# An Approach to Ultrasonic Risk Assessment and an Examination of Selected Experimental Studies

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## INTRODUCTION

It has been more than a decade since the first major efforts to assess the risk of ultrasonic energy have been made (1, 2). Since then there have been numerous reviews and assessments (3-10). And, even today, there are projects which have as their aim an assessment of the health risks from diagnostic ultrasound (11-13). And yet, the basic status regarding risk assessment has remained unchanged during this time viz., the studies necessary to support a reliable assessment of the risks associated with human exposure to ultrasound have not been done. So, rather than present another broad discussion of ultrasonic risk assessment, it seemed to be much more reasonable to focus upon one ultrasonically induced biological observation, viz., fetal weight reduction. But, prior to a discussion of ultrasonically induced fetal weight reduction, let us first consider an approach to risk assessment and then a view of the general trends as they relate to diagnostic level, ultrasonic biological effects. Here, the effect of ultrasound on immunological function and on sister chromatid exchange frequency will be considered as examples.

## APPROACH TO RISK ASSESSMENT

With our current understanding of ultrasonically induced biological effects, it is difficult to argue against statements such as "diagnostic ultrasound is not harmful to the conceptus." Experimental studies can not be used to prove diagnostic ultrasound safe. Rather, what such studies

will provide, if properly planned and executed, are data which will aid in the overall assessment of risk associated with exposure to ultrasound. Safe implies the absence of an effect, not involving risk, or the like. It simply is not possible to prove that ultrasound, or for that matter any agent, does not produce any effect at the levels employed diagnostically. Also, the actual use of the word safe is vague since it almost never refers to the absence of an effect. A more useful and workable approach is to examine the "risk" associated with ultrasonic exposure.

Some thirty-five years after the Curies discovered piezoelectricity in 1880 (14), the first use of ultrasonic energy was developed wherein underwater acoustic echoes were bounced off of submerged objects (15, 16). During the course of this work, the first reported observation was made that ultrasonic energy had a lethal effect upoon small aquatic animals (17). The first extensive investigation of the phenomena confirmed that ultrasonic energy could kill small fishes and frogs within a minute or two (18). In perhaps the first review paper of ultrasonically induced biological effects (19) the physical, chemical and biological effect of ultrasound were evaluated wherein effects on cells, isolated cells, bacteria and tissues were summarized with a view towards identifying the responsible mechanism. The ultrasonic exposure conditions of this early work were not well characterized but the intensity levels were undoubtedly quite high.

In the early pioneering studies where the ultrasonic

exposure conditions were carefully controlled and specified, sciatic nerve paralysis was easily produced in the frog (20, 21) and lesions were produced in central nervous system tissue (22). In addition, high intensity ultrasound was employed to produce lesions in adult cat and rat brain (23-27); adult rat and neonatal mouse spinal cord (22, 28, 29); adult frog muscle (30, 31); rabbit blood vessel (32); rabbit kidney and testicle (33); and rabbit ocular tissue (34-36). The ultrasonic intensities were very much higher than those utilized in diagnostic ultrasound. Mostly, these studies caused rather severe tissue damage, but these studies have been extremely important in the elucidation of fundamental interaction pocesses. In terms of risk assessment, these studies support the view that the currently employed ultrasonic exposure conditions will more than likely not produce acute, gross irreversible damage.

These high intensity studies further aid in recognizing the important fact that, at sufficient energy levels, ultrasound is capable of destroying biological material. Therefore, an approach to the question of assessing the risk from ultrasound is as follows:

(1) what biological systems are most sensitive to ultrasound?

(2) what exposure levels imposed a significant risk on these systems?

Unfortunately, this approach has its difficulties. How does one determine significant risk? Usually, what is meant by

significant risk is that risk which is greater than some upper limit of acceptability. One usually then employes a benefit vs risk concept which, in principal, is quite understandable but, in pratice, poses severe implementation problems.

