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[SAP Report No. 99-04B]
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SAP Report No. 99-04B, September 16, 1999

REPORT:

**FIFRA Scientific Advisory Panel Meeting,
July 21, 1999, held at the Sheraton Crystal City Hotel,
Arlington, Virginia**

*Session II - A Set of Scientific Issues Being Considered by
the Environmental Protection Agency Regarding:*

**A Consultation on Protocol Design to Assess Acute
Neurotoxicity Studies following Oral Administration
of Pesticides**

Mr. Paul I. Lewis,
Designated Federal Official
FIFRA/Scientific Advisory Panel
Date: _____

Ronald J. Kendall, Ph.D
Chair
FIFRA/Scientific Advisory Panel
Date: _____

Federal Insecticide, Fungicide, and Rodenticide Act

**Scientific Advisory Panel Meeting
July 21, 1999**

**SESSION II - A Consultation on Protocol Design to Assess Acute Neurotoxicity Studies
following Oral Administration of Pesticides**

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INTRODUCTION

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Scientific Advisory Panel (SAP) has completed its review of the set of scientific issues being considered by the Agency regarding a consultation on protocol design to assess acute neurotoxicity studies following oral administration of pesticides. Advance notice of the meeting was published in the *Federal Register* on July 6, 1999. The review was conducted in an open Panel meeting held in Arlington, Virginia, on July 21, 1999. The meeting was chaired by Ronald J. Kendall, Ph.D, Professor and Director, The Institute of Environmental and Human Health, Texas Tech University/Texas Tech University Health Sciences Center, Lubbock, TX.

Recently, several acute neurotoxicity studies have been submitted to the Agency employing this protocol design. This novel design deviates from the standard Agency acute developmental neurotoxicity protocols. The primary difference is that the test substance is administered in the diet compared to being administered as a bolus dose in the standard Agency study design. The purpose of this session is to consult with the SAP regarding issues pertaining to this new design. Kathleen Raffaele, Ph.D (Office of Pesticide Programs, EPA) made the Agency's presentation for this session.

CHARGE

The specific issues to be addressed by the Panel are keyed to the background document, *A Consultation on Protocol Design to Assess Acute Neurotoxicity Following Oral Administration of Pesticides*, dated June 30, 1999 and are presented as follows:

Issue #1 - Variability of Total Test Substance Intake Among Animals.

In a gavage study, the desired dose is administered directly to each individual animal. In contrast, in a dietary study the test substance is mixed with the diet, and the dose to an individual animal varies with the amount of food consumed.

A. How much variability in dose/consumption is acceptable within a single dose group (e.g., $\pm 10\%$ of the mean)? If animals are included or excluded from analysis based on actual intake, does this bias the study results?

B. Is it possible to design an acute dietary study which ensures consumption of a uniform dose within each dose group? If so, please give examples of such designs.

Issue #2 - Variability of Test Substance Intake Patterns among Animals and Effects on Kinetic Parameters and Estimation of Time of Peak Effects.

In a gavage study, time and rate of administration are uniform for all animals. In an acute neurotoxicity study, effects are measured at the "time of peak effects," determined with reference to time of administration. In the submitted protocols, a test substance is consumed in the diet

over a one hour period. Variability in consumption patterns during that one hour period is unknown.

A. Does the Panel agree that mixing the test substance in the diet could change the pharmacokinetics of the substance? If so, how might this be assessed?

B. What impact might the temporal pattern of consumption (all at the beginning or the end of the interval, or steady eating throughout the interval) have on the magnitude and time course of the effects? Over what span of time of acute effects might this be a major factor (e.g., if peak effect occurred 6 hours after exposure, would the temporal pattern of consumption be less likely to influence results)?

C. How might these variations influence the estimation of the time of peak effect?

D. Could any such problems be resolved by alterations in protocol design? If so, please suggest appropriate modifications.

