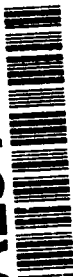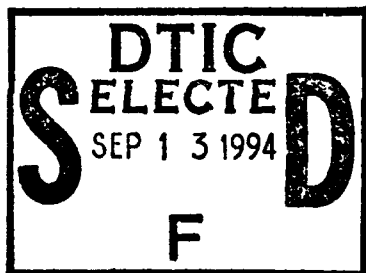# EDGEWOOD

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ERDEC-CR-143

KINEMATIC ANALYSIS OF RABBIT SPERM MOTION IN THE TOXICOLOGICAL ASSESSMENT OF FERTILITY

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13. ABSTRACT (Maximum 200 words)
Rabbit sperm were exposed to three concentrations each of nine compounds known or suspected to be toxic to reproductive function. Sperm motility in the presence and absence of the compounds were recorded on videotape, and the perturbations induced in sperm motions parameters by the compounds were analyzed by the CellTrak/S motion analysis system. The sperm kinematic parameters were used to derive estimates of alterations in fertility due to exposure to the compounds by the utilizing models of the reproductive risk newly developed at the University of California-Davis. The compounds were ranked into three categories of potency by taking into account the differences in the time course of action against spermatozoa and the dose response for each time point. The motion analysis provided a new objective approach to the use of sperm as biomarkers in reproductive risk assessment.

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Sperm	Fertility	
Chemical	Videotape	

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EXECUTIVE SUMMARY

The goal of this project was to translate measurements of perturbations in parameters of sperm motion into estimates of alterations in fertility due to such changes in the sperm. These sperm changes were the consequence of direct exposure to compounds known to be toxic to the reproductive function. The work was organized into several stages and was carried out using the rabbit as a model of the human for such toxic effects. The exposure of rabbit spermatozoa to a series of compounds was performed by Dr. Ronald J. Young, U.S. Army Edgewood Research, Development and Engineering Center, Toxicology Branch. Dr. Young performed replicate experiments in which videotapes were made of suspensions of rabbit sperm exposed to a range of concentrations of each test compound. These tapes were sent to the laboratory at the University of California-Davis (UC-D) for subsequent analysis of sperm kinematic parameters. The analysis was performed using a computer vision instrument, CellTrak/S (version 3.22, Motion Analysis Corporation, Santa Rosa, CA); the generic name used for the analysis is "CASA"- computer aided sperm analysis. An initial validation study was performed to evaluate the accuracy of CellTrak measurements for rabbit spermatozoa as compared to manual frame-by-frame analysis. After the system was validated to UC-D satisfaction, each compound was analyzed, and individual files were imported into Microsoft Excel (version 4.0) for data manipulation and sorting. The data were then inputed into models of reproductive risk developed at UC-D, again using Microsoft Excel.

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PREFACE

The work described in this report was authorized under Contract No. DAAA15-91-C-0130. This work was started in October 1992 and completed in March 1993.

In conducting the work described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," National Institute of Health Publication No. 85-23, 1985, as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, Commission of Life Sciences, National Research Council (Washington, DC). These investigations were also performed in accordance with the requirements of AR 70-18, "Laboratory Animals, Procurement, Transportation, Use, Care, and Public Affairs," and the Laboratory Animal Use and Review Committee (LAURC), U.S. Army Chemical Research, Development and Engineering Center (CRDEC),* which oversees the use of laboratory animals by reviewing for approval all CRDEC research protocols requiring laboratory animals.

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*Now known as the U.S. Army Edgewood Research, Development and Engineering Center.

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KINEMATIC ANALYSIS OF RABBIT SPERM MOTION
IN THE TOXICOLOGICAL ASSESSMENT OF FERTILITY

I.   CASA VALIDATION

Purpose:

To evaluate the accuracy of CellTrak measurements for VCL, VSL and LIN of treated and untreated rabbit sperm, as compared to a manual frame-by-frame analysis.

Methods:

For this study, the Borax Experiment Tape #2 (prepared by Dr. Young on 1/17/91 with rabbit #339) was selected from the available tapes due to the optimal quality of the tape with regard to optics, sperm concentration, and range of percent motilities for the treatment dosages.

A tone-marker was audio-dubbed onto the tape at the beginning of each field after the image was stabilized and focused.   The tape was then analyzed by CellTrak at 60 Hz using one digitized edge and the default-sized window turned on to minimize data overflows. Analysis was triggered by the tone-markers, and the following Calibration Set-up parameters were used (values in ( ) were used for analysis of "plucked" 30 Hz data):

Frame rate (frames/sec)	:	60 (30)
Duration of data capture (frames)	:	30 (15)
Minimum path length (frames)	:	30 (15)
Minimum motile speed (µm/sec)	:	20
Maximum burst speed (µm/sec)	:	500
Distance scale factor (µm/pixel)	:	1.83
Camera aspect ratio	:	1.0000
ALH path smoothing factor (frames)	:	11 (5)
Cent. X search neighborhood (pixels)	:	4
Cent. Y search neighborhood (pixels)	:	2
Cent. cell size minimum (pixels)	:	1
Cent. cell size maximum (pixels)	:	12
Path max. interpolation (frames)	:	1
Path prediction percentage (percent)	:	0
Depth of sample (µm)	:	10
Video processor model	:	VP110

A hardcopy was made of the sperm tracks in each field, and all path and data files were saved (test.pat, vla.now, mad.now). Data were collected for 25-50 sperm for each of the following treatments: Control 0.5 hr., Low dose 0.5 hr., Medium dose 0.5 hr., and High dose 0.5 hr.

Manual analysis was performed on the same sperm at approximately the same time as were analyzed by CellTrak. By using the hardcopy of each field from the CellTrak analysis to identify sperm, and the audio tone-marker for timing, manual sperm tracking began within several frames of the beginning of the CellTrak analysis. Using a 16" B/W Electrohome video monitor and a Panasonic AG6300, the head-midpiece junction of each sperm was tracked frame-by-frame and transferred to an acetate overlay. Each position was then connected by a line to create a path comprised of 15 points (30 Hz). These paths were then measured using a Numonics electronic

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digitizer coupled to an Apple II microcomputer with customized software that calculates VCL, VSL and LIN for each sperm track.

The scale-factor for the manual analysis was computed by measuring the image of a Makler chamber grid on the monitor:
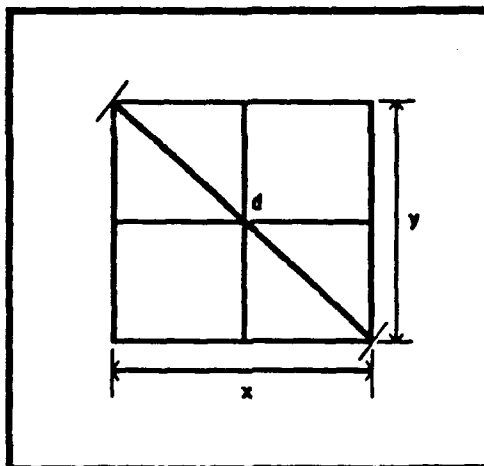
Scale factors were calculated for each direction; x, y and diagonal:

$$X = 17.5 \ \mu m/cm$$
$$Y = 15.7 \ \mu m/cm$$
$$D = 16.6 \ \mu m/cm$$

$$Average = \overline{16.6 \ \mu m/cm}$$

Initially, the average of the three factors was used for the manual analysis. (Previous scale factor calculated for Rat Sperm Validation Study was 16.7 $\mu m/cm$, determined in the X direction only).

Results:

Examples of sperm trajectories for all treatments and both framing rates are shown in Figures 1 (60 Hz) and 2 (30 Hz). The control and the low dose sperm swam with very linear trajectories, while the medium and high dose sperm swam with decreasing linearities and increasing ALH.

Manual vs. CellTrak Analysis:

Summary statistics and correlations for each parameter and all treatment groups are shown in Table 1. Fifty sperm were analyzed for the control and the low dose, while only 33 and 25 sperm were able to be analyzed at the medium and high doses, respectively, due to the lower percent motilities of those samples. CellTrak data shown are for the 30 Hz analysis, to correspond to the manual analyis at 30 Hz. Manual values were calculated with two different scale factors; 16.6 $\mu m/cm$ (average of three scale factors, MANUAL-1), and 17.5 $\mu m/cm$ (x-direction scale factor, MANUAL-2). Results were compared between manual and CellTrak values on a per-sperm basis using a paired-t

test; all parameters were significantly different between Manual-1 and CellTrak for all treatments (p < .01). When the x-direction scale factor was used to compute VCL and VSL (MANUAL-2), the medium and high dose VSL's were not significantly different (p > .05), and the control VSL's approached non-significance at p = .02£. LIN, being a ratio of VSL/VCL, remained the same regardless of the scale factor. When the scale-factor was adjusted on a per-sperm basis according to the primary direction of the sperm's trajectory, all manual values were *still* significantly different from CellTrak's, and in some cases were even *more* significant.

		Manual-1	CellTrak	Correlation (Man-1 v. CT)	Manual-2	Correlation (Man-2 v. CT)
CONTROL	VCL	108.9 ± 4.6	**115.0 ± 4.3**	.97	**114.8 ± 4.9***	.97
	VSL	101.9 ± 5.0	**103.4 ± 4.9***	.98	107.4 ± 5.2	.98
	LIN	.93 ± .02	.88 ± .02	.90	(.93 ± .02)	(.90)
LOW	VCL	109.2 ± 4.1	**116.0 ± 4.0**	.96	**115.1 ± 4.3***	.96
	VSL	101.9 ± 4.4	**103.6 ± 4.6***	.99	107.4 ± 4.6	.99
	LIN	.92 ± .01	.88 ± .02	.68	(.92 ± .01)	(.68)
MEDIUM	VCL	71.2 ± 6.4	**79.6 ± 5.8**	.93	**75.1 ± 6.8***	.93
	VSL	52.3 ± 5.6	**53.0 ± 5.9***	.97	**55.2 ± 6.0***	.97
	LIN	.76 ± .04	.62 ± .04	.41	(.76 ± .04)	(.41)
HIGH	VCL	77.2 ± 5.8	97.4 ± 5.6	.77	81.4 ± 6.1	.77
	VSL	51.3 ± 5.1	**52.9 ± 5.0***	.96	**54.1 ± 5.4***	.96
	LIN	.68 ± .05	.56 ± .05	.78	(.68 ± .05)	(.78)

Table 1. Summary statistics and correlation coefficients for manual and CellTrak values of VCL, VSL, and LIN. Values shown are mean ± SE. Manual-1 values were calculated with the average scale factor, 16.6 μm/cm. Manual-2 values were calculated with the x-direction scale factor, 17.5 μm/cm. Numbers in **bold** and marked by * = **not** significantly different at p > .05.

Regression plots for manual vs. CellTrak analyses for VCL, VSL and LIN are shown in Figures 3 (control sperm), 4 (low dose sperm), 5 (medium dose sperm) and 6 (high dose sperm). The high degrees of correlation between manual and CellTrak datapoints can be seen in these plots for VCL and VSL, however LIN values have much lower correlation. CellTrak VCL values are significantly higher than those obtained by manual analysis; this contributes to the lower LIN values reported by CellTrak, however the differences between manual and CellTrak LIN values are not systematic. There are cases where the manual LIN is lower than the CellTrak LIN for the same sperm. By careful examination of the individual data and sperm tracks for several cases where there was a large discrepancy between manual and CellTrak LIN values, two points became apparent. First, the machine appears to be much better able to discriminate very small differences in the sperm's head position than is discernible by the manual method of tracking the head-midpiece junction (Figure 7). Second, the computer is better able to track erratically moving sperm (Figure 8) because it has the advantage of detecting the head position in relation to the time, whereas by the manual method employed here, the tracking (transferring images to transparencies) and the digitization of those trackpoints are two separate tasks, usually occuring on different days. Solutions to these problems might be to videotape the sperm at higher magnifications, so that minute differences in head position would be more easily seen, and also to utilize a digitizing pen to track the sperm directly off the

monitor (Suarez et al, 1991), rather than manually drawing the tracks and measuring them at a later time.

<u>Effect of Framing Rate</u>:

Summary statistics for each parameter at 60 Hz and 30 Hz are shown in Table 2. A paired t-test was used to compare the results between frame rates. All parameters except VSL were consistently significantly different; VSL was significantly different between the two frame rates for the low dose sperm only (p = .006). This difference is not understood and cannot be explained. Regression plots of the data (not shown) appear to be similar for all treatments, yet only the low dose sperm showed a significant difference between 60 and 30 Hz values.