Another important consideration with respect to risk assessment is the extent to which ultrasound is used. While no statistically based survey has been conducted to document the extent to which ultrasound has been used over the past decade, a number of indicators do, however, support the view that its use is increasing and that a large fraction of the human population will eventually be exposed, especially in utero.

As listed in Table 1, in 1971, the Food and Drug Administration's Bureau of Radiological Health surveyed 301 out of 6306 short-term general hospitals in the United States and found that 12 percent of the hospitals used diagnostic ultrasound (37). The same Federal agency reported on the conduct of its 1974 hospital survey in which 35 percent of the surveyed hospitals used diagnostic ultrasound (38, 39). This represented an almost 200 percent increase in use between 1971 and 1974, or an annualized increase of 43 percent during this time. The survey (38, 39) further showed that an estimated 16 percent of the obstetric services in the United States used diagnostic ultrasound and that about one-third of all U.S. births for 1974 were delivered in these hospitals. Additionally, it was estimated that 470,000 pregnant women were exposed to diagnostic ultrasound in 1974

with about 35-40 percent of these women being examined more than once.

An international mail survey showed that between 1963 and 1971, there was an average annual increase in use of clinical ultrasound of approximately 10 percent (40). And, in the United Kingdom, it had been estimated that in the early 1970's the number of ultrasonic diagnostic examinations was doubling every three years (41), thus representing an annual 26 percent increase.

While more current use information is not available, sales growth information does indicate that in 1976, the ultrasonic industry annual dollar sales was around $30 million (42) and for the following year about $40 million (43). In the next four years, estimates of annual sales were $50, $79, $170, and $214 million (10). Therefore, an increase from $30 to $214 million from 1975 to 1980 represents an average annual increase in sales of approximately 48 percent.

To summarize, if one were to assume that at least one-half of all pregnant women are currently being examined with ultrasound and, additionally, were to assume that its use is increasing at an annual rate of between 10 and 25 percent, then within a few years, virtually every fetus could be diagnostically examined with ultrasound.

## GENERAL TREND

Recent reviews (6, 9, 10, 44-49) of the ultrasonic bioeffect literature appear to suggest that as more sensitive

biological endpoints are studied, the ultrasonic exposure parameters required to produce measurable effects appear to decrease. This is supported by published work (50-57) which describes ultrasonically induced biological effects from ultrasonic diagnostic equipment, that is, ultrasonic energy from a commercial diagnostic device altered the biological system.

It should be noted that at the lower, sometimes diagnostic, levels of ultrasound, there is no consistent indication of the specific ultrasonically induced biological alteration. For the two examples to be discussed, immunological function and sister chromatid exchange, there are considerable conflicting experimental data as to whether these alterations are ultrasonically induced.

In the first study by Anderson and Barrett (50), which reported an effect of ultrasound on immunological function the splenic area of mice was exposed to ultrasound via a pulsed diagnostic device and it was reported that ultrasound exposure reduced the hemaglutinin and hemolysin response after an injection of sheep erythrocytes. However, another laboratory (58) could not reproduce these effects and reported that ultrasound had no significant effect on the hemoglutinin and hemolysin response in mice exposed to ultrasound under very similar conditions to that used in the earlier study (50). Further, in another experiment (58) at higher spatial average, temporal average intensities, no effect was produced.

In a second study, Anderson and Barrett (55) again

exposed mice to diagnostic levels of ultrasound, but this time to the liver area. They reported that ultrasound caused a depression of phagocytosis as measured by carbon clearance, and a depression in the ability of peritoneal macrophages from sonicated animals to phagocytose bacteria. These studies are very interesting from the standpoint that diagnostic levels of ultrasound may affect immune function. There are, however, many problems in translating these findings to the clinical situations, such as the large difference in area of sonicated vs whole body/organ size in mouse vs man.

Liebeskind and colleagues (51) reported that diagnostic levels of ultrasound increased immunoreactivity to antinucleoside antibodies in cultured cells. This type of effect usually suggests unwinding of the DNA helix, or single-strand breaks but the authors were not able to identify DNA strand damage. While this effect is sometimes referred to as an immune effect, it is not an effect on the immune system, but the use of an immune technique as an experimental tool.

