Key Historical Studies Serving as the Basis for the Linear Dose Response Challenged

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INTRODUCTION

The most important publication in the history of risk assessment was the 1956 report of the U.S. National Academy of Sciences (NAS), called the BEAR I report (U.S. National Academy of Sciences 1956). This report lead directly to a dose response revolution, convincing governments worldwide to replace the threshold dose response model for assessing the risks of ionizing radiation on germ cells with the linear dose response model. The key conclusions of the BEAR I report that changed the dose-response default status from threshold to linear at low dose are embodied in the following two quotes on page 17 of that document:

"Any radiation dose, however small, can induce some mutations. There is no minimum amount of radiation dose which might be exceeded before any harmful mutations occur."

"…if we increase the radiation that reaches the reproductive glands by X percent, the number of mutations caused by radiation will also be increased by X percent."

These dose response conclusions were generalized to radiation induced cancer one year later by the National Committee on Radiation Protection (NCRP) and then generalized again for all genotoxic chemical carcinogens by the U.S. NAS Safe Drinking Water Committee (Arch Toxicol, 2009, 83:203; National Academy of Sciences 1977). The presumed safety of a threshold dose response model was replaced with an "acceptable risk" concept of a linear dose response model. With linearity as the guide for cancer risk assessment no exposure to a carcinogen would be without risk.

The present evaluation re-examines key publications directed by the eminent geneticist Curt Stern, upon which the linearity decision was based. The present analysis will demonstrate

- the presence of serious scientific and evaluation flaws in these papers
- a failure of the investigators to provide critical methodological and complementary research findings to validate their conclusions
- that these flawed and unsupported findings were broadly accepted by key leaders in the genetics community, and played an important role in the acceptance of linearity at low dose by the BEAR I Committee its generalization to cancer risk assessment for radiation and chemical carcinogens for use by federal and state regulatory agencies in the context of legislative requirements.

KEY PLAYERS

Curt Jacob Stern (1902 –1981) was born in Hamburg, Germany . He studied zoology at the University of Berlin, received his PhD in 1923 at the age of 21, and was awarded a post-graduate fellowship at Columbia University. Stern was a professor of genetics at the University of Rochester since 1939. He movied to the University of California at Berkeley in August, 1947 where he was a professor until his retirement in 1970. Stern demonstrated crossover of homologous chromosomes in *Drosophila melanogaster* in 1931. He continued to work in the area of genetics and during World War II, doing research for the American government on low-dose radiation safety via the Manhattan Project.

Hermann Joseph Muller (1890 –1967) was born in New York City. At age 16, he entered Columbia College and earned his BA in 1910. He remained at Columbia for his graduate work where he became involved with Drosophila genetics work in Thomas Hunt Morgan's fly lab. In 1926, Muller demonstrated that X-rays caused mutations in male fruit fly germ cells (Science, 1927, 66:84). Nineteen years later he was awarded the Nobel Prize for this finding. Muller took his discovery seriously, trying to determine not just what it meant scientifically but for society as well. Soon after his discovery he expressed strong concerns about the indiscriminate use of X-rays, challenging the medical community to be aware of the benefits and dangers that X-rays may provide. Continued research in Muller's laboratory assessing the nature of the dose response for radiation induced germ cell mutations supported a linear interpretation thereby suggesting that there was no safe dose no matter how low or apparently inconsequential (Environ Mol Mut, 2011, 52:in press; Arch Toxicol, 2009, 83:203).

Warren P. Spencer was a professor on leave from the College of Wooster, with nearly 20 years research experience with Drosophila. He received his Ph.D. from Ohio State University. He published in Drisophila genetics from 1925 through 1949.

Ernst Wolfgang Caspari (1909 - 1984) was born in Berlin. He received his Ph.D. at the University of Gottengen working in genetics under Alfred Kuln. He immigrated to the US in 1938 and was natualized in 1944. He was a senior entomological behavioral geneticist researcher and a professor of biology at Lafayette College, Wesleyan University, and the University of Rochester, until his retirement in 1975.

Delta E. Uphoff (1922 – 1992) was a graduate of Russell Sage College and received a master's degree from the University of Rochester. She went on to have a productive career in genetics and bone marrow transplantation, working for the NIH in Bethesda, MD, and publishing through 1988.

THE EXPERIMENTS

THE TIMELINE

There were three general research projects to assess the nature of the dose response for ionizing radiation under the direction of Stern, each lead by a different person.

