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Isoprene

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TEXAS COMMISSION ON ENVIRONMENTAL QUALITY

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1 ## List of Acronyms and Abbreviations

Acronyms and Abbreviations	Definition
ACGIH	American Conference of Governmental Industrial Hygienists
ADAF	Age-dependent Default Adjustment Factor
AEGL	Acute Exposure Guideline Level
AMCV	Air monitoring comparison values
ATSDR	Agency for Toxic Substances and Disease Registry
BMC	benchmark concentration
BMCL	benchmark concentration lower confidence limit
$BMCL_{10}$	benchmark concentration lower corresponding to the 10% response level
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
BMDS	benchmark dose software
BMR	benchmark response
C	concentration
Cal EPA	California Environmental Protection Agency
CI	confidence interval
CIIT	Chemical Industry Institute of Toxicology
CNS	central nervous system
D	exposure duration, hour per day
d	day
DF	deposition fraction in the target region of the respiratory tract
DAF	dosimetric adjustment factor
DNA	deoxyribonucleic acid
DSD	development support document
E	exposure level or concentration
EC	effective concentration
EC_{10}	effective concentration corresponding to the 10% response level
ET	extrathoracic
ESL	Effects Screening Level
$^{acute}ESL$	acute health-based Effects Screening Level for chemicals meeting minimum database requirements
$^{acute}ESL_{generic}$	acute health-based Effects Screening Level for chemicals not meeting minimum database requirements
$^{acute}ESL_{odor}$	acute odor-based Effects Screening Level
$^{acute}ESL_{veg}$	acute vegetation-based Effects Screening Level

Acronyms and Abbreviations	Definition
$^{chronic}ESL_{nonthreshold(c)}$	chronic health-based Effects Screening Level for linear dose response cancer effect
$^{chronic}ESL_{nonthreshold(nc)}$	chronic health-based Effects Screening Level for linear dose response noncancer effects
$^{chronic}ESL_{threshold(c)}$	chronic health-based Effects Screening Level for nonlinear dose response cancer effects
$^{chronic}ESL_{threshold(nc)}$	chronic health-based Effects Screening Level for nonlinear dose response noncancer effects
$^{chronic}ESL_{veg}$	chronic vegetation-based Effects Screening Level
F	exposure frequency, days per week
h	hour
$H_{b/g}$	blood:gas partition coefficient
$(H_{b/g})_A$	blood:gas partition coefficient, animal
$(H_{b/g})_H$	blood:gas partition coefficient, human
HEC	human equivalent concentration
HQ	hazard quotient
IARC	International Agency for Research on Cancer
IRIS	Integrated Risk Information System
LEC	lowest effective concentration
LEC_{10}	lowest effective concentration corresponding to the 10% response level
LOAEL	lowest-observed-adverse-effect-level
MF	modifying factor
MLE	maximum likelihood estimate
MW	molecular weight
µg	microgram
µm	micrometer
Mm	millimeter
min	minute
MMAD	mass median aerodynamic diameter
MPPD	multiple pass particle dosimetry
MOA	mode of action
MRL	Minimal Risk Level
NA	not applicable
NOAEL	no-observed-adverse-effect-level
NOEL	no-observed-effect-level
NTP	National Toxicology Program
PBPK	physiologically-based pharmacokinetic model
PK	Pharmacokinetic
POD	point of departure

Acronyms and Abbreviations	Definition
POD_{ADJ}	point of departure adjusted for exposure duration
POD_{HEC}	point of departure adjusted for human equivalent concentration
POE	portal of entry
PU	pulmonary
ppbv	parts per billion by volume
ppm	parts per million
RDDR	regional deposited dose ratio
ReV	Reference Value
RfC	Reference Concentration
RfD	Reference Dose
RIVM	Rijksinstituut voor Volksgezondheid en Milieu (Dutch National Institute for Public Health and the Environment)
RTECS	Registry of Toxic Effects of Chemical Substances
SE	Standard Error
σ_g	geometric variance
T	time or exposure duration
TB	trachiobronchial
TCEQ	Texas Commission on Environmental Quality
TD	Toxicology Division
TH	thoracic
TRI	Toxics Release Inventory
TWA	Time-Weighted Average
TWA-TLV	Time-Weighted Average Threshold Limit Value
UCL	upper confidence limit
UF	uncertainty factor
UF_H	interindividual or intraspecies human uncertainty factor
UF_A	animal to human uncertainty factor
UF_{Sub}	subchronic to chronic exposure uncertainty factor
UF_L	LOAEL to NOAEL uncertainty factor
UF_D	incomplete database uncertainty factor
USEPA	United States Environmental Protection Agency
URF	Unit Risk Factor
VE	minute ventilation
VE_{ho}	default occupational ventilation rate for an eight-hour day
VE_h	default non-occupational ventilation rate for a 24-h day
WHO	World Health Organization
WOE	Weight of evidence