Within the last five years, a number of experimental studies have been conducted to examine the effect of ultrasonic energy on sister chromatid exchange (SCE) frequency, an indicator of chromosome damage but of which the biological signficance is unclear. Liebeskind's study (52) has received, perhaps, the greatest attention since the study indicated an increase in human lymphocyte SCE's from a diagnostic ultrasound device. In another study from the same

authors (51), they reported no change in SCE's in a different cell type. There has been only one other positive observation (58) of increased SEC's; here in human lymphocytes at diagnostic levels. But, there have been at least eight other studies (51, 58-64), some at diagnostic levels, some at levels much higher than therapeutic levels which have reported no increase in SCE's. Therefore, it is necessary to put into perspective all of the reported findings, both positive and negative.

One must be caution in assessing the significance of biological alterations such as the immune disturbances or increased SCE's as discussed above, or other such observations. They are simply observations, and whether or not ultrasound will be shown to represent a significant risk will depend upon the types of effects observed, the exposure levels at which these effects occur, their dose-effect responses, and the assessment of the mechanism from dose effect data. These studies are useful, however, in pointing to potentially useful hazardous or potentially therapeutic interactions of ultrasound and to help understand how ultrasound interacts with biological tissue.

## FETAL WEIGHT STUDIES

Over the last few years, and especially within the past year or so, experimental observations have been made which suggest that subtle effects are occurring to rodent embryos and fetuses when exposed to ultrasound in utero. The balance of the article selectively examines ultrasonically induced

fetal weight reduction in experimental animals. In chosing this topic, an attempt has been made to approach the assessment of risk from ultrasound for this single biological endpoint. It is not known whether this biological system is very sensitive (in a chemical and physical sense) to ultrasonic energy but, clearly, it is sensitive in an emotional and political sense. Therefore, it behooves us to understand the experimental data which shows that ultrasonic energy does influence fetal weight when the system is exposed to ultrasound *in utero*.

One of the earliest studies that suggested that *in utero* ultrasonic irradiation affected prenatal growth and development was reported in experimental animals about eight years ago (65). Time-mated mice received continuous wave (1 MHz) ultrasound on the eighth day of gestation under well controlled and documented exposure conditions (66). The fetuses were weighed on the eighteenth day of gestation and a statistically significant weight reduction of up to 17.5 percent relative to the sham was observed. Two hundred and seventy two litters (2866 fetuses) were examined in seven separate ultrasound groups, including a sham group. The spatial average, time average intensity ranged from 0.5 to 5.5 $W/cm^2$ and the exposure time ranged from 10 to 300 seconds as listed in Table 2.

A detail account of the initial finding (65) and an extension of the data analysis (67) showed that a dose-effect response was observed. Here the dose-effect response of the exposure condition versus fetal weight was developed by

defining the dose parameter $I^2t$, where I is the exposure intensity ($W/cm^2$) and t is the exposure time (sec) as listed in Table 2 for each of the seven exposure conditions.

There is a basis for the $I^2t$ dose parameter in the ultrasonic literature and in the literature of other energy forms. Threshold ultrasonic dosages for structural changes in the adult mammalian central nervous system results in a mathematical dependency between the ultrasonic intensity, I, and the exposure duration, t (24, 26, 68-70) which is described by the product of $I^2$ and t equalling a constant value. In other words, if I equals 20 W/cm ($I^2$=400) and t equals 0.5 sec for the threshold of an ultrasonically induced change, then $I^2t$=200. This same biological threshold would occur under the ultrasonic exposure conditions in which I equals 10 W/cm ($I^2$=100) and t equals 2.0 sec, that is $I^2t$=200 again.