Issue #3 - Effects of Restricted Feeding Schedules on Data Interpretation

Normal feeding patterns vary with age and across strains of rats. In addition, altered feeding schedules are known to affect the rat's circadian rhythm, homeostatic indicators, and behavior patterns. In order to achieve acute dietary intake (over one hour period), rats in the submitted protocols were acclimatized to restricted feeding schedules, consisting of two one-hour feeding periods each day (at the beginning and end of the dark cycle). In one study, the light/dark cycle was changed to 10/14 h light/dark, in order to accommodate the altered feeding schedule. Again, in one study, ad libitum feeding was resumed at varying periods following test substance administration.

A. Could these changes in feeding schedules and light/dark cycles differentially alter the study findings in the treated animals? What effect would changing these variables during the study period have on study results?

B. What influence would variations in normal feeding patterns (e.g., due to strain, sex, or age) have on the results of studies using restricted feeding paradigms?

Issue #4 - Appropriateness of the Model.

How well does a one hour dietary exposure period model human eating patterns?

PANEL RECOMMENDATION

The general consensus of the panel was that the protocol submitted for review was inappropriate for acute dietary exposure toxicity testing and that the variability of the data made the results invalid. While there were differences of opinion as to the relative merit of developing a feeding model versus gavage dosing for acute toxicity studies, it was agreed that the present level of scientific knowledge of ingestive behaviors and related physiological factors allows for the development of such a protocol. While the gavage route is established and is used in adult toxicity and developmental toxicity studies, one may be able to move to a dietary approach with caution. This may result in the construct of an experimental protocol that would approach the acute test in a dietary protocol but would require considerable validation of use within a toxicological framework.

DETAILED RESPONSE TO THE CHARGE

Issue #1 - Variability of Total Test Substance Intake Among Animals.

In a gavage study, the desired dose is administered directly to each individual animal. In contrast, in a dietary study the test substance is mixed with the diet, and the dose to an individual animal varies with the amount of food consumed.

A. How much variability in dose/consumption is acceptable within a single dose group (e.g., $\pm 10\%$ of the mean)? If animals are included or excluded from analysis based on actual intake, does this bias the study results?

The amount of variability within any single dose group should be minimal and all efforts should be made to ensure accuracy of delivered dose. Any determination of what might be acceptable within any single dose group depends upon a number of factors, one of which is the lowest level of variability possible for food consumption in the control animals. Finding this level would help to identify the inherent variability in food consumption and assist in making conclusions about the utility of the approach for dosing and determining toxicity. Whether or not the variability would result in the lack of discrete dose groups, when a total consumed dose was calculated, should be examined. The shape of the dose response curve could significantly affect this decision -- if the chemical shows a steep dose response curve, then the span between doses would be a critical factor that could easily be compromised. The variability in the observed effect and its association with dose will also play a role in determining what level of variability is acceptable. Thus, all of these steps will need to be determined on a chemical by chemical basis and any blanket level of acceptable variability should be conservative.

Another way to address this issue is to consider the statistical power (probability of a detecting a true effect) for a given exposure situation and a given expected difference between

control and treated groups. The question (1A) is stated as though the variance around the target response is likely to be both upward and downward; this is true of the mean response, but not the target which is the upper bound of the possible exposure. The variability in feeding can only lower the exposure, not increase it. This is clearly going to result in a drop in statistical power which could be large. Examples of these types of calculations can be found in the literature. The reduction in power could be very small or could be large (e.g., 50% depending upon the variation in the exposure), but it is not likely to ever be zero. These reductions in power can be offset by using larger numbers of animals in the analysis; this issue can be addressed only on a case-by-case basis. In general, one could calculate the power of the current approach when dose is fixed (as in the gavage model) and look at the impact of variation in dose on the power, adding animals to maintain the power of the original assay.