	FRAME RATE	VCL	VSL	LIN	ALH
CONTROL	60	141.9 ± 3.6	103.4 ± 4.9	.72 ± .02	5.78 ± .19
	30	115.0 ± 4.3	103.4 ± 4.9*	.88 ± .02	4.18 ± .19
LOW	60	142.9 ± 4.3	102.5 ± 4.5	.71 ± .02	5.77 ± .18
	30	116.0 ± 4.0	103.6 ± 4.6	.88 ± .02	4.10 ± .19
MEDIUM	60	129.8 ± 6.3	53.2 ± 5.9	.39 ± .04	6.33 ± .29
	30	79.6 ± 5.8	53.0 ± 5.9*	.62 ± .04	4.32 ± .33
HIGH	60	141.3 ± 6.0	52.6 ± 5.1	.37 ± .03	7.08 ± .45
	30	97.4 ± 5.6	52.9 ± 5.0*	.56 ± .05	5.66 ± .44

Table 2. Summary statistics for CellTrak parameters at 60 and 30 Hz. Values shown are mean ± SE. * = not significantly different at p > .05.

Table 3 shows the correlation coefficients between the 60 and 30 Hz data sets.

	VCL	VSL	LIN	ALH
CONTROL	.87	1.0	.91	.57
LOW	.81	1.0	.92	.44
MEDIUM	.90	1.0	.91	.82
HIGH	.92	.99	.91	.87

Table 3. Correlation coefficients for 60 vs. 30 Hz CellTrak data.

<u>Conclusions</u>:

Rabbit sperm are easily digitizable by the CellTrak instrument; however due to their rapid, linear motion, care must be taken to dilute specimens to an acceptable concentration range in order to avoid "losing" sperm to collisions. In spite of the fact that the differences between manual and CellTrak values are significant in most cases, especially for VCL and LIN, there is generally good agreement between the two methods, given the limitation of the manual method of analysis as described earlier.

FIGURE 1
REPRESENTATIVE SPERM TRACKS COLLECTED AT 60 Hz
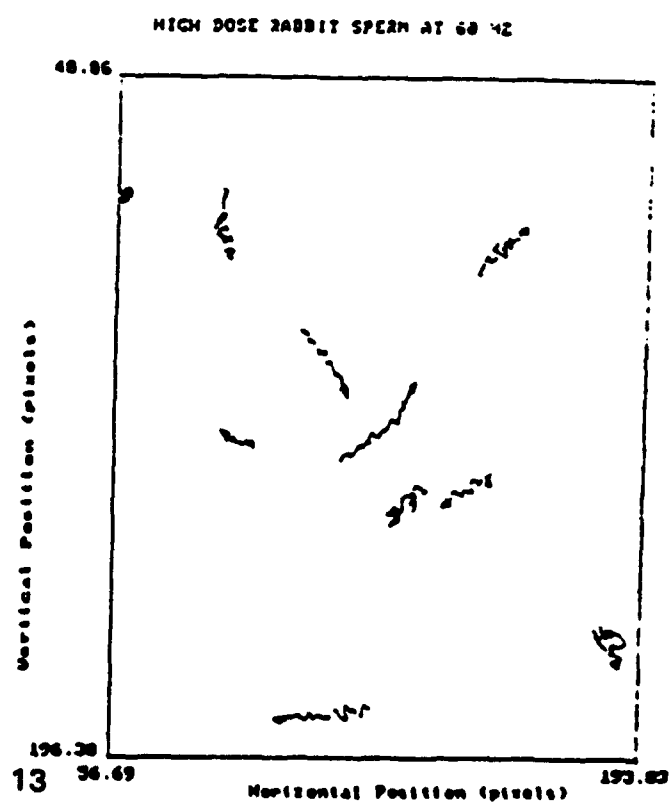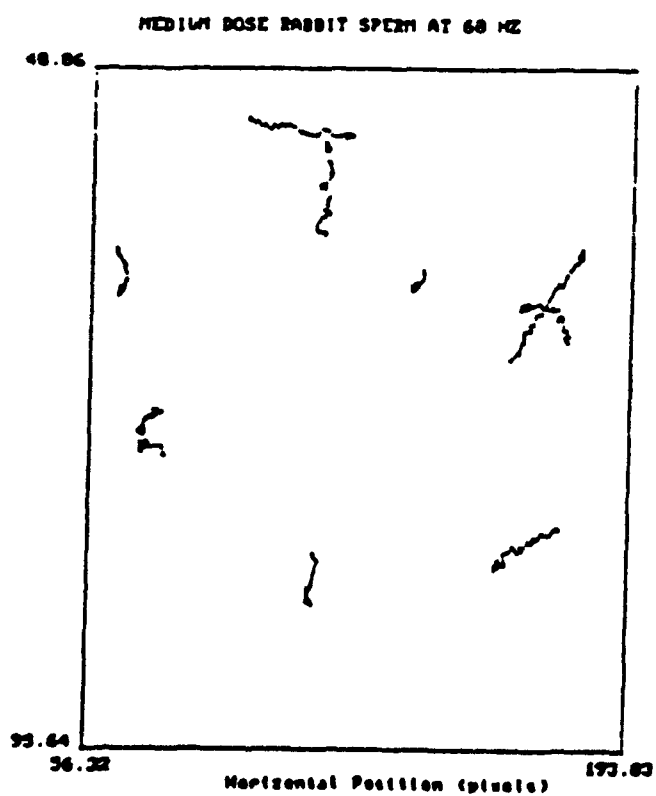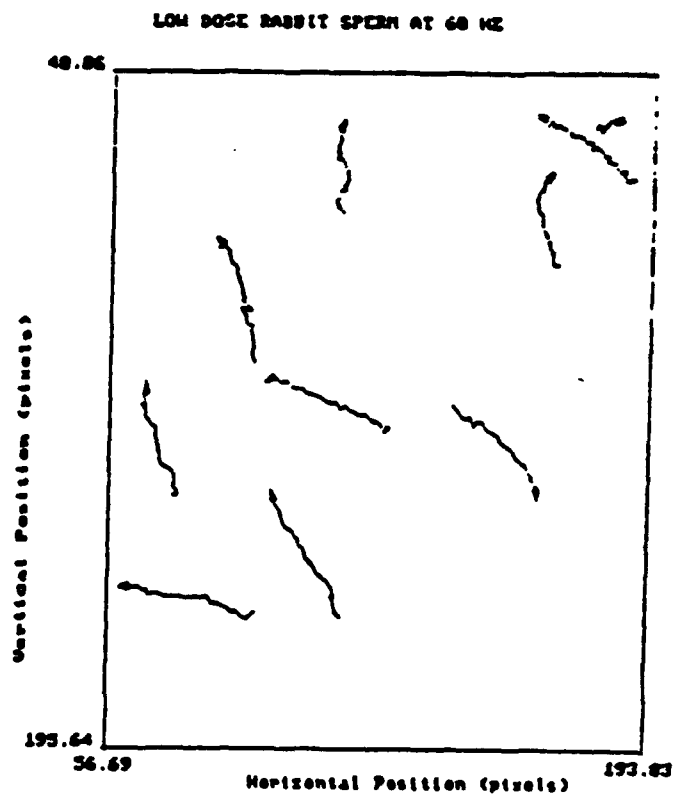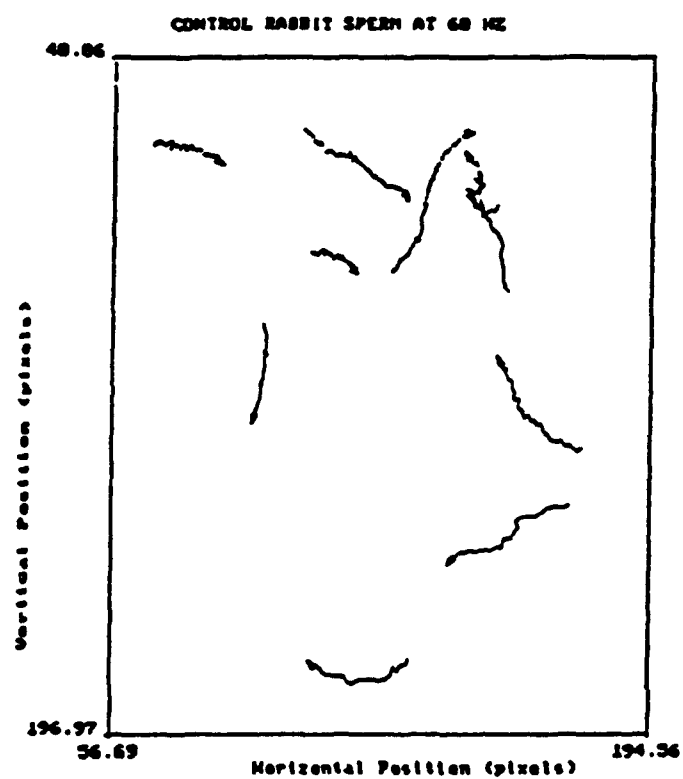
FIGURE 2
REPRESENTATIVE SPERM TRACKS COLLECTED AT 30 Hz

CONTROL RABBIT SPERM AT 30 HZ

LOW DOSE RABBIT SPERM AT 30 HZ

MEDIUM DOSE RABBIT SPERM AT 30 HZ

HIGH DOSE RABBIT SPERM AT 30 HZ
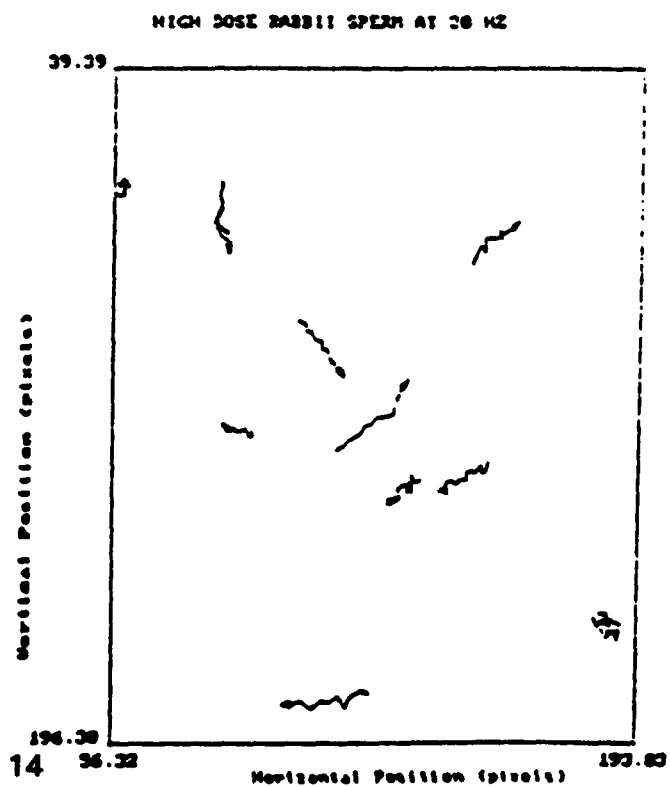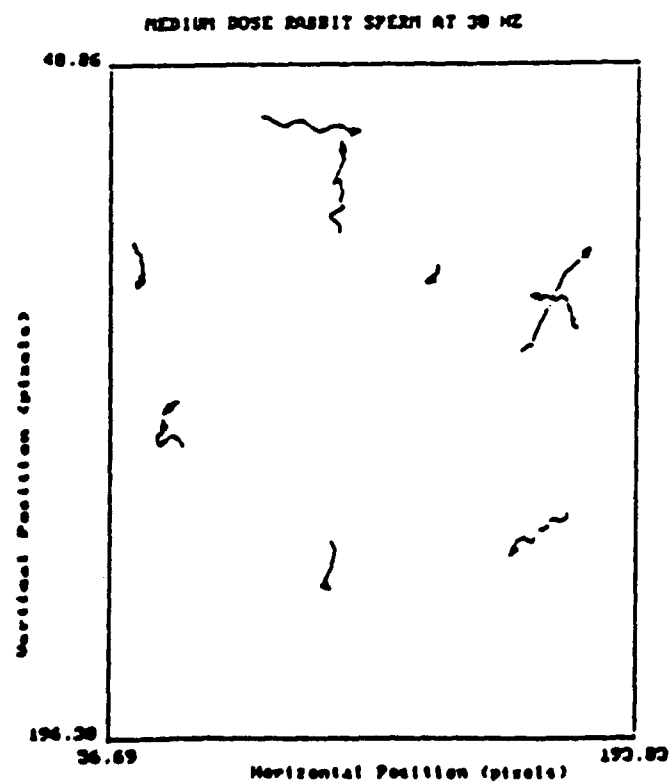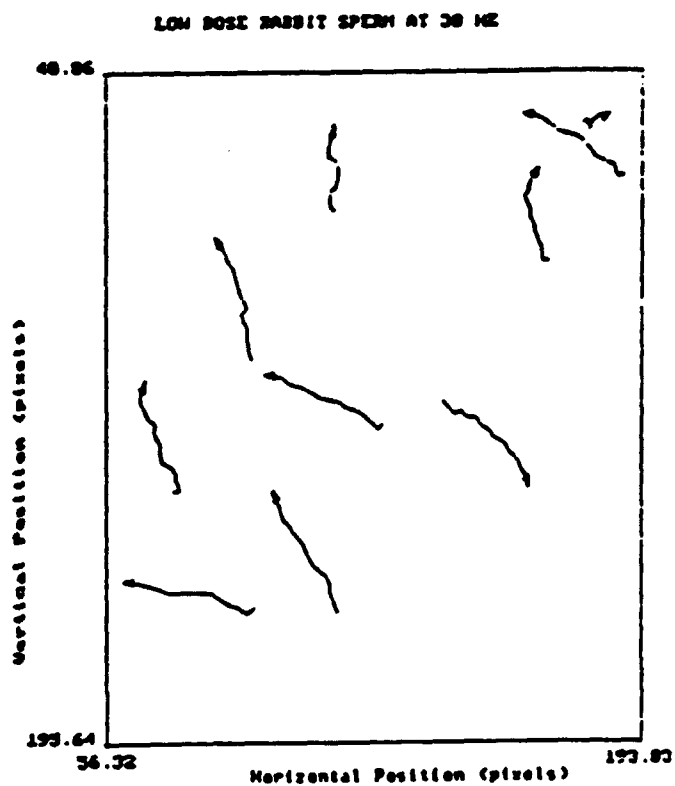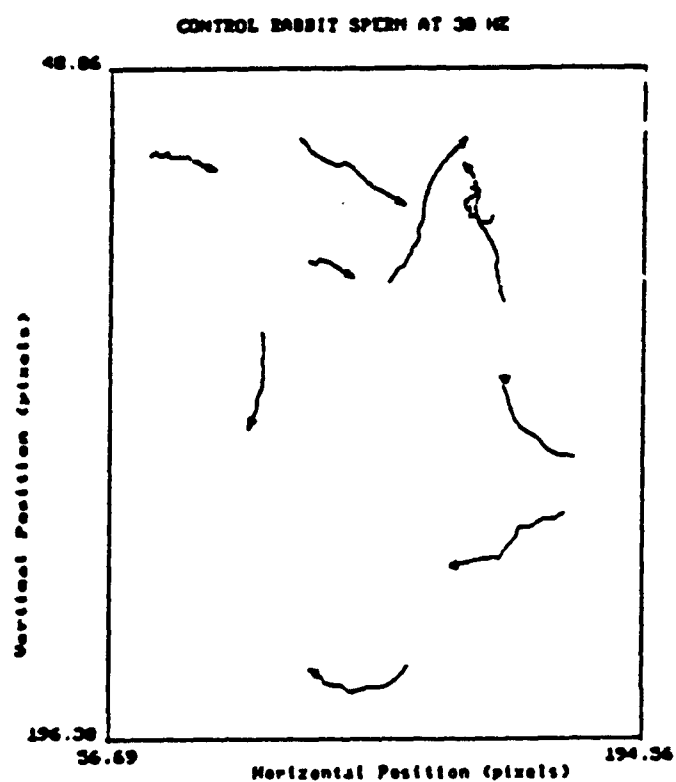
FIGURE 3