A similar type of $I^2t$ dependency has been observed for ultrasonically induced hind limb paralysis of neonatal mice (26, 71), threshold focal lesions in cat liver (72) as well as focal lesions in the rabbit liver, kidney and teste (33). In comparison to other forms of energy, a similar dose dependency has been observed for mammary neoplasms at low ionizing radiation doses wherein two x-ray secondary particles (produced by a single neutron) are required to elicit the effect (73). In photochemical and photobiological studies, at high energy concentrations, biophotonic excitation (two photons required to produce the effect) has been observed (74).

What does an $I^2t$ type of dependency for a biological effect mean? Basically, it means that two energy events are required to produce the biological effect. However, there is not enough fundamental information available at this time to speculate as to whether or not the reduced fetal weight observations reported herein can be explained by this dosimetric model. Nevertheless, such a model begins to provide a basis for extrapolating biological effect observations and such an extrapolation will be done shortly in terms of assessing the potential for ultrasonically induced fetal weight reduction in humans based upon the mouse data. Thus the following fetal weight observations will be presented in terms of their $I^2t$ dependency.

The observation that <u>in utero</u> ultrasonic exposure can cause reduction in the mouse fetuses has been confirmed by two other research groups using two different strains of mice (75-76). For the data listed in Table 3 (75), relatively high level, pulsed ultrasound conditions were employed. Here the mice were exposed to ultrasound on the eighth day of gestation and the fetuses were individually weighed on the eighteenth day of gestation. A statistically significant 18.8 percent weight reduction was observed for spatial peak, temporal average intensities above 50 $W/cm^2$ (spatial peak, temporal peak intensity of 1936 $W/cm^2$ and exposure time of 20 sec) when irradiated on day eight of gestation. At and below a spatial peak, temporal average intensity of 45 $W/cm^2$ no statistically significant change in the fetal weight was observed.

The data listed in Table 4 (76) shows mean fetal weight reductions which range up to 25 percent relative to the sham under continuous wave (2 MHz) exposure conditions at a spatial average, temporal average intensity of 1 $W/cm^2$, for exposure times up to 200 sec at gestation ages of zero, 7 and 12. The fetuses were individually weighed on the seventeenth day of gestation. These results compare most directly with the previous finding (65, 67) in which similar mean fetal weight reductions were observed.

Figure 1 graphically summarizes in a unified way the three published studies (67, 75, 76) which reported statistically significant effects of fetal weight reduction from *in utero* exposure to ultrasound. Here the data from Tables 2, 3 and 4 are represented by the percentage weight change (re: sham) as a function of the calculated dose parameter $I^2t$. All three studies graphically show that as the value of $I^2t$ increases the fetal weight (re: sham) decreases.

## DOSE-EFFECT STUDIES

Dose-effect studies are invaluable for assessing risk (6). Too ofter, a report appears in which a biological effect is reported under a single ultrasonic exposure condition. When the exposure condition arises from a diagnostic device, we tend to question whether the reported observation is real, or whether the experimental set-up produced extraordinary conditions to elicit the effect. But, in either case, there is a tendency to suggest that

diagnostic exposure conditions represent a risk to the patient. When the exposure condition is at levels much in excess of diagnostic conditions, we tend to discount their applicability to the clinical situation. The overall problem is that non dose-effect studies are quite difficult to apply to assessing the risk. They do, however, identify biological endpoint to which dose-effect experimental regimes should then be applied.

Consider the mouse dose-effect fetal weight data shown in Figure 1. If we were to apply this dose-effect curve to a clinical exposure condition for purposes of assessing risk, let us first examine the upper value of the dose parameter $I^2t$ for static pulse echo scanners (77). For a single pulse, the spatial peak, pulse average intensity is about 300 $W/cm^2$ and the exposure time, here the pulse duration, is about 1 microsecond, yielding an $I^2t$ around 0.09. For the time average case, the spatial peak, temporal average intensity is about 200 $mW/cm^2$ and the exposure time, here the length of the exam for maximum effect, is about 30 minutes, yielding an $I^2t$ around 72. Of course, this latter case would require examining the same tissue volume for the entire length of time, which might not be the situation with static pulse echo scanner but is quite possible for a Doppler fetal monitor wherein the spatial peak intensity is about 75 $mW/cm^2$. For an exposure time of one hour, the $I^2t$ dose parameter calculates to be about 20. The point to be made is that with a dose-effect model, one is in a better position of examining what might be the effect under clinical conditions. The

model would have to be validated for such applicability, of course. There is a long way to go with respect to ultrasound.