Chapter 3 Acute Evaluation

3.1.3 Mode-of-Action (MOA) Analysis

Toxicity studies of isoprene indicate the following effects: mutagenicity of the diepoxide metabolites, sister chromatid exchange (SCE) induction in bone marrow cells, increases in MN-PCE and MN-NCE levels in peripheral blood, anemia, and in mice, olfactory epithelial degeneration, testicular atrophy, and forestomach epithelial hyperplasia (Bogaards et al. 2001; Hurst 2007). Developmental effects include decreased mouse fetal body weights, increased incidence of mouse fetal supernumerary ribs, and cleft palate in fetal mice (Mast et al. 1989).

The metabolic reactions of isoprene are similar to those of 1,3-butadiene (Mast et al. 1989). Isoprene is metabolized by cytochrome P450 (P450), a mixed-function oxidase enzyme. In the P450 enzyme family, CYP2E1 is primarily responsible for isoprene metabolism while CYP2B6 metabolizes isoprene to a lesser extent (Bogaards et al. 2001, Hurst 2007). The main metabolites of isoprene are: monoepoxides 3,4-epoxy-3-methyl-1-butene (EPOX I) and 3,4-epoxy-2-methyl-1-butene (EPOX II) and the diepoxide 2-methyl-2,2'-bioxirane (Figure 1). The isoprene diepoxide metabolite is formed from the minor monoepoxide intermediate, whereas the 1,3-butadiene diepoxide is formed from the primary metabolite. Thus, an equivalent exposure to 1,3-butadiene and isoprene would result in greater formation of 1,3-butadiene diepoxides as compared to isoprene diepoxides. Metabolic elimination also plays a role in species differences; in mice, the metabolic elimination rates are much greater than those of rats (approximately two to three times) (Melnick et al. 1994a). Gervasi and Longo (1990) determined that the diepoxide metabolite of isoprene was mutagenic in the Ames Assay, and therefore is presumably responsible for the toxic effects, including SCE, observed in rodents.

The rate of isoprene metabolism is directly proportional to inhalation exposure chamber concentrations of up to approximately 300 ppm, at which point saturation kinetics apply (Peter et al. 1987). While the kinetic characteristics of metabolism between isoprene and 1,3-butadiene are also similar, 1,3-butadiene did not reach saturation kinetics until chamber concentrations were approximately 1,000 ppm (Mast et al. 1989). A radiolabel study using F344 male rats and ^{14}C-labeled isoprene indicated that 75% of the total isoprene metabolites were excreted in urine while 0.0018 – 0.031% of the inhaled ^{14}C-isoprene was tentatively identified as a diepoxide metabolite in blood. Metabolites were observed in the respiratory tract after short exposures while concurrent isoprene concentrations in the blood were low. These data indicate that isoprene may be substantially metabolized in the respiratory tract when inhaled, as well as the liver (Dahl et al. 1987). It was also observed that the concentration of metabolites increased with increasing exposure concentration and duration.

Dahl et al. (1987) detected metabolites of isoprene in the blood, nose, lungs, liver, kidney, and fat of male F344/N rats exposed to 1,480 ppm ^{14}C-labeled isoprene (saturation point). Exposure and duration findings in the study include: when the exposure concentration and duration increased the concentration of metabolites also increased, for lower concentrations the diols and/or diepoxides remained constant over time but increased with increasing exposure. The

blood levels of the parent compound were found to be at their peak after a 2-h exposure to 8200 ppm; increased duration did not increase the levels. The highest diol and diepoxide metabolite concentrations were found in the fat (highest), nose, liver, and lung. The indication is that substantial metabolism is occurring both in the respiratory tract as well as in the liver.

While the differences between the carcinogenicity of 1,3-butadiene and isoprene cannot be explained by the blood levels of the metabolites, the structure differences of the epoxides may (Bond et al. 1991; Dahl 1996; Dahl et al. 1990; Dahl et al. 1987; Watson et al. 2001). Watson et al. (2001) identified significant differences in the reactivities of the metabolites of 1,3-butadiene and isoprene. Isoprene expoxides have an additional methyl group, which may influence the reactivity of the metabolite by suppressing the cross-linking reactivity.