- The first project was lead by Warren P. Spencer. The second project was directed by Ernst Caspari. The third project was given to Delta E. Uphoff, a new master's student at the University of Rochester.
- The data collection of the three projects ran sequentially: Spencer's from December 1944 to June, 1945, Caspari's from October, 1945 to August, 1946, while Uphoff's initial experiment, a partial replication of the Caspari experiment, ran from September, 1946 to April, 1947.
- During the summer of 1947 Uphoff (US AEC, 1947, p 1-6) conducted another experiment at the University of Rochester, a "chronic" (i.e., 21 day) exposure to gamma rays.
- The final Uphoff experiment was performed at the University of California at Berkeley in the first half of 1948.
- Muller was an official consultant to the series of projects, providing the Muller-5 strain flies which were not susceptible to crossing-over genetic alternations. He also guided the group on breeding practices, data interpretation and manuscript refinement.

THE STUDIES

SPENCER AND STERN (ACUTE EXPOSURE STUDY): SUPPORT OF A LINEAR DOSE RESPONSE

- assessed the effects of X-rays on sex-linked recessive lethality in Drosophila males from short term (2-40 minutes) exposures (10-96 r/hour)
- cumulative doses ranged from a high of 4,000 r to a low of 25 r
- indicated a dose response relationship that supported a linearity interpretation

CASPARI AND STERN (CHRONIC EXPOSURE STUDY): SUPPORT FOR A THRESHOLD DOSE RESPONSE

- assessed the effects of gamma rays on Drosophila sex-linked recessive lethality.
- cumulative doses were similar between the studies, both ~50 r
- females were mated and exposed to radiation (2.5 r/day) for 21 days with sperm stored in the female's spermatheca, were fed a diet that suppressed egg laying during the irradiation exposure and then were placed on a diet and altered environmental conditions to facilitate egg laying and development
- the aged sperm showed no statistically significant treatment effect, supporting a threshold rather than linear model.

UPHOFF AND STERN (ACUTE AND CHRONIC EXPOSURE STUDIES): INCONCLUSIVE FINDINGS

The first study, Uphoff and Stern (US AEC, 1947, MDDC-1492, p 1-6) replicated the Caspari and Stern (US AEC, 1947, MDDC-1200, p 1-18) work as closely as possible except that

- the exposure to the gamma rays was over 24 hours rather than the 21 days of the Caspari and Stern (US AEC, 1947, MDDC-1200, p 1-18) study.
- the data evaluation Uphoff and Stern (US AEC, 1947, MDDC-1492, p 1-6) adjusted the control and treatment group responses of Caspari and Stern (US AEC, 1947, MDDC-1200, p 1-18) for lethal clusters resulting in a decreased control and treatment group mutation rate.
- These changes were performed to make the two studies as comparable as possible.
- A significant treatment effect was reported by Uphoff and Stern (US AEC, 1947, MDDC-1492, p 1-6), but the control group mutation rate was low at 0.1682.

The second of Uphoff's experiments attempted to closely replicate the earlier Caspari study.

- The study used aged sperm with a chronic exposure of 21 days.
- The treated flies showed a 0.2834 mutation rate which was similar to the other 52.5 r exposures, whether the exposure was acute or chronic.
- The control group mutation rate was again low at 0.1765.

The third and final Uphoff experiment followed the Caspari design except that the dose was about double (100 r vs 52.5 r) that used by Caspari.

- This experiment displayed a control mutation rate of 0.2352, a value similar to the original Caspari and Stern (US AEC, 1947, MDDC-1200, p 1-18) mutation rate.
- There was a significant treatment effect consistent with a linear interpretation.

Table 1: Mutation rates of treatments and controls for each of the studies*.

Study	Mutation rate percent Controls	Mutation rate percent Treatment	Difference
Spencer and Stern 50r acute exposure, sperm not aged	0.0974	0.2440	0.1466
Caspari and Stern 52.5 r over 21 days, aged sperm	0.2489	0.2848	0.0359
Uphoff and Stern (I) 50 r over 24hr exposure, aged sperm	0.1682	0.2834	0.1152
Uphoff and Stern (II) 52.5 r over 21 days, aged sperm	0.2352	0.4658	0.0777
Uphoff and Stern (III) 100 r over 21 days, aged sperm	0.1765	0.2542	0.2306

*Science, 1949, 106:609

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Male (left) and Female (right) Drosophila

