Behavioral Testing as a Method for Assessing Risk

by Richard E. Butcher*

Behavioral effects have been found to result from the prenatal administration of substances known to be teratogenic to the CNS. These effects occur at dose levels lower than those producing gross malformations and when the agent is administered at times other than that optimal for CNS teratogenesis. These findings have led to the belief that behavioral testing can be a sensitive and relevant technique for detecting adverse consequences of prenatal exposure to drugs and chemicals. Behavioral testing, however, also appears to have attributes that dictate a thoughtful approach to its role as a method for assessing risk, and additional research is needed to obtain usable techniques. The need for such research is intensifies by the present inability to identify potential behavioral teratogens by means other than laboratory investigation.

Information about the behavioral effects of drugs administered during development is now required by the British and Japanese governments as part of their reproduction testing procedures. The United States guidelines also contain an optional provision calling for such behavioral testing, but to the best of my knowledge this option has never been exercised. These government actions have focused attention upon behavioral testing and its role as a device for assessing the undesirable consequences of environmental stress during development. Within this context, I would like to review a sample of the literature in this area, to offer what appear to be the implications of this research, and finally to offer some comments on behavioral testing as a tool for assessing risk.

I will limit my remarks to those circumstances usually denoted by the term "behavioral teratology;" that is, a set of circumstances in which an organism is exposed to a test agent at some time during its development and the consequences of that exposure is looked for in the behavior of the subject at a later time when the immediate effects of the agent, if any, have passed. In the usual procedure, the agent is administered during gestation, and the test subject is examined postnatally to determine if there are enduring effects. The six examples of research I have chosen are all of this type and although each undoubtedly has some faults, I believe they are a reasonably representative sample of experiments in this area. In each study (Table 1) the agent, vitamin A, was administered during gestation to the rat and behavioral tests were administered postnatally. Hypervitaminosis A is known to have a teratogenic effect, and, in addition, has been demonstrated to malform the CNS. The immediate impression made by these studies is one of great diversity—a wide variety of dose levels, times of administration, strains, and behavioral tests have been used. Perhaps this diversity has some importance, for at least we know that the phenomenon under study is rather general and is not strain specific or task specific.

Closer scrutiny of these experiments, however, reveals a common logic in the apparently diverse procedures. That logical thread is an attempt to

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Table 1. Summary of behavioral studies on the subteratogenic effect of hypervitaminosis A.

| Author       | Vitamin A dose, IU/kg/day | Time of administration, day(s)* | Strain       | Physical effect | Tests                  | Result           |
|--------------|---------------------------|---------------------------------|--------------|----------------|------------------------|------------------|
| Malakhovskii (1) | 60,000 300,000            | 9                               | Albino rat   | No report       | Shock avoidance escape| Impaired learning|
| Malakhovskii (2) | 150,000                   | 9                               | Albino rat   | No gross No microscopic | Activity Shock avoidance escape aggression | Hypoactive Improved Reduced |
| Butcher (3)   | 100,000                   | 8, 9, 10                        | Sprague-Dawley | +2% in gross malformations | Swimming maze | Reduced learning |
| Hutchings (4) | 180,000                   | 13, 14                          | Wistar       | No gross Growth retardation Small brains | Discriminative operant | Poorer discrimination |
| Hutchings (5) | 270,000                   | 16, 17                          | Wistar       | No gross No microscopic | Discriminative operant | Lower response rates |
| Vorhees (6)   | 100,000 40,000 25,000 10,000 | 8, 9, 10                        | Fischer 344  | Growth retardation in 100,000 IU level | Y-maze Shock avoidance Activity | Impaired avoidance No difference |

* Date of sperm = day 0.

detect a behavioral deficit in the absence of anatomic deformities. In almost every case the investigator(s) have offered some evidence indicating that anatomic defects are either absent or that the incidence of such malformations is low. In the Hutchings (4, 5) studies an attempt has been made to demonstrate behavioral impairments resulting from exposure at a time during gestation when anatomic malformations are rare. Incidentally, not only do these effects appear in the absence of malformation, but the effects of exposure on days 13-14 are different from those resulting from exposure on days 16-17.

On taking these studies in combination, what do we know about the effect of prenatal exposure to large amounts of vitamin A that we would not have known had the behavioral studies not been done? First, it would appear that hypervitaminosis A has an effect at doses lower than those that produce noteworthy anatomic defects. Stated in other terms, hypervitaminosis A has a functional (in this case behavioral) effect that precedes anatomic defects on the dose/response curve. In addition, the vitamin has an effect when administered at a time later in gestation, when the rat has been found to be more resistant to gross malformation. Neither of these findings is revolutionary—it would be unusual that all effects of an agent would end abruptly with the disappearance of gross anatomic effect, and it would be equally surprising if the CNS which differentiates over a long period would be vulnerable for just a few days during gestation.

From a more general perspective, what appear to be the implications from studies such as these for the role of behavioral testing as a tool in assessment of risk? The discovery of behavioral effects in the absence of morphological alteration of the CNS implies that behavioral testing is a sensitive technique for the detection of adverse consequences of prenatal environmental stresses. Where minor or infrequent anatomic abnormalities are found, alterations in behavior provide a demonstration of the functional significance of what might otherwise be regarded as anatomic "variants" having no functional consequences. The temporal relationship between the administration of the agent and the testing procedures also suggests an enduring effect.