CellTrak vs Manual Plots for VCL, VSL and LIN

Control sperm analyzed at 30 Hz
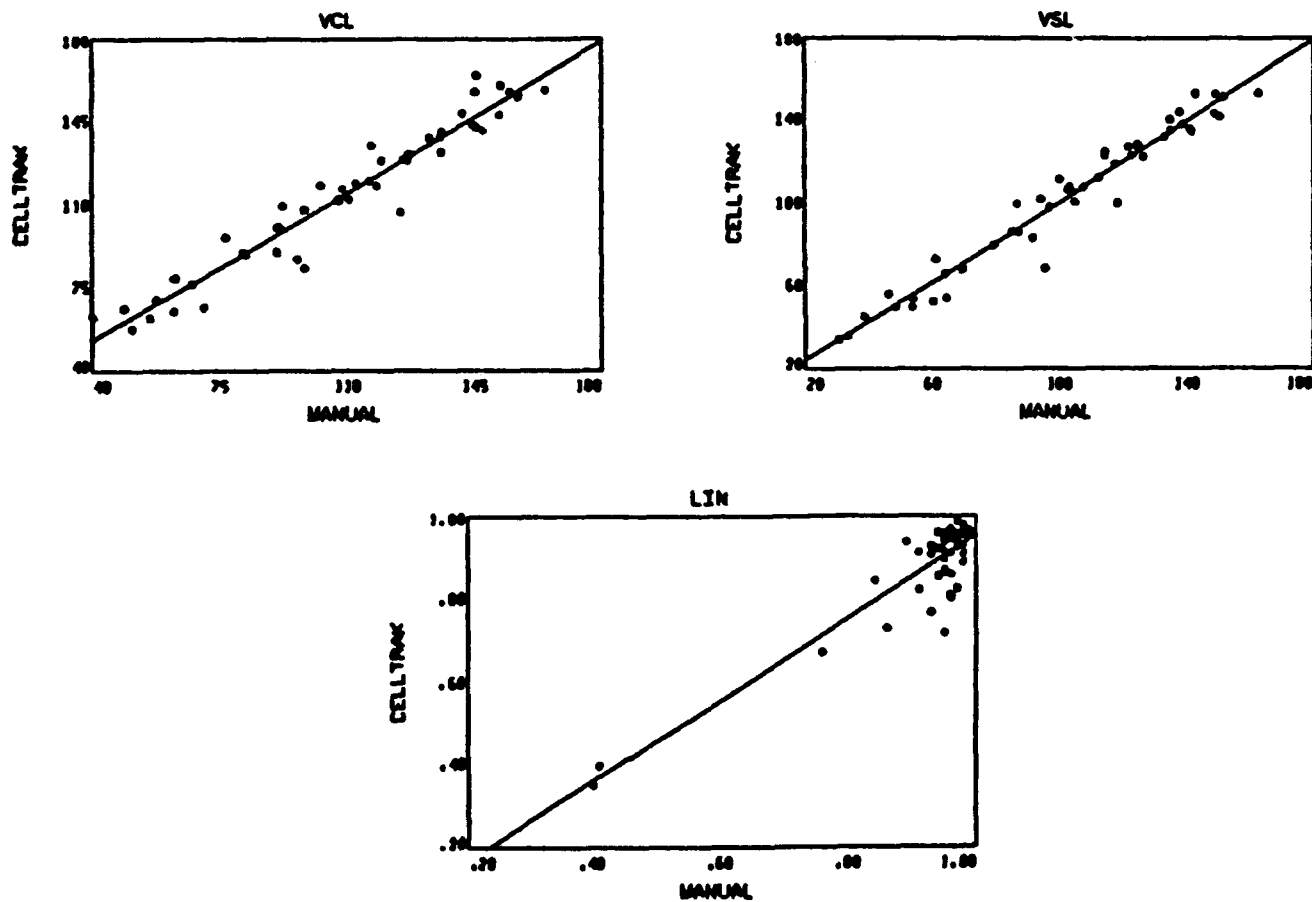
FIGURE 4

CellTrak vs Manual Plots for VCL, VSL and LIN

Low dose sperm analyzed at 30 Hz
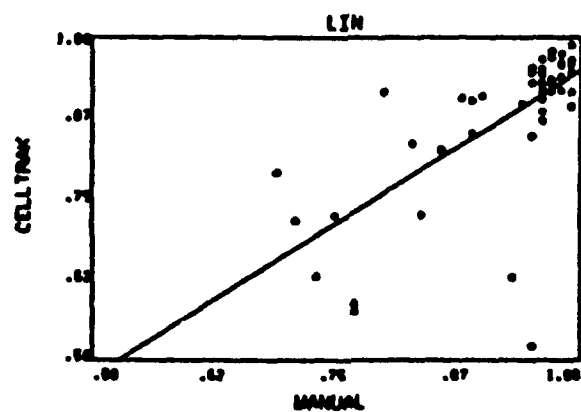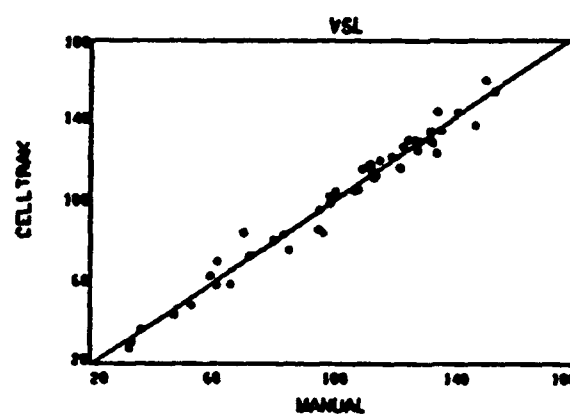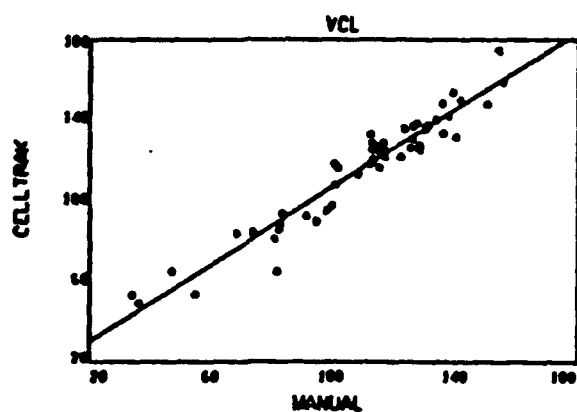
FIGURE 5

CellTrak vs Manual Plots for VCL, VSL and LIN

Medium dose sperm analyzed at 30 Hz
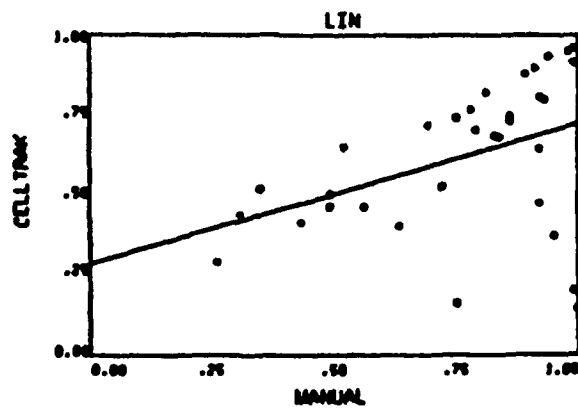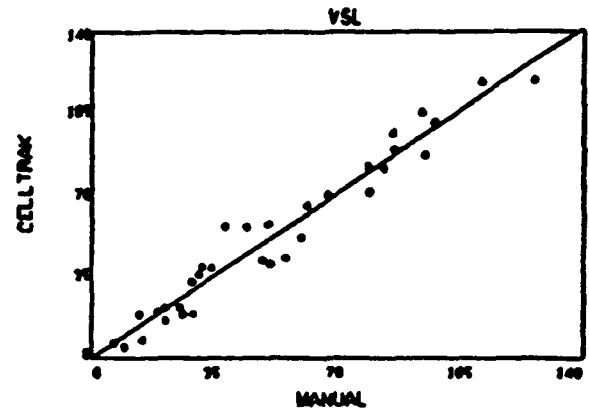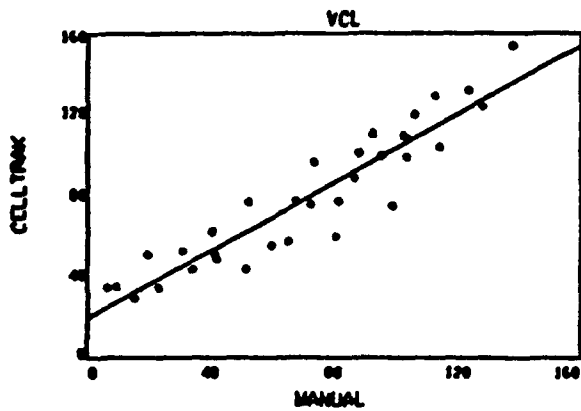
FIGURE 6

CellTrak vs Manual Plots for VCL, VSL and LIN

High dose sperm analyzed at 30 Hz
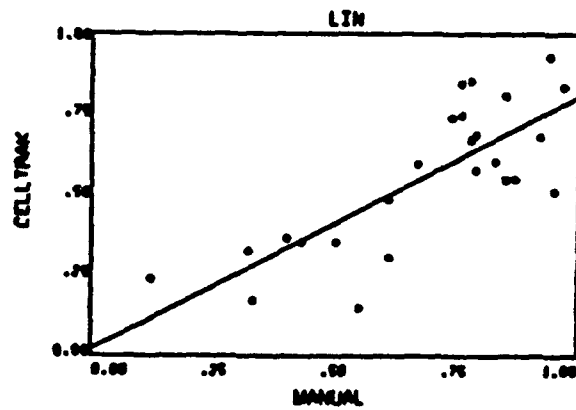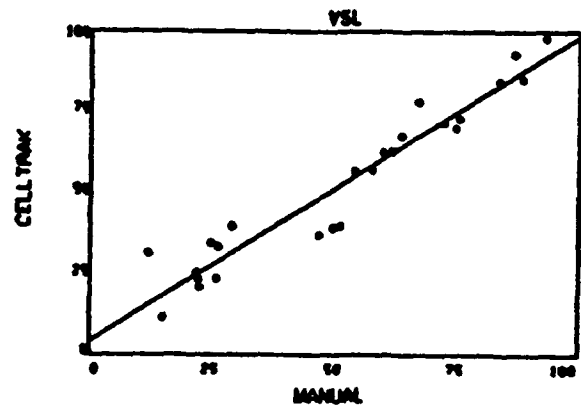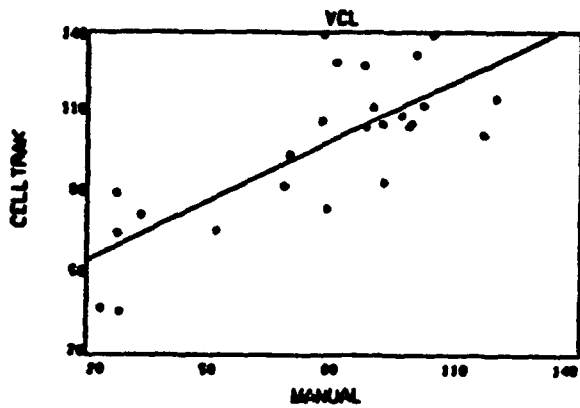
FIGURE 7

boraxh30.pat   HIGH DOSE SPERM #3
Paths: 13

First: 13
Last: 13

119.83

Vertical Position (pixels)