The feeding studies could create some problems in data analysis. It is possible that animals in the higher exposure groups will eat less of the food than the lower exposure groups because of palatability. In a chronic assay, this issue becomes less critical since, in most cases, the animals eventually will adjust to the diet. But in a single feeding dose experiment, the animal may choose not to eat at all for this one feeding. It is possible to have high-dose animals receiving less chemical than the low-dose animals. Grouping of responses by amount ingested becomes necessary and dose-response trends may become less informative.

If a gavage experiment has been done prior to the feeding study, then it is advised to target one of the feeding doses to the gavage dose to assess the possibility of variability in feed consumption affecting the power of the statistical methods used in the analysis.

The dynamics of ingestion need to be taken into consideration. For example, when the gavage method of dose delivery is used, the administered dose is controlled but the internal dose may be more variable. Fluids injected directly into the stomach via gavage distribute extremely rapidly into the intestine within minutes of its administration. In comparison, when a comparable amount of substance is ingested orally, the vast majority of the dose is confined to the stomach within a comparable amount of time. Concern was raised that a dose administered via gavage will be absorbed much more rapidly than what would occur in the normal exposure and may produce a distorted pharmacodynamic profile. This concern was raised both for the consideration of using oral dosing and with the relevance of comparing toxicity data from gavage versus ingestion route of exposure in acute or short term exposure studies.

If animals are included or excluded from analysis based on actual intake, does this bias the study results?

There was a consensus among the Panel members that animals not be excluded from the study based on actual intake. Dietary intake is variable by nature; investigators studying drug or chemical effects on feeding behavior often encounter this problem. It is very important that this potential source of selection bias be eliminated or minimized in the protocol. It may well be that the animals most sensitive to the chemical are the ones that consume less. With large individual

animal differences in consumption, mean or median data collected from such groups would be too variable to be interpretable. If animals were excluded from groups because of “too much” variability in consumption, there would definitely be the opportunity to bias the results unless this was done carefully and objectively. It would be difficult to design a strategy for appropriate incorporation or removal of a particular animal from the group. One possibility would be to “normalize” the individual animals based on calculated consumption instead of combining them into groups. This has statistical concerns but it has been argued that multiple, individual observations can be more powerful for dose-response analysis than evaluation of fewer dosing groups with multiple replicates.

A specific outcome with regard to the behavioral data could be the elimination of more sensitive or emotional animals and the generation of an unacceptable bias with regard to open-field behavior. The animals that refuse to eat or eat an amount far lower than normal could very well be the most sensitive or neophobic animals. These are the animals that would be expected to have the most emotional responses in the open field.

B. Is it possible to design an acute dietary study which ensures consumption of a uniform dose within each dose group? If so, please give examples of such designs?

While gavage is certainly the most accurate and uniform procedure for oral dosing of animals, it is still impossible to ensure uniform dosing; this is especially true when the animals self-administer the chemical as in a feeding study. However, it is possible to markedly improve the uniformity of dosing from that presented by the Agency. A number of techniques were presented for consideration. These procedures may be more complicated to develop and more costly to implement than gavage. The choice of procedure would depend on the type of diet, test agent, and test animal (strain, species, etc.) used in the study.

It may be possible to train the animals to eat their allocation of food within a shorter time period each day, e.g., 30 minutes. However, caution must be taken in that dry food can impact the stomach, adjust chemical and nutrient absorption, and alter normal hormonal cycles. Often, rats are fasted prior to dosing in oral toxicity studies. It might be possible to remove or temporarily alter the earlier feeding opportunity on the day of dosing to ensure that the animal is highly motivated to obtain the food rapidly, without undue stress. While the level of food restriction should not exceed 20%, a 10% reduction in ad lib food intake of rats is probably more representative of the natural situation. With a 10% food restriction level, one would observe that animals would adjust relatively quickly, there would be an increase in the uniformity between animals, and there would be no expected changes in spontaneous behavior.

