* and by Nelson *et al.* discussed above. To reiterate, the results of toxicity studies on part of a chemical structure cannot be extrapolated to the whole chemical structure, and oral or vapor modes of administration are not representative of microcapsules in aerosol application.

GENERAL CONCLUSIONS

Upon reviewing several government documents and a number of independently publisher papers, It became apparent to this reviewer that no chronic toxicity study has been conducted in a mammalian species by any route of administration and certainly not involving exposures to the product to be employed by the method of application (aerosol spray of microcapsules). Claims of safety are based on extrapolation from acute toxicity studies and one sub-acute, 90-day study that employed the oral route of administration. These are no guarantee that longer-term, repeated exposures of humans are without risk. A chronic toxicity study of at least 90 days and preferably six months duration, employing daily exposure to aerosol of the product in question (Check®Mate) at a high exposure level is required. It is customary in such studies to use a much higher exposure level in order not to miss adverse reactions that might occur too infrequently to be detected at lower exposures. Using the intact product addresses questions of the safety of so-called "inert" ingredients, the chemical nature of which is not available to the public.

In none of the documents discussed above, including the USDA environmental assessment, is there any mention of previous experience with aerial spraying of populated, urban areas. If one wishes to convince the populace of the safety of such a practice, it would seem obvious that presentation of past experiences with it, documenting a lack of adverse reactions in the exposed population, would be key evidence. Previous efforts to control LBAM in the treatment area employed ground application techniques. Pheromone baited traps were placed throughout the State of California to monitor the moth population and distribution. Isolated populations in Napa and Oakley were treated using ground equipment with *Bacillus thuringiensis kurstaki* (Btk) (USDA Environmental Assessment, 2007, p2, para 2). This is a bacterial product that attacks the early larval stages of most lepidopterans.

There is ample evidence that many pheromones and semiochemicals possess significant toxicity for aquatic species. This suggests that aerial spraying carries an increased environmental risk given the difficulty in confining the spray to the target area.

In summary, the USDA and EPA documents are filled with contradictory statements regarding the toxicity testing of pheromones, inappropriate extrapolations from irrelevant toxicity studies, and are suggestive of a poor understanding of basic pharmacological and toxicological principles.

RECOMMENDATIONS

Since the decision to use aerial spraying as the method of application appears to have been made entirely on economic grounds, the decision should be revisited given the lack of adequate evidence for its safety in the long term. Either

ground-based methods of application should be employed or an adequate chronic toxicity study should be conducted. Ground-based technology has the added advantage of posing less risk to the environment.