13

CellTrak values:
VCL 65
VSL 33
LIN .51

130.10
80.09   95.82

Horizontal Position (pixels)
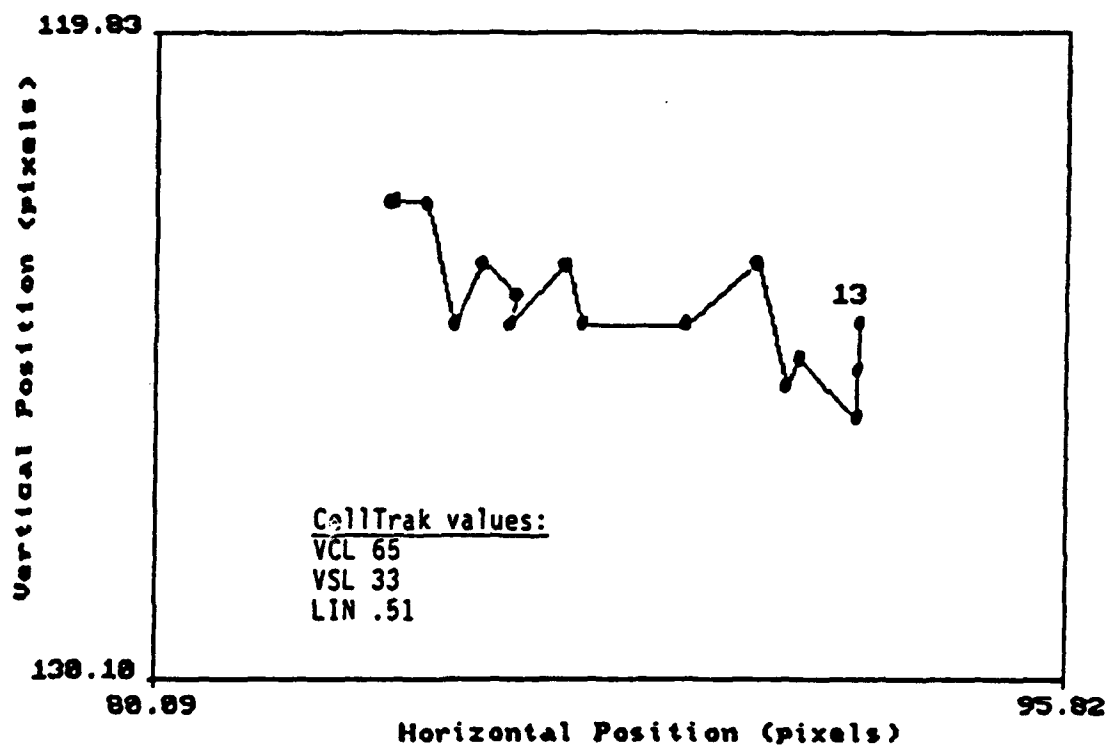
Manual track:

Manual values:
VCL 27
VSL 26
LIN .95

NOTE: Manual and CellTrak paths not shown at the same
      scale!!!

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boraxh38.pat
Paths: 48

First: 48
Last: 48

162.87

48

Vertical Position (pixels)

CellTrak values:
VCL 131
VSL 31
LIN .24

178.43
179.57   193.46
Horizontal Position (pixels)
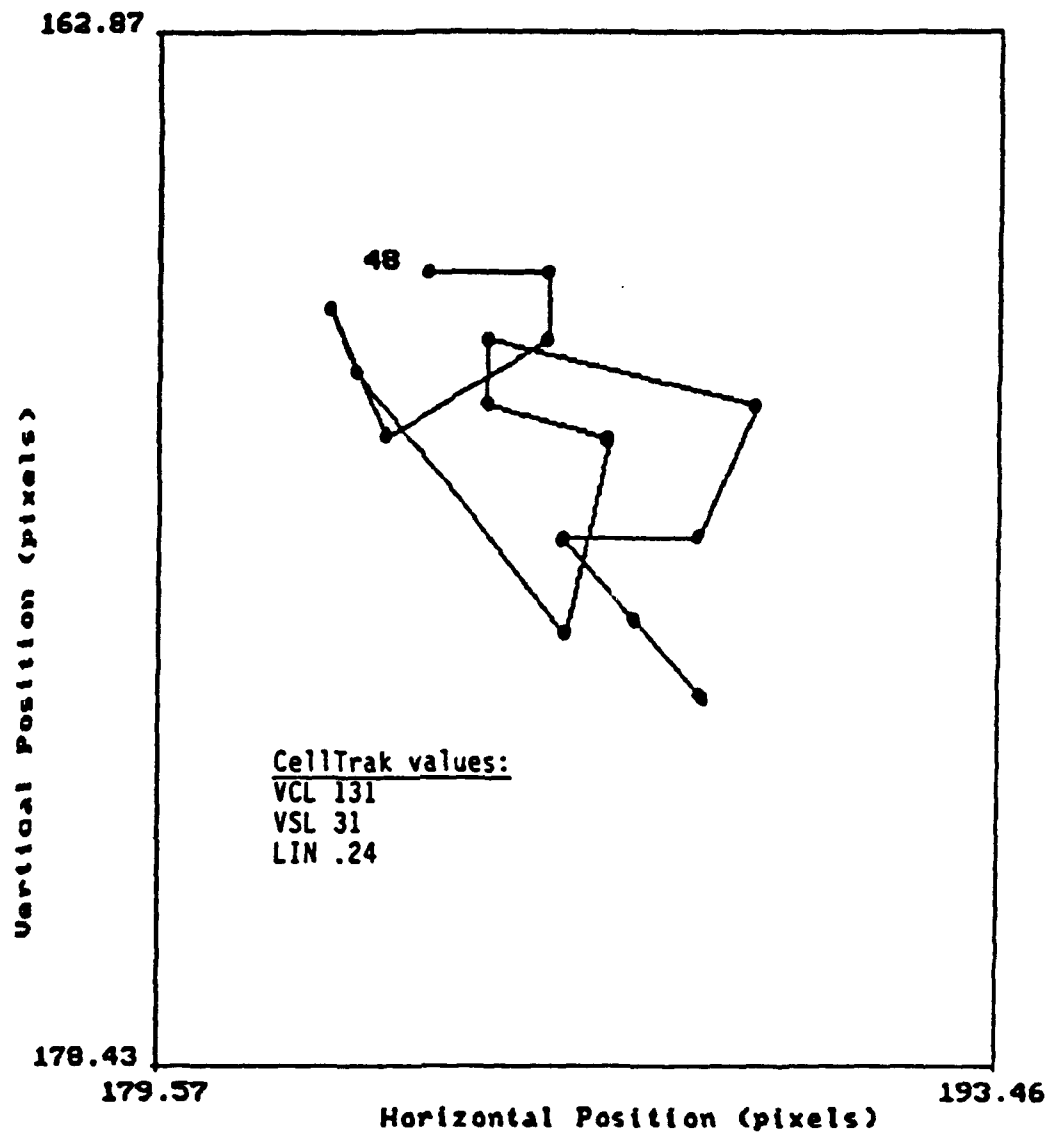
Manual track:

Manual values:
VCL 99
VSL 12
LIN .12

NOTE: Manual and CellTrak paths are not at the same
       scale!!!

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II. CASA METHODS

CASA was performed using the CellTrak system (Motion Analysis Corp., Santa Rosa, CA). Sperm images were digitized using a single right edge (threshold set by visually matching the size of the digitized image, with all four edges on, to the size of videotaped images of progressively motile sperm heads). Sperm were tracked at 60 frames/sec for 305 frames (or 1/2 second). Other CellTrak calibration setup parameters were the same as described earlier for the validation study without the "window" turned on to allow the maximum number of sperm to be tracked per field.

All videotaped fields were analyzed for each treatment in order to maximize the number of sperm tracked. However, due to the nature of the experiments, the number of motile sperm varied according to dosage, and therefore the total number of motile sperm analyzed varied as well. The number of motile sperm in each analysis ranged from 0 to approximately 570, and averaged approximately 200. Parameters measured were % motility, straight-line velocity (VSL), curvilinear velocity (VCL), linearity (LIN), amplitude of lateral head displacement (ALH), and mean angular deviation (MAD).

Following is a list of each compound tested and videotaped. Not all compounds or timepoints were analyzed by CASA. In general, only compounds where three experiments were repeated at the same dosage were analyzed, at .5 and 1 hour timepoints. For several compounds, a 2 hour timepoint was also analyzed if no effect was noted at the earlier timepoints.

COMPOUND	EXPT.#	DATE	RABBIT#	DOSAGES	CASA
Propranolol	1	10/10/90	465	.5,.1,.01 mM	X
	2	12/07/90	485	.4,.1,.05 mM	(X)
	3	02/05/91	465	.3,.1,.03 mM	X*
	4	04/28/92	210	.0003,.0001,.00001M	X*
	5	04/30/92	772	.0003,.0001,.00001M	X*
Borax	1	12/10/90	-	32.5,13.0,6.5 mM	not analyzed
	2	01/17/91	339	26.2,13.1,6.55 mM	X*
	3	11/18/91	688	26.2,13.1,6.55 mM	X*
Epichlorohydrin	1	01/29/91	263	12.7,6.35,3.175 mM	X*
	2	02/12/91	465	12.7,6.35,3.175 mM	X*
	3	02/13/91	556	12.7,6.35,3.175 mM	X*
PERC	1	01/14/91	556	3.91,1.96,0.978 mM	not analyzed

Nonoxynol-9	1	11/05/91	712	.00081,.0000405,.0000162 M*	X*
	2	11/07/91	793	.00081,.0000405,.0000162 M	X*
	3	11/12/91	779	.00081,.0000405,.0000162 M	X*
(hyperactivated)	4	04/16/92	038	.00081,.0000405,.0000162 M	X*
	5	04/22/92	779	.00081,.0000405,.0000162 M	X*
	6	08/11/92	772	.00081,.0000405,.0000162 M	X*
Chlorhexidine	1	10/17/91	688	.0236,.0118,.0059 mM	not analyzed
	2	10/22/91	793	.0147,.0059,.00295 mM	not analyzed
	3	10/31/91	854	.0000118,.0000059,.00000295 M	X*
	4	05/04/92	967	.0000118,.0000059,.00000295 M	X*
	5	05/06/92	972	.0000118,.0000059,.00000295 M	X*
Alpha-Chlorohydrin	1	01/02/92	793	59.8,29.9,11.96 mM	not analyzed
	2	01/07/92	712	29.9,11.96,5.98 mM	X*
	3	01/09/92	772	29.9,11.9,5.98 mM	X*
2,5-Hexanedione	1	01/13/92	688	12.8,8.52,4.26 mM	not analyzed
	2	01/23/92	038	8.52,4.26,1.704 mM	X*
	3	01/28/92	779	8.52,4.26,1.704 mM	X*
Dinitrobenzene	1	01/30/92	712	3.567,1.78,1.19 mM	X*
	2	02/03/92	213	3.567,1.78,1.19 mM	X*
	3	02/10/92	793	3.567,1.78,1.19 mM	X*
	4	05/27/92	772	3.56,1.78,1.189 mM	X*
	5	06/09/92	779	3.56,1.78,1.189 mM	X*
EGME	1	02/13/92	779	0.634,0.317,0.127 M	X*
	2	02/18/92	210	0.634,0.317,0.127 M	X*
	3	02/20/92	712	0.634,0.317,0.127 M	X*
Lead	1	06/11/92	038	.000025,.0000125,.000005 M	X*
	2	06/18/92	712	.000025,.0000125,.000005 M	X*
	3	07/16/92	772	.000025,.0000125,.000005 M	X*
	4	07/21/92	864	.000025,.0000125,.000005 M	X*
	5	07/28/92	793	.000025,.0000125,.000005 M	X*

All samples were taped at 0.5, 1, 2, 4 and 6 hours after addition of the compound. Control samples were diluted in T6 medium and were also taped at t=0 hours.