One simple method is to use a low level of dietary restriction (DR) such as a 10%-20% reduction in food consumption. DR animals usually eat their food as soon as it is presented when given as meals of different quantities during the active (dark) phase. By this procedure all of the test agent may be eaten in a short period. To accomplish this, a small increment of food (33% or less) could be given at the time of dosing at the end of the dark period. This will ensure that the

animals are hungry and eat all of their food at the beginning of the feeding interval. The largest increment of food (67% or more) could be given at the beginning of the dark cycle. The duration of the late dark feeding interval would be increased or decreased to promote the rapid and timely consumption of the test agent. Total food consumption would be controlled by altering the time period for the second feeding interval, (early dark phase). In all cases, high levels of food restriction (<20%) should be avoided.

A double feeder design could be used. Food treated with test-agent could be placed in one feeder, and food without agent could be placed in a second feeder. Access to both of the adjacent feeders would be blocked until the onset of the two feeding intervals. At the start of the feeding interval, the feeder with the agent and a small amount of food would be opened, thereby ensuring that all of the agent is eaten immediately. Then after all of the food is consumed in the agent feeder, the second feeder would be opened to supply the rest of the food for that interval. Simple photocells or strain gages would automatically sense when the agent feeder was empty. This event would then open the second feeder. Rats would rapidly adapt to this regimen during the acclimation period.

Variability among test subjects and groups can be further reduced by the following procedure. The concentration of agent in the diet could be increased or decreased based on the amount of food consumed by each individual rat. This would ensure that all rats in a group get the same dose. The concentration of test agent per gram of food would be determined by food consumption measurements during the acclimation period before dosing.

Proper food rationing can also be accomplished in a single feeder design. This can be done by placing food treated with test agent at the bottom of the feeder or at the location that is most accessible to the rat. This would ensure that the ration with the agent would be eaten first in a relatively short period of time. Diet without agent would be placed on top of the treated food (in feeders with food access at the bottom) so that the rat gets the full compliment of food after the test agent has been eaten.

Testing should be done during the active (dark) phase of the circadian rhythm and could be accomplished by reversing the animal room light/dark cycle. This would not require that the animals be tested in the dark or under red light, they could be moved into the light for testing. This type of procedure would be more similar to human exposure conditions.

Intragastric co-administration is a method that could potentially produce accurate dosing in conjunction with relatively natural intake would be to simply inject the substance via an pre-implanted indwelling gastric catheter. The injection could be made directly into the stomach at a precisely defined point into the ingestion of a naturally occurring meal. Rats, with a good deal of regularity, naturally ingest a meal very soon after lights off, at the beginning of the dark phase. One minute after the initiation of the meal, the substance could be pumped into the stomach at a rate that would complete dosing before the natural end of the meal. However, it was noted that this may be impractical considering the cost and time required for a large number of animals.

Rats tend to eat most rapidly at the beginning of a meal and become more variable later in the meal. If this tendency were taken advantage of, a more precise dosing could be applied via ingestion. In particular, if the amount of food that is scheduled for consumption at the meal was broken into thirds (and the animal not allowed access to the next third until the first "course" is completely ingested), then dosing could be accomplished in the first third. This could markedly reduce the interanimal variability in amount of dose consumed and time to fully administer the dose.

The Agency reported that some animals refused the food due to changes in palatability. This could be due to palatability or neophobia. In either case, it can be minimized by routinely mixing in the daily diet a strong highly palatable flavorant to better obscure (mask) the new and potentially unpalatable substance. There may still be difficulties with the higher dose levels mixed in the feed.

The final decision on light/dark cycles will depend on both scientific and practical considerations as on scientific basis. The ideal method would have the animals consume the feed containing the chemical consistently in as short a time as possible. However, there is also the practical consideration of making sure that the time of exposure coincides with the time that the parameters of interest are efficiently obtained and are not confounded to any significant degree by protocol changes. One suggestion was that the light/dark schedule could be "reversed" so that the animals were exposed to chemical-laced feed at the start of the laboratory work day. This would be a time that would coincide with the start of the "dark" or active cycle for the animals in which they consume the majority of their food for the day. As mentioned above, a minor food restriction could be employed during the light cycle to maximize the animal's "appetite". Such an exposure would be compatible with the normal working hours of the scientists and technicians and would allow for data to be collected in a practical manner.