Table 2. Differences between Spencer/Stern and Caspari/Stern

Spencer/Stern (Genetics, 1948, 33:43)	Caspari/Stern (Genetics, 1948, 33:75)
Exposure: X-rays	Exposure: gamma rays (radium needle – 10 mg radium)
Animal Model: males exposed prior to mating	Animal Model: females exposed after mating
Not aged sperm	Aged Sperm
Exposure Duration: acute exposure (minutes)	Exposure Duration: chronic exposure (21 days)
Dose Rate: ~15,000-fold greater than Caspari	Dose Rate: ~1/15,000 of Spencer
Plastic vials to hold flies	Glass vials to hold flies
Temperature: 24^{0}C	Temperature: 18^{0}C
Diet: cornmeal molasses	Diet: honey yeast agar to suppress egg laying during the 21 day treatment period
Age (males): $\leq$ 7 days, most 2-4 days old	Age (males): $\geq$ 5 days (may yield sperm with higher mutations rates)
Controls poorly matched with treatment exposure period. One treatment subgroup was exposed for 15.7% and the other for 61% of the time the control group was kept.	Controls closely matched with treatment exposure period.
Temperature Control: poor, highly variable based on external conditions. Certain mutants can behave as semilethals at one temperature and lethals at another	Temperature Control: good
50 r treatment group: 2 groups with different dose rates and exposure periods all combined.	A single 50 r treatment group; all treated similarly.
Mold Control: used Moldex throughout study.	Possibly less Moldex used in the 21 day radiation exposure period due to the lower temperature (18^{0}C vs 25^{0}C).
Not corrected for lethal clusters. If so, the treatment group (50 r) used would have had its mutation rate decrease by -8% versus 4% for controls.	Corrected for lethal clusters. No differences between control and treatment.
Control radiation exposure not given.	Control radiation exposure reported as 0.6 r.
50 r treatment group had 20,400 less flies than the Caspari experiment.	
The study was not designed to affect the occurrence of lethal clusters.	The study was designed to minimize the possibility of lethal clusters.
F_2 Breeding Protocol Differed: 40 females,/40 males; females – 2 days old	F_2 Breeding Protocol Differed: 50 females/ 100 males; females $\leq$ 16 hours old.
Radiation Exposure Condition Differed: 20 males/capsule; no food in capsule	Radiation Exposure Condition Differed: 50 females/capsule; food in capsule.
Lethal Designation Protocol Differed: Used 6 heterozygote females in F_2 generation to identify lethality.	Lethal Designation Protocol Differed: Used 2 female heterozygotes in F_2 to identify lethality.
A single wild type male offspring lead to a designation of a viable culture.	A single wild type male offspring lead to a designation of a semi-lethal.

EQUIVOCAL RESULTS

➢The findings of Spenser and Stern (Genetics, 1948, 33:43) supported a linear model over the range of doses tested.

➢The follow up study of Caspari and Stern (Genetics, 1948, 33:75) provided support for the threshold dose response model at the lowest dose rate of ionizing radiation yet tested.

➢Uphoff and Stern's first replication of the Caspari and Stern study resulted in the author's conclusion that the results were un-interpretable because of the long control. For the same reason the second experiment would have yielded un-interpretable results

➢Differences in control group rate of mutation.

- ➢The Casperi/Stern study control mutation rates were questioned as possibly being aberrantly high resulting in no treatment affect.
  - ➢ A literature search by Caspari indicated that his mutation frequency was in agreement with the observations of others, including very experienced Drosophila geneticists.
  - ➢Muller provided Caspari with a large body of control group data for aging Drosophila sperm, confirming the observations.
- ➢Low control mutation rates in the first and second Uphoff/Stern experiments were reinterpreted in Uphoff and Stern (Science, 1949, 109:609) as normal, resulting in a treatment effect and supporting the Spencer and Stern results.

➢Validity of the Spencer/Stern study called into question.

- ➢Lack of precise temperature control in the Spencer/Stern study could have affected lethal mutation rates.
  - ➢Certain mutants can behave as semi-lethals at one temperature and lethals at another.
- ➢Averaging of all control data by Spencer/Stern rather than matching of control to treatment data over time could have affected results.
  - ➢It was observed that control mutation rate varied from month to month.
  - ➢In the Caspari and Stern (Genetics, 1948, 33:75) study, the control group showed higher mutation rates than the 50.r treatment group during three of the eight treatment months, indicating that natural background variation for this mutational endpoint can be larger than a possible treatment effect at low dose.
- ➢Data of two separate treatment groups (50r) were combined.

➢Uphoff replications do not provide any resolution.