X = CASA complete for .5 and 1 hour (and 2 hour for propranolol and epichlorohydrin; all timepoints for lead);

(X) = need to reanalyze to increase sperm numbers. * = experiments used for fertility models.

After each analysis, *motile.ind* and *motile.mad* ASCII files were saved and stored on floppy disks for permanent records and further statistical analysis. These files contain VCL, LIN, ALH, and MAD values for each motile (>20 μm/sec VSL) sperm analyzed. VSL is derived by multiplying VCL x LIN. *Test.pat* (binary) was also saved for a permanent record of the paths.

III. THE RISK ASSESSMENT MODEL

The reproductive risk model derives from ideas initially proposed by C. van Duijn for predicting the fertility of cattle and sheep after artificial insemination (van Duijn, 1965). van Duijn developed a simple model relating insemination dose to the probability of fertility. We have extended his ideas in two basic ways, and then recast them into a model of risk assessment rather than fertility itself. First, we have utilized a body of knowledge related to sperm motility and transport in the female, and to fertilization itself, that was obtained after van Duijn's pioneering efforts (cf. Katz et al, 1989). On the basis of these studies, we have modified the criteria for functional spermatozoa to include thresholds in measures of the vigor and pattern of sperm motion. Thus, our "insemination dose" is the number of sperm that have a velocity > 100 μm/sec and a linearity of motion > 0.8 (see below). We have also utilized more modern data on minimum insemination doses necessary to achieve fertility. Here, we considered a range of values, in order to account for the difference between a species of high relative fertility (the rabbit) and a species with lower relative fertility (the human). We have retained the value of the parameter $k_f / k_q = 1.8$, obtained experimentally by van Duijn for cattle and sheep. This parameter is the ratio of the temporal decline in egg fertilizability divided by the rate of decline of sperm fertility with time (for a given number of sperm). We believe, on the basis of our own and others' studies, that this ratio is very nearly conserved among mammals. We did conduct parametric studies of our data which demonstrated a relative lack of sensitivity to small perturbations in the exact value of this parameter. Our model of reproductive risk computes the ratio of the altered probability of fertility (due to exposure to a toxicant) divided by the initial, unperturbed fertility. This ratio is akin to the odds ratio frequently used in risk assessment, but is not identical to it.

1)   For a fixed delivery flux of sperm in the female tract to the egg:
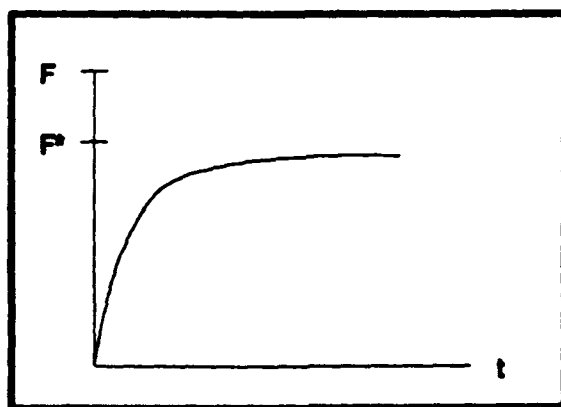
$$\frac{dF}{dt} = k_F (F^* - F) \tag{1}$$

or

$$\frac{F}{F^*} = 1 - e^{-k_F t} \tag{2}$$

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F = probability of fertilization

F^* = maximum possible value of F; $F^* \leq 1$

t = time

k_F = rate constant for change in fertilization probability with time for fixed supply of sperm

2)   Let $Q = Nv$ be the net transport flux or supply rate of fertile sperm in the female tract

N = number of fertile sperm

v = average straight-line velocity of fertile sperm

$$\frac{dQ}{dt} = -k_Q Q \qquad (3)$$

or

$$Q = Q_o e^{-k_Q t} \qquad (4)$$

Q_o = initial flux of functional sperm

k_Q = rate constant for decline of sperm flux with time

3)   We assume that there is a limiting or threshold value of Q, call it Q_T, at or below which there is no chance of fertilization, i.e.

$$F = 0 \text{ and } Q \leq Q_T \qquad (5)$$

Now combine equations (1) and (3):

$$\frac{dF}{dQ} = \frac{k_F}{k_Q} \frac{(F^* - F)}{Q} \qquad (6)$$

Integrating, using the boundary condition (5)

$$\frac{F}{F^*} = 1 - \left(\frac{Q}{Q_T}\right)^{-k_F/k_Q}$$

or

$$\frac{F}{F^*} = 1 - \left(\frac{Q_T}{Q}\right)^{k_F/k_Q} \qquad (7)$$

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We shall apply equation (7) with respect to the initial value of Q, call it Q_o, at insemination.

Note that the value $\dfrac{Q_T}{Q_o}$ can be obtained from experimental data for a species under given

conditions of insemination. So can the value of k_F/k_Q .

Now suppose there is a perturbation in sperm numbers and quality due to exposure to some toxicant. Let a prime symbol ' refer to these conditions, i.e

$$\frac{F}{F'} = 1 - (\tfrac{Q_T}{Q})^{k_F/k_Q} \qquad (8)$$

Combining equations (7) and (8):

$$\frac{F'}{F} = \frac{1 - \left[(\tfrac{Q_o}{Q})^{k_F/k_Q}\right](\tfrac{Q_T}{Q})^{k_F/k_Q}}{1 - (\tfrac{Q_T}{Q})^{k_F/k_Q}} \qquad (9)$$

$\dfrac{F'}{F}$ is the reduction in fertility due to exposure.

$\dfrac{Q_T}{Q_o}$ and k_F/k_Q are the empirical constraints for the species.

$\dfrac{Q_o'}{Q_o}$ can be determined experimentally from exposure data.

van Duijn calculated the values k_F/k_Q ~ 1.7 - 1.8 for different data sets for the bovine. These

values imply that the decline in fertilization probability is 1.7 - 1.8 X the decline in sperm flux. We

still have to input the ratio $\dfrac{Q_T}{Q_o}$ for the species.

Based on limited data in the human,

$$\frac{1}{20} < \frac{Q_T}{Q_o} < \frac{1}{10}$$

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For species with higher fertility rates (such as the rabbit), $\frac{Q_T}{Q_o} \approx \frac{1}{100}$

For our exposure data for the rabbit, we compute Q_o as the percentage of sperm with VSL > 100 µm/sec and LIN > 0.8, multiplied by v, the average value of VSL for this subpopulation. Thus, for each dosage value for a compound we compute Q_o'. Our control value Q_o is also obtained. These are substituted into equation (9) to determine $\frac{F'}{F}$.

IV. APPLICATION OF THE RISK ASSESSMENT MODEL

In order to apply the fertility model described above to our experimental data, *motile.ind* files were imported into Excel, where the percent of progressive sperm (VSL > 100 μm/sec and LIN > .8) was calculated for each sample. The model was applied by two different methods:

Method 1

For all compounds, $\frac{Q_o}{Q_e}$ was calculated for each treatment (low, medium and high doses) and experiments as follows:

$$\frac{Q_o}{Q_e} = \frac{(P_C)(VSL_C)}{(P_T)(VSL_T)}$$

P_C = % progressive for Control group

VSL_C = VSL of progressive sperm in the Control group

P_T = % progressive for Treated group

VSL_T = VSL for progressive sperm in the Treated group

Then, using $\frac{Q_T}{Q_e}$ ratios of $\frac{1}{20}$ (or .05) and $\frac{1}{100}$ (or .01), and $\frac{k_F}{k_Q} = 1.8$, $\frac{F}{F}$ was calculated for each treatment from formula (9):

$$\frac{F}{F} = \frac{1 - \left[(\frac{Q_T}{Q_e})^{k/k_Q}\right](\frac{Q_o}{Q_e})^{k/k_Q}}{1 - (\frac{Q_T}{Q_e})^{k/k_Q}}$$

Results of $\frac{F}{F}$ calculated by this method for each compound follow.

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ACHFERT.XLS

ALPHACHLOROHYDRIN DOSE RESPONSE OF F'/F FOR Qt/Qo = 1/20 AND 1/100

.5 HR

EXPT. #	CONTROL % PROG, VSL	LOW DOSE % PROG, VSL	MEDIUM DOSE % PROG, VSL	HIGH DOSE % PROG, VSL	Qt/Qo RATIO	LOW DOSE F'/F	MEDIUM DOSE F'/F	HIGH DOSE F'/F
2	52% 125.3	41% 122.1	42% 122.5	12% 111.5	0.05 0.01	1.00 1.00	1.00 1.00	0.93 1.00
3	32% 123.1	34% 124.4	36% 125.3	5% 110.9	0.05 0.01	1.00 1.00	1.00 1.00	0.85 0.99

1 HR

EXPT. #	CONTROL % PROG, VSL	LOW DOSE % PROG, VSL	MEDIUM DOSE % PROG, VSL	HIGH DOSE % PROG, VSL	Qt/Qo RATIO	LOW DOSE F'/F	MEDIUM DOSE F'/F	HIGH DOSE F'/F
2	44% 126.9	32% 119.8	18% 114.9	0%	0.05 0.01	1.00 1.00	0.98 1.00	0 0
3	32% 130.1	30% 122.5	12% 112.6	0%	0.05 0.01	1.00 1.00	0.97 1.00	0 0

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BORAX DOSE RESPONSE OF F'/F FOR Qt/Qo = 1/20 AND 1/100

.5 HR

EXPT. #	CONTROL % PROG, VSL	LOW DOSE % PROG, VSL	MEDIUM DOSE % PROG, VSL	HIGH DOSE % PROG, VSL	Qt/Qo RATIO	LOW DOSE F'/F	MEDIUM DOSE F'/F	HIGH DOSE F'/F
2	30% 140.7	29% 130.7	3% 123.1	0%	0.05 0.01	1.00 1.00	0.64 0.98	0 0
3	40% 133.6	23% 123.2	18% 117.1	1% 103.9	0.05 0.01	0.99 1.00	0.98 1.00	0 0.70

1 HR

EXPT. #	CONTROL % PROG, VSL	LOW DOSE % PROG, VSL	MEDIUM DOSE % PROG, VSL	HIGH DOSE % PROG, VSL	Qt/Qo RATIO	LOW DOSE F'/F	MEDIUM DOSE F'/F	HIGH DOSE F'/F
2	32% 141.7	28% 127.1	8% 122.9	0%	0.05 0.01	1.00 1.00	0.93 1.00	0 0
3	36% 134.9	19% 119.9	19% 116.2	0.05% 107.2	0.05 0.01	0.99 1.00	0.99 1.00	0 0

CHLORHEXIDINE DOSE RESPONSE OF F'/F FOR Qt/Qo = 1/20 AND 1/100

.5 HR

EXPT. #	CONTROL % PROG, VSL	LOW DOSE % PROG, VSL	MEDIUM DOSE % PROG, VSL	HIGH DOSE % PROG, VSL	Qt/Qo RATIO	LOW DOSE F'/F	MEDIUM DOSE F'/F	HIGH DOSE F'/F
4	11% 122.8	7% 125.3	17% 128.6	0.02% 111.4	0.05 0.01	0.99 1.00	1.00 1.00	0 0
5	30% 120.3	21% 125.8	26% 122.5	8% 115.6	0.05 0.01	1.00 1.00	1.00 1.00	0.95 1.00

1 HR

EXPT. #	CONTROL % PROG, VSL	LOW DOSE % PROG, VSL	MEDIUM DOSE % PROG, VSL	HIGH DOSE % PROG, VSL	Qt/Qo RATIO	LOW DOSE F'/F	MEDIUM DOSE F'/F	HIGH DOSE F'/F
4	9% 126.2	4% 140.6	13% 128.1	0%	0.05 0.01	0.99 1.00	1.00 1.00	0 0
5	27% 125.6	22% 124.9	14% 120.2	0%	0.05 0.01	1.00 1.00	0.99 1.00	0 0

DINITROBENZENE DOSE RESPONSE OF F'/F FOR Qt/Qo = 1/20 AND 1/100

.5 HR

EXPT. #	CONTROL % PROG, VSL	LOW DOSE % PROG, VSL	MEDIUM DOSE % PROG, VSL	HIGH DOSE % PROG, VSL	Qt/Qo RATIO	LOW DOSE F'/F	MEDIUM DOSE F'/F	HIGH DOSE F'/F
1	27% 113	0%	.4% 108.4	1% 105	0.05 0.01	0 0	0.85 0.99	0 0.89
3	24% 110.2	2% 107	2% 109.9	0.03% 103	0.05 0.01	0.58 0.98	0.60 0.98	0 0
4	18% 115.4	2% 115.2	2% 106.7	2% 103.8	0.05 0.01	0.77 0.99	0.73 0.99	0.72 0.98

1 HR

EXPT. #	CONTROL % PROG, VSL	LOW DOSE % PROG, VSL	MEDIUM DOSE % PROG, VSL	HIGH DOSE % PROG, VSL	Qt/Qo RATIO	LOW DOSE F'/F	MEDIUM DOSE F'/F	HIGH DOSE F'/F
1	19% 113.9	2% 103.3	1% 107.2	0%	0.05 0.01	0.69 0.98	0 0.94	0 0
3	19% 110	1% 102.3	0.03% 109.3	0.00%	0.05 0.01	0 0.94	0 0	0 0
4	15% 116	4% 110.8	2% 109.1	0.03% 105.6	0.05 0.01	0.95 1.00	0.81 0.99	0 0