As with any toxicity test, the final design of a "testing" protocol is a product of three principles and often represents a compromise of the three. First and foremost, the question being asked should be critical in the design of the "test". If the design does not answer the scientific question in a way that provides meaningful and necessary data, then it is of limited practical value. The second principle concerns the concept of route of exposure. Ideally, the route of exposure should mimic the route of human exposure. In this case, for many reasons as noted in other sections of this document, exposure in the animals' food is superior to delivery by oral gavage. However, if confounders exist that make this route of exposure questionable in the animal model, the route of exposure to mimic human exposure needs to be considered within the framework of generating data that can be interpreted in light of public health concerns. Simply mimicking the human route may not be the appropriate approach to take in all animal studies. Third, all scientific variables need to be taken into consideration when constructing a protocol to test a chemical for its potential toxicity. An important aspect of any protocol design is to allow for the collection of useful and valid data in an efficient and practical manner. If a protocol is so complicated that it is impractical for general testing, it is of little use in satisfying the needs of the Agency. In regard to the use of "restricted feeding" as a motivational factor to elicit food

consumption in a short period of time, the final method of accomplishing this will require careful consideration of the advantages and disadvantages. No method will be "ideal" in the classic sense but will be dictated by various considerations. Any method under serious consideration will require validation as with any other testing protocol.

Issue #2 - Variability of Test Substance Intake Patterns among Animals and Effects on Kinetic Parameters and Estimation of Time of Peak Effects.

In a gavage study, time and rate of administration are uniform for all animals. In an acute neurotoxicity study, effects are measured at the "time of peak effects," determined with reference to time of administration. In the submitted protocols, test substance is consumed in the diet over a one hour period. Variability in consumption patterns during that one hour period is unknown.

A. Does the Panel agree that mixing the test substance in the diet could change the pharmacokinetics of the substance? If so, how might this be assessed?

There is no doubt that the kinetics of absorption, distribution, elimination, and biotransformation could be different if a chemical is given as a bolus dose compared to when it is mixed with feed and consumed over time. The amount absorbed is dependent on the amount consumed. In addition, the presence of the food in dietary exposure could alter absorption compared to when it is given by gavage in a vehicle, e.g., corn oil. The absorption rate would be affected by the nature and composition of the ingested diet thus, it is important to standardize the diet, especially in its macronutrient composition.

With gavage exposure, one would expect a peak of chemical in the blood after absorption at a certain time, followed by a decay phase. However, since dosing by ingestion produces a different pattern of stomach emptying, the absorption of the substance into the bloodstream would in turn be quite different. The time over which the animal receives the dose is critical -- for example, a 6 hour delivery of the chemical is different than a 1 hour delivery. With dietary exposure over a relatively long time period (60 minutes), there could be multiple times of peak blood levels, occurring at different times in different animals because of different rates of consumption. A rapidly consumed, high dose would produce earlier toxicity. For example, an anticholinesterase organophosphate would inhibit butyrylcholinesterase in blood which would, in turn, reduce its circulating dose and that which reach synapses within the nervous system.

Depending on the kinetic characteristics of biotransformation enzymes, differences in metabolic handling of the chemical might also be prominent between these two methods of exposure. Again, substantial variability in the intake of the chemical among animals would be expected to contribute to variability among these kinetic processes. Also, because of the existence in some cases of "spare" target sites, a lower dose may not produce an adverse effect until a certain amount of target is affected. Many of these possibilities could be studied directly by measuring blood, plasma or urinary levels of the chemical or a metabolite at various times and

comparison with such measurements after gavage exposure.

B. What impact might the temporal pattern of consumption (all at the beginning or the end of the interval, or steady eating throughout the interval) have on the magnitude and time course of the effects? Over what span of time of acute effects might this be a major factor (e.g., if peak effect occurs 6 hours after exposure, would the temporal pattern of consumption be less likely to influence results?)