- ➢Methodological details were no published.
- ➢Control mutation rates were believed to be aberrantly low.
- ➢Positive treatment effect in the third study was abnormally high based on the linear dose response prediction.

BIAS DIRECTED SUPPORT FOR THE LINEAR MODEL

➢Curt Stern assumed that the linear dose response model was accurate and critically important for public policy and directed his scientific efforts to ensure that experiments challenging a linear at low dose perspective would need a higher degree of scientific proof, being subjected to greater efforts at replication and more scrutiny than results that supported a linearity perspective. This conclusion is supported by

- ➢the decision to only replicate the findings of Caspari and Stern (Genetics, 1948, 33:75) and not the Spencer and Stern (Genetics, 1948, 33:43) paper which supported the linearity perspective,
- ➢the assertion that the Caspari and Stern findings could not be accepted until it could be determined why they differed from that of Spencer and Stern,
- ➢the repeated attempts to challenge the findings of Caspari under the assumption that the control group data was spuriously high despite substantial data to the contrary,
- ➢attempts to enhance the credibility, mask the criticism and further the acceptance of the series of Uphoff experiments and
- ➢ failure to adjust the Spencer and Stern (Genetics, 1948, 33:43) study for lethal clusters as was the case for the Caspari and Stern (Genetics, 1948, 33:75) research.

➢The actions displayed by Stern raise questions about whether and to what extent philosphopical/ideological perspectives may have influenced his science. The present analysis suggests that he used his very elevated reputation, his associations with other leaders in the genetics field, his relationship with key journals such as *Science* and the complexity of his research to mask his intentions and activities. He was successful in achieving his goal of ensuring acceptance of the linear model via these multiple manipulations and obfuscations as they reinforced similar biases within the genetics community.

➢The data from Spencer and Stern (Genetics, 1948, 33:43) and Caspari and Stern (Genetics, 1948, 33:75) were actually in close agreement on the nature of the dose response in the low dose zone, even though one strongly supported linearity and the other a threshold interpretation.

- ➢ In both studies it was clear that at the low doses tested they were close to the limits of detection of a treatment effect.
- ➢ In fact, Spencer and Stern (Genetics, 1948, 33:43) noted that it was not uncommon for control mutation rates to exceed those seen at 25 and 50 r, due to background variation.
- ➢In a similar fashion, in three of the eight months of the Caspari study the controls displayed a higher mutation rate than the treatment group.
- ➢ These observations indicate that in this low dose area both studies found it difficult to distinguish treatments from controls.
- ➢ A treatment effect could become statistically significant when a control group yielded an uncharacteristically low value, something that could happen by chance.
  - ➢This possibly happened in the Uphoff replication of Caspari where the control response was about 40% lowered than expected, leading to the significant treatment effect.
  - ➢ Although the control mutation rate was so low in the Uphoff replication experiment Stern was initially committed to using it.
  - ➢The literature research of Caspari which disputed the Stern position and the surprising and copious data of Muller forced him to back down, even though only temporarily.

➢While the evidence is circumstantial, it appears that Stern was determined to suppress the acceptance of the Caspari study.

- ➢ The discussion of Caspari and Stern (Genetics, 1948, 33:75) was, in retrospect, a professional oddity despite its scholarliness.
- ➢This discussion was endorsed by Muller, another strong proponent of linearity.
- ➢When viewed within the framework of promoting the acceptance of linearity at low dose the decision was another example of Stern placing a road block in the path of acceptance of the Caspari data while trying to appear reasonable and objective.

➢Even the case of Spencer and Stern (Genetics, 1948, 33:43), a study that most geneticists of that era could support, had serious methodological issues that challenge the validity of its low dose findings. Nonetheless, both the authors themselves and the genetics community failed to note weaknesses that are obvious in retrospect.

➢The Stern papers represent a case-study for assessing scientific findings within a broader societal context. Stern was an accomplished scientist but his actions suggest strong ideological tendencies. While this historical reassessment has academic interest, the principal significance is that the actions of Stern manipulated the scientific appraisal and the quality of the scientific record on the issue of the dose-response default model, the results of which were to change the course of risk assessment throughout the world for the next 60 years.

CONCLUSIONS

BEAR I support for linearity is challenged. The Spencer and Stern paper, used to support the linearity conclusion., was shown to have critical methodological flaws that affect an interpretaion of low dose effects. The methodologically superior study of Caspari and Stern strongly supported a threshold model. Follow up replication studies by Uphoff and Stern used to support a linearity conclusion, revealed aberrant control group response and a failure of the investigators to publish their methods and documentation.

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Others available upon request