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EGME DOSE RESPONSE OF F'/F FOR Qt/Qo = 1/20 AND 1/100

.5 HR

EXPT. #	CONTROL % PROG, VSL	LOW DOSE % PROG, VSL	MEDIUM DOSE % PROG, VSL	HIGH DOSE % PROG, VSL	Qt/Qo RATIO	LOW DOSE F'/F	MEDIUM DOSE F'/F	HIGH DOSE F'/F
1	27% 130.8	23% 125	7% 115.5	1% 112.6	0.05 0.01	1.00 1.00	0.94 1.00	0 0.88
2	27% 114	27% 114.1	27% 114.2	7% 108.2	0.05 0.01	1.00 1.00	1.00 1.00	0.95 1.00
3	45% 118.8	42% 123.7	27% 115.7	27% 112.1	0.05 0.01	1.00 1.00	0.99 1.00	0.99 1.00

1 HR

EXPT. #	CONTROL % PROG, VSL	LOW DOSE % PROG, VSL	MEDIUM DOSE % PROG, VSL	HIGH DOSE % PROG, VSL	Qt/Qo RATIO	LOW DOSE F'/F	MEDIUM DOSE F'/F	HIGH DOSE F'/F
1	15% 121.6	16% 124.2	5% 111.1	1% 109.5	0.05 0.01	1.00 1.00	0.97 1.00	0.28 0.96
2	32% 116.7	20% 113.1	10% 110.2	1% 102.1	0.05 0.01	0.99 1.00	0.96 1.00	0 0.84
3	41% 128.4	16% 110	22% 111.3	23% 111.7	0.05 0.01	0.97 1.00	0.99 1.00	0.99 1.00

EPICHLOROHYDRIN DOSE RESPONSE OF F'/F FOR Qt/Qo = 1/20 AND 1/100

.5 HR

EXPT. #	CONTROL % PROG, VSL	LOW DOSE % PROG, VSL	MEDIUM DOSE % PROG, VSL	HIGH DOSE % PROG, VSL	Qt/Qo RATIO	LOW DOSE F/F	MEDIUM DOSE F/F	HIGH DOSE F/F
1	12% 119.1	8% 109.2	14% 122.8	3% 111.3	0.05 / 0.01	0.99 / 1.00	1.00 / 1.00	0.94 / 1.00
2	17% 149.4	21% 126	27% 128.5	11% 125.3	0.05 / 0.01	1.00 / 1.00	1.00 / 1.00	0.99 / 1.00
3	34% 136	26% 127.4	21% 120	30% 119	0.05 / 0.01	1.00 / 1.00	0.99 / 1.00	1.00 / 1.00

1 HR

EXPT. #	CONTROL % PROG, VSL	LOW DOSE % PROG, VSL	MEDIUM DOSE % PROG, VSL	HIGH DOSE % PROG, VSL	Qt/Qo RATIO	LOW DOSE F/F	MEDIUM DOSE F/F	HIGH DOSE F/F
1	11% 129.8	11% 115.7	6% 111.1	7% 138.8	0.05 / 0.01	1.00 / 1.00	0.99 / 1.00	1.00 / 1.00
2	25% 133.1	17% 135	26% 140.1	16% 117.7	0.05 / 0.01	1.00 / 1.00	1.00 / 1.00	0.99 / 1.00
3	37% 133.9	43% 137	32% 128.1	9% 115.1	0.05 / 0.01	1.00 / 1.00	1.00 / 1.00	0.93 / 1.00

2 HR

EXPT. #	CONTROL % PROG, VSL	LOW DOSE % PROG, VSL	MEDIUM DOSE % PROG, VSL	HIGH DOSE % PROG, VSL	Qt/Qo RATIO	LOW DOSE F/F	MEDIUM DOSE F/F	HIGH DOSE F/F
1	20% 133	8% 109.2	0%	0%	0.05 / 0.01	0.97 / 1.00	0 / 0	0 / 0
2	23% 142.3	12% 103.8	7% 115	0%	0.05 / 0.01	0.98 / 1.00	0.95 / 1.00	0 / 0
3	25% 139	29% 130	15% 118.8	0%	0.05 / 0.01	1.00 / 1.00	0.99 / 1.00	0 / 0

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2,5 - HEXANEDIONE DOSE RESPONSE OF F'/F FOR Qt/Qo = 1/20 AND 1/100

.5 HR

EXPT. #	CONTROL % PROG, VSL	LOW DOSE % PROG, VSL	MEDIUM DOSE % PROG, VSL	HIGH DOSE % PROG, VSL	Qt/Qo RATIO	LOW DOSE F'/F	MEDIUM DOSE F'/F	HIGH DOSE F'/F
2	39% 118.7	24% 119.5	22% 116.7	28% 115.6	0.05 0.01	0.99 1.00	0.99 1.00	1.00 1.00
3	20% 129.2	17% 124.1	17% 129	12% 120.8	0.05 0.01	1.00 1.00	1.00 1.00	0.99 1.00

1 HR

EXPT. #	CONTROL % PROG, VSL	LOW DOSE % PROG, VSL	MEDIUM DOSE % PROG, VSL	HIGH DOSE % PROG, VSL	Qt/Qo RATIO	LOW DOSE F'/F	MEDIUM DOSE F'/F	HIGH DOSE F'/F
2	30% 123.5	24% 116.2	19% 118.7	14% 110.2	0.05 0.01	1.00 1.00	0.99 1.00	0.98 1.00
3	26% 133.1	20% 131.9	17% 127.6	17% 120.6	0.05 0.01	1.00 1.00	0.99 1.00	0.99 1.00

LEAD DOSE RESPONSE OF F/F FOR Qt/Qo = 1/20 AND 1/100

.5 HR

EXPT. #	CONTROL % PROG, VSL	LOW DOSE % PROG, VSL	MEDIUM DOSE % PROG, VSL	HIGH DOSE % PROG, VSL	Qt/Qo RATIO	LOW DOSE F/F	MEDIUM DOSE F/F	HIGH DOSE F/F
3	9% 119.3	25% 129.9	59% 138.4	14% 121.9	0.05 0.01	1.00 1.00	1.00 1.00	1.00 1.00
4	28% 126.4	50% 130.9	39% 129.3	30% 127.9	0.05 0.01	1.00 1.00	1.00 1.00	1.00 1.00
5	5% 110.6	17% 118.4	20% 115.3	45% 121.6	0.05 0.01	1.00 1.00	1.00 1.00	1.00 1.00

1 HR

EXPT. #	CONTROL % PROG, VSL	LOW DOSE % PROG, VSL	MEDIUM DOSE % PROG, VSL	HIGH DOSE % PROG, VSL	Qt/Qo RATIO	LOW DOSE F/F	MEDIUM DOSE F/F	HIGH DOSE F/F
3	20% 125.6	40% 130	35% 135	51% 135.9	0.05 0.01	1.00 1.00	1.00 1.00	1.00 1.00
4	38% 125.5	44% 131.9	44% 132.9	50% 140.5	0.05 0.01	1.00 1.00	1.00 1.00	1.00 1.00
5	22% 112.6	27% 122.6	30% 123.6	20% 112.3	0.05 0.01	1.00 1.00	1.00 1.00	1.00 1.00

LEADFERT.XLS

2 HR

EXPT. #	CONTROL % PROG, VSL	LOW DOSE % PROG, VSL	MEDIUM DOSE % PROG, VSL	HIGH DOSE % PROG, VSL	Qi/Qo RATIO	LOW DOSE F/F	MEDIUM DOSE F/F	HIGH DOSE F/F
3	41% 127.2	38% 132.3	43% 132.8	48% 125.7	0.05 0.01	1.00 1.00	1.00 1.00	1.00 1.00
4	27% 131.6	40% 129.6	43% 139.9	41% 137.8	0.05 0.01	1.00 1.00	1.00 1.00	1.00 1.00
5	18% 112.3	41% 124.5	58% 125.3	48% 123.8	0.05 0.01	1.00 1.00	1.00 1.00	1.00 1.00

4 HR

EXPT. #	CONTROL % PROG, VSL	LOW DOSE % PROG, VSL	MEDIUM DOSE % PROG, VSL	HIGH DOSE % PROG, VSL	Qi/Qo RATIO	LOW DOSE F/F	MEDIUM DOSE F/F	HIGH DOSE F/F
3	24% 121.3	34% 127.7	21% 136.1	20% 118.1	0.05 0.01	1.00 1.00	1.00 1.00	1.00 1.00
4	47% 129.7	34% 129.1	29% 135.7	37% 131	0.05 0.01	1.00 1.00	0.99 1.00	1.00 1.00
5	36% 118.3	34% 122.9	21% 115.3	20% 118	0.05 0.01	1.00 1.00	0.99 1.00	0.99 1.00

6 HR

EXPT. #	CONTROL % PROG, VSL	LOW DOSE % PROG, VSL	MEDIUM DOSE % PROG, VSL	HIGH DOSE % PROG, VSL	Qi/Qo RATIO	LOW DOSE F/F	MEDIUM DOSE F/F	HIGH DOSE F/F
3	29% 118.9	12% 116.5	5% 110.6	4% 115.8	0.05 0.01	0.98 1.00	0.88 0.99	0.83 0.99
4	38% 130.9	28% 130.9	18% 121.2	21% 120.3	0.05 0.01	1.00 1.00	0.98 1.00	0.99 1.00
5	21% 113.3	3% 119.5	3% 107.4	2% 137.5	0.05 0.01	0.87 0.99	0.84 0.99	0.78 0.99

NONOXYNOL-9

NOFERT.XLS

DOSE RESPONSE OF F'/F FOR Qt/Qo = 1/20 AND 1/100

.5 HR

EXPT. #	CONTROL % PROG, VSL	LOW DOSE % PROG, VSL	MEDIUM DOSE % PROG, VSL	HIGH DOSE % PROG, VSL	Qt/Qo RATIO	LOW DOSE F'/F	MEDIUM DOSE F'/F	HIGH DOSE F'/F
1	47% 115.1	36% 110.7	5% 103	0%	0.05 0.01	1.00 1.00	0 0.98	0 0
2	38% 122.2	25% 122.6	5% 114.5	0%	0.05 0.01	0.99 1.00	0.00 0.99	0 0
3	20% 128.1	6% 119.3	3% 115.4	0%	0.05 0.01	0.96 1.00	0.84 0.99	0 0
4	33% 118.8	16% 119.3	2% 111.5	1% 116.3	0.05 0.01	0.99 1.00	0.21 0.96	0 0
5	22% 121.1	14% 128.7	5% 124.5	0%	0.05 0.01	1.00 1.00	0.94 1.00	0 0
6	30% 126.1	29% 123.8	21% 121	10% 112.7	0.05 0.01	1.00 1.00	1.00 1.00	0 0

EXPT. #	CONTROL % PROG, VSL	LOW DOSE % PROG, VSL	MEDIUM DOSE % PROG, VSL	HIGH DOSE % PROG, VSL	Qi/Qo RATIO	LOW DOSE F/F	MEDIUM DOSE F/F	HIGH DOSE F/F
1	39% 123.8	14% 116	2% 113.7	0%	0.05 0.01	0.97 1.00	0 0.94	0 0
2	39% 121.6	20% 122.9	3% 115.3	0%	0.05 0.01	0.99 1.00	0.50 0.97	0 0
3	16% 127.6	9% 126.7	0%	0%	0.05 0.01	0.99 1.00	0 0	0 0
4	36% 117.5	21% 114.4	1% 104.6	0%	0.05 0.01	0.99 1.00	0 0.80	0 0
5	20% 125.8	11% 123.5	3% 117.6	0%	0.05 0.01	0.99 1.00	0.85 0.99	0 0
6	26% 124.3	29% 126.4	15% 123.2	2% 114.3	0.05 0.01	1.00 1.00	0.99 1.00	0.47 0.97

PROPRANOLOL DOSE RESPONSE OF F'/F FOR Qt/Qo = 1/20 AND 1/100

.5 HR

EXPT. #	CONTROL % PROG, VSL	LOW DOSE % PROG, VSL	MEDIUM DOSE % PROG, VSL	HIGH DOSE % PROG, VSL	Qt/Qo RATIO	LOW DOSE F'/F	MEDIUM DOSE F'/F	HIGH DOSE F'/F
1	10% 132.2	10% 124.8	11% 129.5	0%	0.05 0.01	1.00 1.00	1.00 1.00	0 0
3	13% 139.2	12% 131.3	10% 133.8	0.02% 112.9	0.05 0.01	1.00 1.00	1.00 1.00	0 0
4	25% 115.4	21% 117.2	2% 106.5	0.03% 111.5	0.05 0.01	1.00 1.00	0.51 0.97	0 0
5	19% 122.1	15% 118.5	5% 119	2% 110.4	0.05 0.01	1.00 1.00	0.95 1.00	0.69 0.98

1 HR

EXPT. #	CONTROL % PROG, VSL	LOW DOSE % PROG, VSL	MEDIUM DOSE % PROG, VSL	HIGH DOSE % PROG, VSL	Qt/Qo RATIO	LOW DOSE F'/F	MEDIUM DOSE F'/F	HIGH DOSE F'/F
1	17% 129.8	20% 134	14% 125.7	0%	0.05 0.01	1.00 1.00	1.00 1.00	0 0
3	21% 137.7	17% 133.9	8% 124.1	0%	0.05 0.01	1.00 1.00	0.97 1.00	0 0
4	23% 114.8	20% 115.2	3% 111	0.04% 103	0.05 0.01	1.00 1.00	0.81 0.99	0 0
5	17% 122.3	9% 115.7	5% 118.5	2% 110.3	0.05 0.01	0.99 1.00	0.96 1.00	0.75 0.99

PROPFERT.XLS

2 HR

EXPT. #	CONTROL % PROG, VSL	LOW DOSE % PROG, VSL	MEDIUM DOSE % PROG, VSL	HIGH DOSE % PROG, VSL	Qt/Qo RATIO	LOW DOSE F/F	MEDIUM DOSE F/F	HIGH DOSE F/F
1	23% 138.8	20% 126.7	9% 124.5	0%	0.05 0.01	1.00 1.00	0 0	0 0
3	29% 134.4	13% 135.4	5% 113.8	0%	0.05 0.01	0.99 1.00	0.86 0.99	0 0
4	38% 118.7	15% 112.7	2% 104	0%	0.05 0.01	0.98 1.00	0 0.94	0 0
5	34% 126.2	4% 127.5	3% 117	0.05% 115.4	0.05 0.01	0.79 0.99	0.59 0.98	0 0

Dosages differ for each experiment: Expt. 1 = .5,.1,.01mM; Expt. 3 = .3,.1,.03 mM; Expt 4,5 = .3,.1,.01mM.