The temporal pattern of food consumption could have a major impact on the magnitude of the effects and the time course of the effects dependent upon the chemical under study. In previous experiments, the restriction of feeding to a short period of time (3-4 hrs.) has been used to induce caloric restriction and weight loss. Therefore, nutritional parameters that modulate toxicity, such as temporal patterns of food consumption, must be carefully controlled. The consumption of the agent-treated food must be confined to as short an interval as possible (15-30 min.).

The stomach, in natural ingestion situations, acts as a reservoir with only a fraction of the ingestate emptying every minute. A naturally ingested meal may take several hours to fully empty from the stomach. The rat stomach is rarely empty and a newly ingested meal would not be filling an empty stomach, but supplementing the undigested foods remaining in the stomach from the prior meal. To look at this issue, a model of rat stomach emptying (de Castro, 1981) was presented to look at a hypothetical intake situation. One half of an average intake for a rat (~50 Kcal) was assumed to be ingested either in the first 10 min of the 1-hr feeding period, uniformly over the 1-hr period, or during the last 10 min of the 1-hr feeding period. Under an empty stomach condition, the 50% emptying time was 150, 167, 200 min for the first 10 min, uniform, and last 10 min ingestion model, respectively. Using the 175-min midpoint, the amount the model indicated that had emptied from the stomach was 28.5, 26.2, and 21.4 Kcal, respectively. This analysis suggests that the difference in the time to 50% empty, and also the amount emptied at a constant time since the initiation of the 1-hr interval from the early and late ingestion models, can be as much as 33%.

Looking at the data produced by the 10-Kcal beginning stomach content model, the time to empty the original 10 KCal and 50% of the new ingestate was 196, 210, 228 min for the first 10 min, uniform, and last 10 min ingestion model, respectively. Using the 212-min midpoint, the amount the model indicated that had emptied from the stomach was 27.3, 25.4, and 22.7 Kcal, respectively. This analysis suggests with the 10-Kcal stomach condition that the difference in the time to 50% empty and also the amount emptied at a constant time since the initiation of the 1-hr interval from the early and late ingestion models ranges from 16% to 20%. This is a substantially reduced error in comparison to the stomach empty model.

This simulation suggests that ensuring that the target substance is ingested at a reasonably reproducible time is important. It also suggests that the degree of importance decreases when the animal has food remaining in the stomach at the time of the test meal ingestion. The temporal

pattern of feeding would have the greatest effect on agents that have a short response time (peak within 30 min. after dosing). Rapid consumption of the test compound, either at the beginning or the end of the feeding period, would most likely lead to a higher peak blood level compared to slow, steady consumption throughout the entire period of food access. Given these possible eating patterns, the time to peak effect would be problematic with rapid onset toxicants. For example, with a chemical that produced peak effects within 30 minutes of exposure, these changes might not be noted if some animals consume their dietary exposure rapidly at the beginning of the eating period. With “slow consumers” or those eating throughout the time interval, the relative time to peak effect would potentially be less of a concern than differences in the magnitude of peak effects. If the time and duration of feeding and the time of peak response are not closely synchronized among the test animals, the peak effects could easily be missed. If animals eat at a constant rate during the dosing interval, the magnitude of the effect may be reduced. Also, the duration of the effect might be increased and the time course of the effects may be delayed in these animals.

The temporal pattern of feeding would have a smaller effect on agents that have a long response time (6 hours or more). Therefore, nutritional parameters may be less critical. However, these temporal effects may still be significant. The amplitude and duration of the response is highly dependent on the type of agent being used.

Changes in the temporal patterns of food consumption may change the relative amount of food in the gastrointestinal tract at critical times when the agent is being absorbed, when the agent is having its maximum effect, and/or when the drug is being metabolized. These factors may significantly alter the amplitude, time to onset, and the duration of the drug effect.