ACKNOWLEDGMENTS

One of the authors (WDO) acknowledges gratefully the partial support by a grant from the National Institute of General Medical Sciences, National Institutes of Health.

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TABLE 1

Selected Assessment of Diagnostic Ultrasound Useage

| Assessment Period | Assessment |
|---|---|
| 1963-1971 | Annualized increase in useage *via* a mail survey about 10 percent (40). |
| early 1970's | In the United Kingdom, the number of diagnostic exams doubles every three years (26 percent annualized) (41). |
| 1971 | Twelve percent of hospitals use ultrasound (37). |
| 1974 | Thirty five percent of hospitals use ultrasound (38, 39). |

TABLE 2

Summary of $CF_1$ mouse fetal weight study (65, 67). The mice were ultrasonically exposed (continuous wave of 1 MHz) *in utero* on day eight of gestation and the fetuses were examined on the eighteenth day of gestation.

| Isata ($W/cm^2$) | t(sec) | $I^2t$ | Percentage weight change (re: sham) |
|---|---|---|---|
| sham | | 0 | 0 |
| 0.5 | 300 | 75 | - 5.3 |
| 2.0 | 20 | 80 | - 6.1 |
| 3.0 | 10 | 90 | - 6.1 |
| 0.7 | 300 | 147 | - 8.8 |
| 3.0 | 20 | 180 | - 7.9 |
| 5.5 | 10 | 303 | -17.5 |