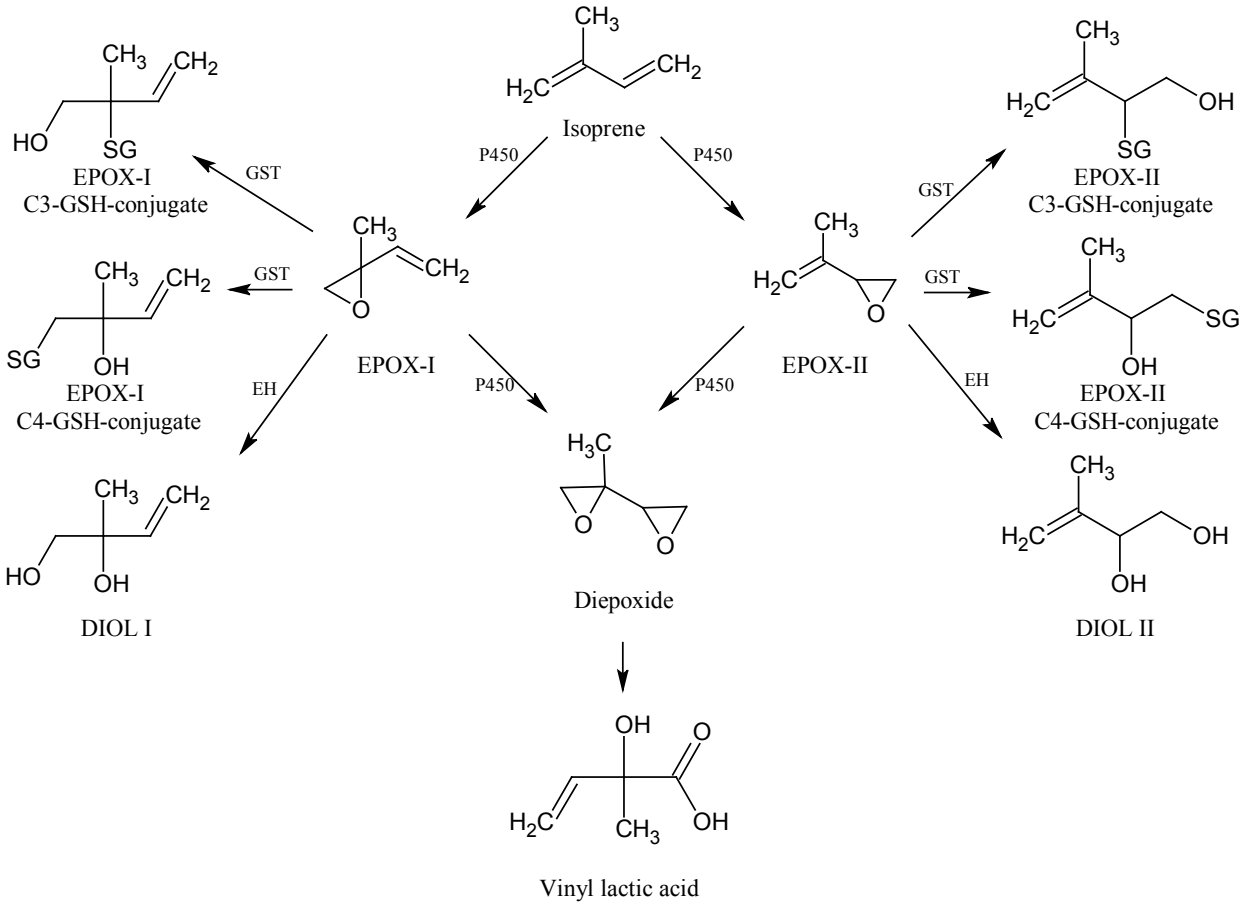

Figure 1. Metabolic Pathways of Isoprene.
EPOX-I = 3,4-epoxy-3-methyl-1-butene; EPOX-II = 3,4-epoxy-2,methyl-1-butene; GSH = glutathione; P450 = cytochrome P450; GST = glutathione-S-transferase; EH = epoxide hydrolase. Figure adapted from: Bogaards et al. (2001); Gervasi and Longo (1990); Melnick et al. (1999).