Under conditions where access to food is limited, the presentation of food becomes the dominant timing mechanism that controls the circadian rhythms for many behavioral, physiological, biochemical, and neurological parameters. The photoperiod cycle is no longer the dominant environmental synchronizer. When animals are placed on scheduled meals they develop an “expectancy” for each meal during which metabolic features are altered. For example, insulin levels increase 30 minutes prior to a scheduled meal. Other biological factors that can be related to circadian rhythm, such as hormonal or melatonin levels, need to be taken into consideration as they may alter the neurotoxicity of any specific chemical. Therefore, the shifting of feeding patterns away from normal regimens may have a significant effect on neurotoxicity and behavioral testing, as well as the pharmacokinetics and pharmacodynamics of drugs and chemicals.

C. How might these variations influence the estimation of the time of peak effect?

As is clear from the simulation presented above, the time to peak could be influenced considerably. If some animals eat primarily at the beginning of the eating period while others eat primarily at the end of the period, this might alter the time to peak effect slightly. A problem will arise if the restricted feeding has more than one distinct feeding period which result in two peaks of exposure and changes in the physiological effects of the animal e.g., body temperature.

The buffering and relatively slow emptying from the stomach would result in a flattening of the absorption curve. In general, however, differences due to eating rapidly (regardless of when) or slowly would probably contribute more to variability in peak effects and their timing. The degree of influence is dependent upon the chemical under study and its target effect. The onset and duration of effect is dependent upon the mechanism of action of the chemical. Several chemicals to be tested, Type II pyrethroids, are degraded by digestive enzymes and modifying the route of exposure to feed may change the dynamics of the exposure.

D. Could any such problems be resolved by alterations in protocol design? If so, please suggest appropriate modifications.

As noted above, it might be possible to alter the feeding availability on the day of testing to ensure high motivation for eating. If such changes could make animals consume their dose rapidly (e.g., within 30 minutes), this might make more uniform rates of consumption and time to peak effects.

Issue #3 - Effects of Restricted Feeding Schedules on Data Interpretation

Normal feeding patterns vary with age and across strains of rats. In addition, altered feeding schedules are known to affect the rat's circadian rhythm, homeostatic indicators and behavior patterns. In order to achieve acute dietary intake (over one hour period), rats in the submitted protocols were acclimatized to restricted feeding schedules, consisting of two one-hour feeding periods each day (at the beginning and end of the dark cycle). In one study, the light/dark cycle was changed to 10/14 h light/dark, in order to accommodate the altered feeding schedule. Again, in one study, ad libitum feeding was resumed at varying periods following test substance administration.

A. Could these changes in feeding schedules and light/dark cycles differentially alter the study findings in the treated animals? What effect would changing these variables during the study period have on study results?

Some of these exogenous factors are known to modify physiological functions, basal metabolism, and response to toxicants. For example, the light cycle can have a variety of influences on response to chemicals. Any modifications in a normal schedule require an acclimation period for the animal. Given that such acclimation can be stressful on the animal and that toxicity of a chemical could be modified, it is strongly advised that such variables should not be changed during the study period.

Any alteration in the normal light/dark cycle of the animals could shift the toxicity response. For example, differences in the length of the dark cycle may mimic seasonal changes and influence various growth parameters. A change in the length of the light cycle could alter melatonin levels.

Since the treated and control animals are affected equally by the alteration in feeding schedule, the internal validity of the findings would not be affected. However, the study's external validity is affected. When an animal ingests food after a 12-hour fast, it has a completely empty stomach. This is a very unusual circumstance for the rat. Under these conditions, the rat is forced to eat an abnormally large amount (gorge) in order to ingest sufficient nutrients to maintain body weight. This can produce an abnormal level of stomach distension. Hence, the 2 feedings per day schedule is not a good replica of natural eating.