Method 2

In order to eliminate some of the variation between experiments seen when $\frac{F}{F}$ was calculated

by Method 1, a second method was employed. This time $\frac{Q_t}{Q_o}$ was calculated for each treatment as

described for Method 1 and then *averaged* across all experiments. Then, a *single* value of $\frac{F}{F}$ was

calculated from formula (9) for each treatment, using $\frac{Q_t}{Q_o} = \frac{1}{20}$ only. Results using this method follow.

ALPHACHLOROHYDRIN

DOSE RESPONSE OF F'/F FOR Qt/Qo = 1/20

.5 HR

EXPT. #	CONTROL % PROG, VSL	LOW DOSE % PROG, VSL	MEDIUM DOSE % PROG, VSL	HIGH DOSE % PROG, VSL	LOW DOSE Qo/Qo'	MEDIUM DOSE Qo/Qo'	HIGH DOSE Qo/Qo'
2	52% 125.3	41% 122.1	42% 122.5	12% 111.5	1.30	1.27	4.87
3	32% 123.1	34% 124.4	36% 125.3	5% 110.9	0.93	0.87	7.10

AVERAGE Qo/Qo'= 1.12   1.07   5.99

	LOW	MEDIUM	HIGH
F'/F=	1.00	1.00	0.89

ALHPACHLOROHYDRIN

DOSE RESPONSE OF F'/F FOR Qt/Qo = 1/20

1 HR

EXPT. #	CONTROL % PROG. VSL	LOW DOSE % PROG. VSL	MEDIUM DOSE % PROG. VSL	HIGH DOSE % PROG. VSL	LOW DOSE Qo/Qo'	MEDIUM DOSE Qo/Qo'	HIGH DOSE Qo/Qo'
2	44% 126.9	32% 119.8	18% 114.9	0%	1.46	2.70	0
3	32% 130.1	30% 122.5	12% 112.6	0%	1.13	3.08	0

AVERAGE Qo/Qo':   1.29   2.89   0

	LOW	MEDIUM	HIGH
F'/F=	1.00	0.97	0

BORAX

DOSE RESPONSE OF F'/F FOR Qt/Qo = 1/20

.5 HR

EXPT. #	CONTROL % PROG. VSL	LOW DOSE % PROG. VSL	MEDIUM DOSE % PROG. VSL	HIGH DOSE % PROG. VSL	LOW DOSE Qo/Qo'	MEDIUM DOSE Qo/Qo'	HIGH DOSE Qo/Qo'
2	30% 140.7	29% 130.7	3% 123.1	0%	1.11	11.43	0
3	40% 133.6	23% 123.2	18% 117.1	1% 103.9	1.89	2.54	51.43

AVERAGE Qo/Qo': 1.50   6.98   51.43

	LOW	MEDIUM	HIGH
F'/F=	1.00	0.85	0

BORX1MDL.XLS

BORAX

DOSE RESPONSE OF F'/F FOR Qt/Qo = 1/20

1 HR

EXPT. #	CONTROL % PROG. VSL	LOW DOSE % PROG. VSL	MEDIUM DOSE % PROG. VSL	HIGH DOSE % PROG. VSL	LOW DOSE Qo/Qo'	MEDIUM DOSE Qo/Qo'	HIGH DOSE Qo/Qo'
2	32% 141.7	28% 127.1	8% 122.9	0%	1.27	4.61	0
3	36% 134.9	19% 119.9	19% 116.2	0.05% 107.2	2.13	2.20	906.04

AVERAGE Qo/Qo': 1.70   3.41   906.04

F/F=	LOW 0.99	MEDIUM 0.96	HIGH 0

46

CHLORHEXIDINE

DOSE RESPONSE OF F'/F FOR Qt/Qo = 1/20

.5 HR

EXPT. #	CONTROL % PROG. VSL	LOW DOSE % PROG. VSL	MEDIUM DOSE % PROG. VSL	HIGH DOSE % PROG. VSL	LOW DOSE Qo/Qo'	MEDIUM DOSE Qo/Qo'	HIGH DOSE Qo/Qo'
4	11% 122.8	7% 125.3	17% 128.6	0.02% 111.4	1.54	0.62	606.28
5	30% 120.3	21% 125.8	26% 122.5	8% 115.6	1.37	1.13	3.90

AVERAGE Qo/Qo':   1.45   0.88   305.09

LOW	MEDIUM	HIGH
F'/F = 1.00	1.00	0

47

CHLORHEXIDINE

DOSE RESPONSE OF F'/F FOR Qt/Qo = 1/20

1 HR

EXPT. #	CONTROL % PROG. VSL	LOW DOSE % PROG. VSL	MEDIUM DOSE % PROG. VSL	HIGH DOSE % PROG. VSL	LOW DOSE Qo'/Qo'	MEDIUM DOSE Qo'/Qo'	HIGH DOSE Qo'/Qo'
4	0% 126.2	4% 140.6	13% 128.1	0%	2.02	0.68	0
5	27% 125.6	22% 124.9	14% 120.2	0%	1.23	2.02	0

AVERAGE Qo/Qo':

	LOW	MEDIUM	HIGH
	1.63	1.35	0

	LOW	MEDIUM	HIGH
F'/F=	0.99	1.00	0

DNB

DOSE RESPONSE OF F'/F FOR Qt/Qo = 1/20

.5 HR

EXPT. #	CONTROL % PROG. VSL	LOW DOSE % PROG. VSL	MEDIUM DOSE % PROG. VSL	HIGH DOSE % PROG. VSL	LOW DOSE Qo/Qo'	MEDIUM DOSE Qo/Qo'	HIGH DOSE Qo/Qo'
1	27% 113	0%	4% 108.4	1% 105	NA	7.04	29.06
3	24% 110.2	2% 107	2% 109.9	0.03% 103	12.36	12.03	855.92
4	18% 115.4	2% 115.2	2% 106.7	2% 103.8	9.02	9.73	10.01

AVERAGE Qo/Qo':   10.69   9.60   298.33

	LOW	MEDIUM	HIGH
F'/F=	0.68	0.74	0

49

DNB1MDL.XLS

DNB

DOSE RESPONSE OF F'/F FOR Qt/Qo = 1/20

1 HR

EXPT. #	CONTROL % PROG. VSL	LOW DOSE % PROG. VSL	MEDIUM DOSE % PROG. VSL	HIGH DOSE % PROG. VSL	LOW DOSE Qo/Qo'	MEDIUM DOSE Qo/Qo'	HIGH DOSE Qo/Qo'
1	19% 113.9	2% 103.3	1% 107.2	0%	10.47	20.19	0
3	19% 110	1% 102.3	0.03% 109.3	0%	20.43	637.39	0
4	15% 116	4% 110.8	2% 109.1	0.03% 105.6	3.93	7.97	549.24

AVERAGE Qo/Qo's: 11.61   221.85   549.24

F'/F=	LOW 0.63	MEDIUM 0	HIGH 0

50

EGME

DOSE RESPONSE OF F'/F FOR Qv/Qo = 1/20

.5 HR

EXPT. #	CONTROL % PROG, VSL	LOW DOSE % PROG, VSL	MEDIUM DOSE % PROG, VSL	HIGH DOSE % PROG, VSL	LOW DOSE Qo/Qo'	MEDIUM DOSE Qo/Qo'	HIGH DOSE Qo/Qo'
1	27% 130.8	23% 125	7% 115.5	1% 112.8	1.23	4.37	31.36
2	27% 114	27% 114.1	27% 114.2	7% 108.2	1.00	1.00	4.06
3	45% 118.8	42% 123.7	27% 115.7	27% 112.1	1.03	1.71	1.77

AVERAGE $Qo/Qo's$ 1.09   2.36   12.40

	LOW	MEDIUM	HIGH
F/F=	1.00	0.98	0.58

EGME

DOSE RESPONSE OF F'/F FOR Qv/Qo = 1/20

1 HR

EXPT. #	CONTROL % PROG, VSL	LOW DOSE % PROG, VSL	MEDIUM DOSE % PROG, VSL	HIGH DOSE % PROG, VSL	LOW DOSE Qo/Qo'	MEDIUM DOSE Qo/Qo'	HIGH DOSE Qo/Qo'
1	15% 121.6	16% 124.2	5% 111.1	1% 109.5	0.92	3.28	16.66
2	32% 116.7	20% 113.1	10% 110.2	1% 102.1	1.65	3.39	36.58
3	41% 128.4	16% 110	22% 111.3	23% 111.7	2.99	2.15	2.05

AVERAGE Qo/Qo's   1.85   2.94   18.43

	LOW	MEDIUM	HIGH
F'/F=	0.99	0.97	0.14

52

EPICHLOROHYDRIN

DOSE RESPONSE OF F/F FOR Qt/Qo = 1/20

.5 HR

EXPT. #	CONTROL % PROG. VSL	LOW DOSE % PROG. VSL	MEDIUM DOSE % PROG. VSL	HIGH DOSE % PROG. VSL	LOW DOSE Qo/Qo'	MEDIUM DOSE Qo/Qo'	HIGH DOSE Qo/Qo'
1	12% 119.1	8% 109.2	14% 122.8	3% 111.3	1.64	0.83	4.28
2	17% 149.4	21% 126	27% 128.5	11% 125.3	0.96	0.73	1.84
3	34% 136	26% 127.4	21% 120	30% 119	1.40	1.83	1.30

AVERAGE Qo/Qo'=   1.33   1.13   2.47

	LOW	MEDIUM	HIGH
F/F=	1.00	1.00	0.98

EPICHLOROHYDRIN

DOSE RESPONSE OF F'/F FOR Qt/Qo = 1/20

1 HR

EXPT. #	CONTROL % PROG, VSL	LOW DOSE % PROG, VSL	MEDIUM DOSE % PROG, VSL	HIGH DOSE % PROG, VSL	LOW DOSE Qo/Qo'	MEDIUM DOSE Qo/Qo'	HIGH DOSE Qo/Qo'
1	11% 129.8	11% 115.7	6% 111.1	7% 138.8	1.12	2.14	1.47
2	25% 133.1	17% 135	26% 140.1	16% 117.7	1.45	0.91	1.77
3	37% 133.9	43% 137	32% 128.1	9% 115.1	0.84	1.21	4.76

AVERAGE Qo/Qo'=   1.14   1.42   2.67

	LOW	MEDIUM	HIGH
F'/F=	1.00	1.00	0.98

54

EPICHLOROHYDRIN

DOSE RESPONSE OF F'/F FOR Qt/Qo = 1/20

2 HR

EXPT. #	CONTROL % PROG. VSL	LOW DOSE % PROG. VSL	MEDIUM DOSE % PROG. VSL	HIGH DOSE % PROG. VSL	LOW DOSE Qo/Qo'	MEDIUM DOSE Qo/Qo'	HIGH DOSE Qo/Qo'
1	20% 133	8% 109.2	0%	0%	3.04	0	0
2	23% 142.3	12% 103.8	7% 115	0%	2.63	4.07	0
3	25% 139	29% 130	15% 118.8	0%	0.92	1.95	0