TABLE 3

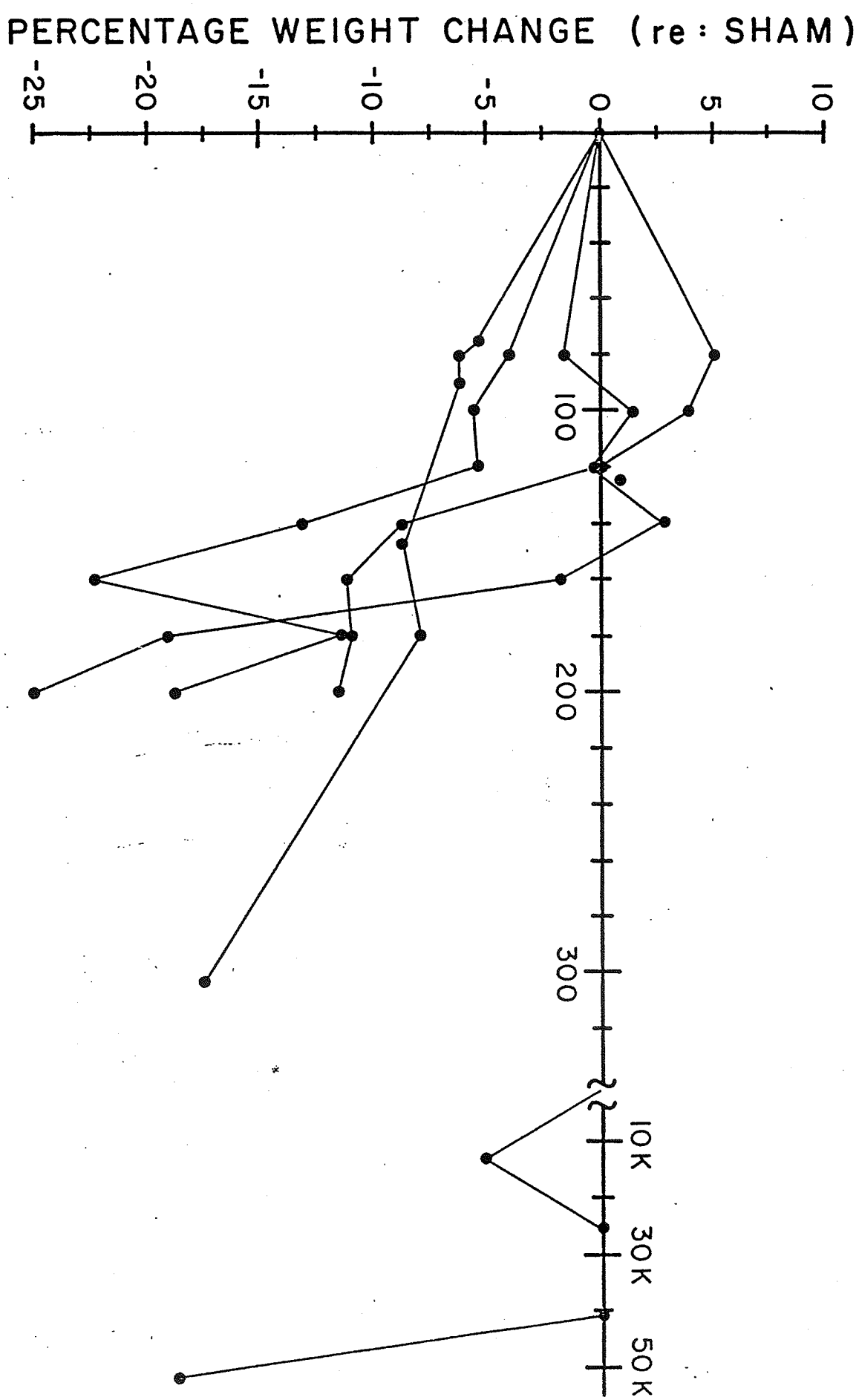
Summary of $LAF_1/J$ mouse fetal weight study (~~70~~ 75). The mice were ultrasonically exposed (pulsed, $I_{sptp}$ = 1936 $W/cm^2$, 1 MHz, pulsed repetition frequency = 1 kHz) *in utero* on the eighth day of gestation and the fetuses were weighed on the eighteenth day of gestation.

| $I_{spta}$ ($W/cm^2$) | t(sec) | $I^2t$ | Percentage weight change (re: sham) |
|---|---|---|---|
| sham | | 0 | 0 |
| 25.6 | 20 | 13,100 | - 5.4 |
| 35.3 | 20 | 24,900 | 0 |
| 45.0 | 20 | 40,500 | 0 |
| 50.8 | 20 | 51,600 | -18.8 |

TABLE 4

Summary of CFW Swiss Webster mouse fetal weight study (~~71~~ 76). The mice were ultrasonically exposed (continuous wave of 2 MHz) in utero on days 0, 7 or 12 of gestation and the fetuses were weighed on the seventeenth day of gestation.

| $I_{sata}$ ($W/cm^2$) | t(sec) | $I^2t$ | Percentage weight change (re: sham) day 0 | day 7 | day 12 |
|---|---|---|---|---|---|
| sham | | 0 | 0 | 0 | 0 |
| 1 | 80 | 80 | - 2.7 | + 5.1 | - 3.9 |
| 1 | 100 | 100 | + 1.6 | + 3.9 | - 5.6 |
| 1 | 120 | 120 | - 0.4 | - 0.4 | - 5.3 |
| 1 | 140 | 140 | + 2.9 | - 8.7 | -13.3 |
| 1 | 160 | 160 | - 1.8 | -11.2 | -22.2 |
| 1 | 180 | 180 | -19.1 | -11.0 | -11.3 |
| 1 | 200 | 200 | -25.1 | -11.5 | -18.4 |


PERCENTAGE WEIGHT CHANGE (re: SHAM)
-25
-20
-15
-10
-5
0
5
10
100
200
300
10K
30K
50K