On the other hand, in the human environment, intake is not completely ad libitum. It is scheduled and constrained to some extent by external factors such as family and work schedules and socially defined occasions. In fact, we have found that scheduling feeding times for rats, in some ways, produces a better model of human ingestion patterns than one which allows complete ad libitum access to food (de Castro, 1988).

B. What influence would variations in normal feeding patterns (for example due to strain or sex or age) have on results of studies using restricted feeding paradigms?

There are very large individual differences in meal patterns that can be measured within strains. This within-strain variance is far larger than the across-strain variance. Hence any difference produced by variation in feeding patterns would already be built into the results found with a single strain.

As one panel member indicated, results of studies using restricted feeding paradigms may be less variable than in normal feeding patterns due to strain, sex, or age.

Age is an important variable in the characterization of pesticide toxicity. Recent enactment of the Food Quality Protection Act addressed concerns regarding potential higher sensitivity of infants and children to certain pesticides. Oral exposures in very young animals may be difficult to model by a feeding paradigm similar to that proposed.

Issue # 4 - Appropriateness of the Model

A. How well does a one hour dietary exposure period model human eating patterns?

In general, this model attempts to simulate better the exposure of humans to pesticides through the diet. If humans were exposed to dietary pesticides by consumption of foods over a one-hour period, this model may be appropriate. However, dietary intake of pesticides most likely occurs at different rates with different foodstuffs, and a one hour exposure may be more representative of a poisoning. For example, a glass of juice or a piece of fruit may contain pesticide residues and it may be consumed over a brief time, whereas other foods may be consumed throughout a meal over a longer time. The variability in exposure due to differential rates of consumption appears to be a major flaw in this model. Variability decreases the ability to

detect significant differences. Thus, data derived from such studies would have low capacity for determining effect levels.

Normal humans in affluent environments ingest, on average, about 4 meals per day lasting about 30 minutes. The only meal of the day similar to the one hour model is the first meal of the day. It is ingested after a prolonged fast and the stomach is usually empty. However, this tends to be the smallest major meal of the day and thus is unlike the oversized meal ingested by animals fed twice a day. The larger meals that humans eat are generally in the evening and are eaten with food still remaining in the stomach from prior meals. Considering the variability in short-term consumption patterns, possibly a better approach to acute dietary exposure would incorporate 24-hour consumption. While this would pose problems for determining such parameters as time to peak effect, this approach might yield useful information on the acute dietary exposure to a particular compound. Used in conjunction with the standard acute gavage data to obtain time-to-peak effect data, this approach may yield data on more realistic rates of exposure. In particular, if acute neurotoxicity studies are "used to determine an acute NOAEL for estimation of the 24-hour dietary risk," the longer-term dietary exposures (8-12 hours) may be a better model for acute dietary pesticide exposures.

The Agency posed another question "Do we have a dietary model from which a protocol can be derived or is more research needed before such a protocol should be undertaken?" that elicited a dialogue among the Panel members. There appeared to be a consensus that the level of basic science research on ingestion behaviors and on the physiological factors associated with such behaviors is sufficient, such that one may be able to move to a dietary approach and construct an experimental protocol. However, these procedures or any specific protocol would require set performance criteria and considerable validation within a toxicological framework.

Given the inherent differences in ingestive behaviors, circadian rhythms, and associated factors between the rat and the human, as well as the experimental variability of administered dose, the question was raised by a Panel member as to what additional information would a dietary model of acute toxicity offer in determining potential human risk? If we want to maximize the opportunity to see an adverse effect, is there a need for doing an acute study by feed versus gavage? While one other member of the Panel agreed with this argument, other members of the panel felt that a dietary exposure would more accurately mimic human exposure. It was mentioned that exposure constricted to a distinct period of time would offer data conservative for public health but not as conservative as the data generated with gavage dosing. Discussions were based on the assumption that any acute feeding study would be conducted only after other data were available; thus, no comparison of sensitivity was discussed. It appeared as if the concern was more for the non-physiological route of exposure, (i.e., gavage) that is currently used for oral dosing.