So we may claim for the psychological test procedures two attributes not possessed in these instances by morphological examination, sensitivity, and relevance. It is, I believe, the quality of sensitivity that has provided the primary impetus for behavioral testing and some comments on the way in which this sensitivity is developed methodologically could be informative. To generalize again, it appears that behavioral tests are usually procedures that assay the performance of a test subject in situations requiring the use and integration of several primary functional systems. The overall ability of the subject to adapt to the experimental situation is what is judged, and deficits in any subsystem that contribute to the overall performance become apparent. Such a test is often referred to as apical, a single test re-
quiring the successful integration of intact sub-
systems. The performance of even a simple maze
 task, for example, involves the interplay of moti-
vational, sensory, learning, and motor capaci-
ties—and this is the most gross level of analysis
possible.

Under conditions of this type of apical testing,
it is less surprising that behavioral testing is a
-sensitive evaluation technique because the tasks
summarize in a single measure of performance
the contributions of a number of systems that
may have been harmed by the test agent. The
psychological testing process is also a nonde-
structive technique and such summary perform-
ance can not only be tested once, but again and
again. To go even further along these lines, you
will notice that in many such tests each succes-
vie step is contingent upon the preceding one,
and that flaws in performance are carried for-
ward.

So there appears to be some logical basis for
the sensitivity of behavioral testing which is in-
herent in the methods. The sensitivity of these
methods, however, does not come without cost
for just as a particular weakness in a system will
be reflected in a reduction in overall perform-
ance, so can particular strengths compensate. As
Rodier (7) has correctly pointed out in her re-
view, behavioral testing can illustrate the sub-
jects ability to perform despite injury. It is a con-
tinual source of wonder how much as animal can
do with the little bit of brain tissue left to it after
surgery, and the literature of psychology is re-
plete with examples of compensation for brain in-
jury. Just what sorts of injury are likely to escape
detection despite the sensitivity of the behavioral
test and what kinds of damage are particularly
likely to be revealed is not known. This appears
to be an area toward which some research might
profitably be directed. In any case, the sensitivity
of the behavioral test is a one-way judgement, the
results can indict, but not acquit. Deficient per-
formance means "something is wrong," but ade-
quate performance does not mean "nothing is
wrong."

Unfortunately for the investigator, the behav-
ioral performance of a subject is also a sensitive
indicator of influences other than the test agent
under consideration. The number of these influ-
ences form an intimidating list of variables to be
controlled. All aspects of selecting, housing, and
handling of the dam including those surrounding
the administration of the agent, the housing,
handling, fostering, size, and sexual composition
of the litter must be carefully controlled before
the first subject is examined. Testing then brings
with it another long list of influences that can in-
trude upon the detection of a treatment effect.
The necessity of controlling these numerous in-
fluences over a considerable length of time is a
time consuming and expensive proposition when
compared to the techniques presently used in re-
productive testing.

These characteristics of behavioral testing
have implications for the way a psychological
evaluation might be used in the assessment of
risk process. For example, until more efficient
behavioral techniques in this area are developed,
it appears likely that behavioral testing would be
among the final tests administered. Thus, the
apical tests would be used in the apical situa-
tion—after something is known about the mor-
phological and physiological effects of the agent
studied—and the question asked, "has something
been missed?" A benefit of using a psychological
test series in later stages would be the possibility
of examining the effects of an agent when admin-
istered in an amount closer to the anticipated
therapeutic dose. The sensitivity of the behav-
ioral test could be exploited in a way that would
provide direct information about moderate expo-
sure levels and a more realistic estimate of the
range of response than would be provided by ex-
trapolations from teratologic studies.

Examining the functional capacity of the test
subject would also engage a new and compelling
set of health problems. Functional deficits and
behavioral defects in particular constitute a
chronic public health problem, and insofar as we
can imagine that they may result from a prenatal
insult, we should endeavor to identify and elim-
inate these insults. If we allow the possibility of a
behavioral impairment from prenatal exposure to
an environmental agent, we must also consider
the possibility of detecting that effect. Under
present circumstances our ability to identify the
relationship between a prenatal stress and its
effect on behavior is extraordinarily limited. In-
deed, almost all human teratogenic effects have
been identified clinically rather than in the labo-
atory. Many of you are aware of the difficulty
with which thalidomide was identified as a tera-
togen under circumstances in which the abnor-
mality produced was gross, rather unique, and
obvious at birth. Consider, in light of that history,
how small would be the probability of detecting
the cause-and-effect relationships between an
agent administered during pregnancy and an im-
pairment in learning ability. Such an impairment
would not be an uncommon event, would repre-
sent a rather subtle effect, and would be diag-
nosed almost certainly after the fourth year of

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postnatal life. It would seem that we must place increased reliance upon laboratory investigation, because our ability to identify possible behavioral teratogens is so very limited.

I have tried in this brief review to provide a very general perspective on behavioral testing as it might be used in reproductive studies designed to assess risk. Such investigations can have benefits and I have tried to point some of the cautions that seem appropriate. If such testing is to be used, however, we must very rapidly become specific about the design, method and interpretation of such studies. This sort of specific information is not available and it is going to take some hard work to develop usable, meaningful tests. The final comment must be a call for research.

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