AVERAGE Qo/Qo: 2.20   3.01   0

	LOW	MEDIUM	HIGH
F'/F=	0.99	0.97	0

2,5-HEXANEDIONE

DOSE RESPONSE OF F'/F FOR Qt/Qo = 1/20

.5 HR

EXPT. #	CONTROL % PROG. VSL	LOW DOSE % PROG. VSL	MEDIUM DOSE % PROG. VSL	HIGH DOSE % PROG. VSL	LOW DOSE Qo/Qo'	MEDIUM DOSE Qo/Qo'	HIGH DOSE Qo/Qo'
2	39% 118.7	24% 119.5	22% 116.7	28% 115.6	1.61	1.80	1.43
3	20% 129.2	17% 124.1	17% 129	12% 120.8	1.22	1.18	1.78

AVERAGE Qo/Qo': 1.42 1.49 1.61

	LOW	MEDIUM	HIGH
F'/F=	1.00	1.00	0.99

2,5-HEXANEDIONE

DOSE RESPONSE OF F'/F FOR Qt/Qo = 1/20

1 HR

EXPT. #	CONTROL % PROG. VSL	LOW DOSE % PROG. VSL	MEDIUM DOSE % PROG. VSL	HIGH DOSE % PROG. VSL	LOW DOSE Qo/Qo'	MEDIUM DOSE Qo/Qo'	HIGH DOSE Qo/Qo'
2	30% 123.5	24% 116.2	19% 118.7	14% 110.2	1.33	1.64	2.40
3	26% 133.1	20% 131.9	17% 127.6	17% 120.6	1.31	1.60	1.69

AVERAGE Qo/Qo': 1.32   1.62   2.04

	LOW	MEDIUM	HIGH
F'/F=	1.00	0.99	0.99

57

LEAD

DOSE RESPONSE OF F'/F FOR Qt/Qo = 1/20

.5 HR

EXPT. #	CONTROL % PROG, VSL	LOW DOSE % PROG, VSL	MEDIUM DOSE % PROG, VSL	HIGH DOSE % PROG, VSL	LOW DOSE Qo/Qo'	MEDIUM DOSE Qo/Qo'	HIGH DOSE Qo/Qo'
3	9% 119.3	25% 129.9	59% 138.4	14% 121.9	0.33	0.13	0.63
4	28% 126.4	50% 130.9	39% 129.3	30% 127.9	0.54	0.70	0.92
5	5% 110.6	17% 118.4	20% 115.3	45% 121.6	0.27	0.24	0.10

AVERAGE Qo/Qo'=   0.38   0.36   0.55

	LOW	MEDIUM	HIGH
F'/F=	1.00	1.00	1.00

58

LEAD

DOSE RESPONSE OF F'/F FOR Qt/Qo = 1/20

1 HR

EXPT. #	CONTROL % PROG. VSL	LOW DOSE % PROG. VSL	MEDIUM DOSE % PROG. VSL	HIGH DOSE % PROG. VSL	LOW DOSE Qo/Qo'	MEDIUM DOSE Qo/Qo'	HIGH DOSE Qo/Qo'
3	20% 125.6	40% 130	35% 135	51% 135.9	0.48	0.53	0.36
4	38% 125.5	44% 131.9	44% 132.9	50% 140.5	0.82	0.82	0.68
5	22% 112.6	27% 122.6	30% 123.6	20% 112.3	0.75	0.67	1.10

AVERAGE Qo/Qo'=   0.68   0.67   0.71

F'/F=	LOW 1.00	MEDIUM 1.00	HIGH 1.00

LEAD

DOSE RESPONSE OF F'/F FOR Qt/Qo = 1/20

2 HR

EXPT. #	CONTROL % PROG. VSL	LOW DOSE % PROG. VSL	MEDIUM DOSE % PROG. VSL	HIGH DOSE % PROG. VSL	LOW DOSE Qo/Qo'	MEDIUM DOSE Qo/Qo'	HIGH DOSE Qo/Qo'
3	41% 127.2	38% 132.3	43% 132.8	48% 125.7	1.04	0.91	0.66
4	27% 131.6	40% 129.6	43% 139.9	41% 137.8	0.69	0.59	0.63
5	18% 112.3	41% 124.5	58% 125.3	48% 123.8	0.40	0.28	0.34

AVERAGE Qo/Qo=   0.71   0.59   0.61

	LOW	MEDIUM	HIGH
F'/F=	1.00	1.00	1.00

60

LEAD

DOSE RESPONSE OF F'/F FOR Qt/Qo = 1/20

4 HR

EXPT. #	CONTROL % PROG, VSL	LOW DOSE % PROG, VSL	MEDIUM DOSE % PROG, VSL	HIGH DOSE % PROG, VSL	LOW DOSE Qo/Qo'	MEDIUM DOSE Qo/Qo'	HIGH DOSE Qo/Qo'
3	24% 121.3	34% 127.7	21% 136.1	20% 118.1	0.67	1.02	1.23
4	47% 129.7	34% 129.1	29% 135.7	37% 131	1.39	1.55	1.26
5	36% 118.2	34% 122.9	21% 115.3	20% 118	1.02	1.76	1.80

AVERAGE Qo/Qo'=   1.03   1.44   1.43

	LOW	MEDIUM	HIGH
F'/F=	1.00	1.00	1.00

LEAD

DOSE RESPONSE OF F/F FOR Qt/Qo = 1/20

6 HR

EXPT. #	CONTROL % PROG, VSL	LOW DOSE % PROG, VSL	MEDIUM DOSE % PROG, VSL	HIGH DOSE % PROG, VSL	LOW DOSE Qo/Qo'	MEDIUM DOSE Qo/Qo'	HIGH DOSE Qo/Qo'
3	29% 118.9	12% 116.5	5% 110.6	4% 115.8	2.47	6.24	7.44
4	36% 130.9	28% 130.9	18% 121.2	21% 120.3	1.36	2.28	1.97
5	21% 113.3	3% 119.5	3% 107.4	2% 137.5	6.64	7.36	8.65

AVERAGE Qo/Qo'=   3.49   5.30   6.02

	LOW	MEDIUM	HIGH
F/F=	0.98	0.91	0.89

62

NONOXYNOL-9

DOSE RESPONSE OF F'/F FOR Qt/Qo = 1/20

.5 HR

EXPT. #	CONTROL % PROG. VSL	LOW DOSE % PROG. VSL	MEDIUM DOSE % PROG. VSL	HIGH DOSE % PROG. VSL	LOW DOSE Qo/Qo'	MEDIUM DOSE Qo/Qo'	HIGH DOSE Qo/Qo'
1	47% 115.1	36% 110.7	5% 103	0%	1.36	10.50	0
2	38% 122.2	25% 122.6	5% 114.5	0%	1.52	8.11	0
3	20% 128.1	6% 119.3	3% 115.4	0%	3.58	7.40	0
4	33% 118.8	16% 119.3	2% 111.5	1% 116.3	2.05	17.58	33.71
5	22% 121.1	14% 128.7	5% 124.5	0%	1.48	4.28	0
6	30% 126.1	29% 123.8	21% 121	10% 112.7	1.05	1.49	3.36

AVERAGE Qo/Qo'   1.84   8.23   18.53

	LOW	MEDIUM	HIGH
F'/F=	0.99	0.80	0.13

63

NONOXYNOL-9

DOSE RESPONSE OF F'/F FOR Qt/Qo = 1/20

1 HR

EXPT. #	CONTROL % PROG. VSL	LOW DOSE % PROG. VSL	MEDIUM DOSE % PROG. VSL	HIGH DOSE % PROG. VSL	LOW DOSE Qo/Qo'	MEDIUM DOSE Qo/Qo'	HIGH DOSE Qo/Qo'
1	39% 123.8	14% 116	2% 113.7	0%	2.97	21.23	0
2	39% 121.6	20% 122.9	3% 115.3	0%	1.93	13.71	0
3	16% 127.6	9% 126.7	0%	0%	1.79	0	0
4	36% 117.5	21% 114.4	1% 104.6	0%	1.76	40.44	0
5	20% 125.8	11% 123.5	3% 117.6	0%	1.85	7.13	0
6	26% 124.3	29% 126.4	15% 123.2	2% 114.3	0.88	1.75	14.14

AVERAGE Qo/Qo': 1.86 14.04 14.14

	LOW	MEDIUM	HIGH
F'/F=	0.99	0.47	0.47

64

PROPRANOLOL

NOTE: DOSAGES DIFFER FOR EACH EXPERIMENT!!

EXPT. 1 = .5, .1, .01mM; EXPT. 3 = .3, .1, .03mM; EXPT. 4,5 = .3, .1, .01mM

DOSE RESPONSE OF F'/F FOR Qt/Qo = 1/20

.5 HR

EXPT. #	CONTROL % PROG. VSL	LOW DOSE % PROG. VSL	MEDIUM DOSE % PROG. VSL	HIGH DOSE % PROG. VSL	LOW DOSE Qo/Qo'	MEDIUM DOSE Qo/Qo'	HIGH DOSE Qo/Qo'
1	10% 132.2	10% 124.8	11% 129.5	0%	1.06	0.93	0
3	13% 139.2	12% 131.3	10% 133.8	0.02% 112.9	1.15	1.35	801.42
4	25% 115.4	21% 117.2	2% 106.5	0.03% 111.5	1.17	13.54	862.48
5	19% 122.1	15% 118.5	5% 119	2% 110.4	1.31	3.90	10.51

AVERAGE Qo/Qo':   1.17   4.93   558.14

	LOW	MEDIUM	HIGH
F'/F=	1.00	0.92	0

PROPRANOLOL

DOSE RESPONSE OF F'/F FOR Qt/Qo = 1/20

1 HR

EXPT. #	CONTROL % PROG. VSL	LOW DOSE % PROG. VSL	MEDIUM DOSE % PROG. VSL	HIGH DOSE % PROG. VSL	LOW DOSE Qo/Qo'	MEDIUM DOSE Qo/Qo'	HIGH DOSE Qo/Qo'
1	17% 129.8	20% 134	14% 125.7	0%	0.82	1.25	0
3	21% 137.7	17% 133.9	8% 124.1	0%	1.27	2.91	0
4	23% 114.8	20% 115.2	3% 111	0.04% 103	1.15	7.93	640.87
5	17% 122.3	9% 115.7	5% 118.5	2% 110.3	2.00	3.51	9.42

AVERAGE Qo/Qo':   1.31   3.90   325.15

F'/F=	LOW 1.00	MEDIUM 0.95	HIGH 0

PROPRANOLOL

DOSE RESPONSE OF F'/F FOR Qt/Qo = 1/20

2 HR

EXPT. #	CONTROL % PROG. VSL	LOW DOSE % PROG. VSL	MEDIUM DOSE % PROG. VSL	HIGH DOSE % PROG. VSL	LOW DOSE Qo/Qo'	MEDIUM DOSE Qo/Qo'	HIGH DOSE Qo/Qo'
1	23% 138.8	20% 126.7	9% 124.5	0%	1.26	2.85	0
3	29% 134.4	13% 135.4	5% 113.8	0%	2.21	6.85	0
4	38% 118.7	15% 112.7	2% 104	0%	2.67	21.69	0
5	34% 126.2	4% 127.5	3% 117	0.05% 115.4	8.41	12.22	743.64

AVERAGE Qo/Qo':   3.64   10.90   743.64

F'/F=	LOW 0.96	MEDIUM 0.67	HIGH 0

V. CONCLUSIONS

The compounds studied exhibited a range of effects upon sperm motion and subsequent fertility. Our selection of dose levels was designed to span a range which just attained a threshold in visually obvious diminution of sperm motility. That is, we sought to study the onset of deleterious effects on sperm and fertility. The objective analysis of sperm motion and risk assessment produced a range of effects for the different toxic compounds. This range exemplifies the importance of such objective analysis, as compared to visual estimates of cessation of sperm motility: according to the latter, the doses for all compounds produced relatively similar effects. Given that the concentration ranges of the different compounds applied to sperm varied, we can rank their level of potency as follows:

Highly potent	Dinitrobenzene
	Nonoxynol-9
Potent	Borax
	Propranolol
	EGME
Moderately potent	Alphachlorohydrin
	Epichlorohydrin
	2,5-Hexanedione
	Lead

This ranking takes into account differences in the time course of action against the spermatozoa, as we' as the dose responses for each time point. For example, lead exhibited virtually no effect until extended incubation with sperm.

It should be appreciated that these results are for an in vitro bioassay of effects of compounds applied topically to spermatozoa. Such effects may not be identical to those that occur when the entire organism is exposed to such compounds. In addition, effects on the human (in vitro as well as in vivo) may not be the same as those in the rabbit. However, the rabbit is a commonly used model in testing compounds that are chemical contraceptives. Thus, its use in this context is reasonable. Overall, this study has presented a new, objective approach for using sperm as biomarkers of reproductive risk assessment. It has provided estimates of the onset of risk, with increasing exposure, that would not have been possible with traditional visual methods of sperm assessment and statistical analysis. We hope that this approach will be of value in further analysis of reproductive risk after toxic exposure of a male, e.g. in screening potentially deleterious workplace or environmental exposures.