3.1.4 Dose Metric

Data on the exposure dose of the parent chemical are available in the key study (Mast et al. 1989) and supporting studies. Since data on other more specific dose metrics are not available (e.g., blood concentration of parent chemical, area under blood concentration curve of parent chemical, or putative metabolite concentrations in blood or target tissue), exposure concentration of the parent chemical will be used as the default dose metric.

Chapter 4 Chronic Evaluation

4.2 Carcinogenic Potential

4.2.3 Carcinogenic Weight-of-Evidence

Currently, there are two entities that have classified the carcinogenic potential of isoprene, the National Toxicology Program (NTP) and the International Agency for Research on Cancer (IARC). NTP, in their Report on Carcinogens (ROC), has listed isoprene as reasonably anticipated to be a human carcinogen (NTP 2011). IARC has classified isoprene as 2B; possibly carcinogenic to humans (IARC 1999). Both classifications are based on evidence of tumor formation at multiple organ sites in multiple species of experimental animals (Melnick et al. 1994a; Melnick et al. 1999; Placke et al. 1996). In mice, inhalation exposure to isoprene induced increased incidences of neoplasms in the following organs: lung, liver, harderian gland, forestomach, the hematopoietic system, and the circulatory system. In rats, inhalation exposure to isoprene induced increased incidence of neoplasms in the following organs: mammary gland, kidney, and testis (IARC 1999; NTP 1999). Although there are currently no human studies that indicate isoprene exposure may increase the risk of cancer, the 2005 USEPA Cancer Guidelines (USEPA 2005a) recommend that tumors observed in animals are an indication of the potential for tumor production in humans. TCEQ considers isoprene likely to be carcinogenic to humans. According to the TCEQ Guidelines (TCEQ 2012), TCEQ will perform a carcinogenic dose-response assessment for chemicals considered “likely to be carcinogenic to humans” or “carcinogenic to humans.”

4.2.4 Carcinogenic MOA

Since isoprene is the 2-methyl analogue of 1,3-butadiene, they have similar oxidative metabolic pathways, which includes the formation of a mutagenic diepoxide metabolite, mechanisms of detoxification, and toxic effects such as induction of SCE in bone marrow and anemia. One of the most reactive butadiene metabolites is the diepoxide 2,2'-bioxirane (DBE). The mutagenic metabolite of isoprene is the diepoxide 2-methyl-2,2'-bioxirane (Hurst 2007). Hurst (2007) notes that Gervasi and colleagues (Del Monte et al. 1985; Gervasi et al. 1985; Gervasi and Longo 1990) observed that the diepoxides of both isoprene and butadiene were equivalent in mutagenic potential. Although these tumor sites aren't applicable to humans, point mutations in K-ras and H-ras genes have been observed in forestomach and harderian tumors produced in mice after exposure to isoprene (Hong et al. 1997; Sills et al. 2001). Similar to butadiene, isoprene has been

shown to induce tumors at multiple sites in mice and rats; although, isoprene is not as potent a carcinogen as butadiene (Shelby 1990; Tice et al. 1988; Watson et al. 2001).

Among rodents, isoprene toxicity is varied, with mice showing the most sensitivity. It has been shown that tissue concentrations of isoprene metabolites are higher than metabolites of butadiene in mice and rats. It has been suggested that the differences observed in the carcinogenic potency of butadiene versus isoprene are most likely due to differences in the reactivities of their metabolites. The isoprene diepoxide is the 2-methyl analogue of the butadiene diepoxide. It is likely that the presence of the methyl group on the diepoxide has a substantial suppressive effect on the cross-linking reactivity of this metabolite compared with that of the butadiene diepoxides (Watson et al. 2001).

Scientific evidence suggests that carcinogenic effects observed from isoprene exposure are mediated by its genotoxic metabolite, the diepoxide 2-methyl-2,2'-bioxiran (Section 3.1.3 and Figure 1). It has been suggested by Cox et al. (1996) that isoprene does not follow a traditional dose-response relationship, but rather a nonlinear MOA where exposure duration is not as much of a factor as exposure intensity based on data from Placke et al. (1996) (discussed in Section 4.2.5.3). The purpose of the Placke et al. (1996) study was to investigate the effects of various concentrations and durations of exposure to isoprene in B6C3F$_1$ mice. Based on the analyses conducted by Sielken et al. (2012) (Appendix A and B) on behalf of TCEQ, they conclude that, for isoprene exposure, exposure intensity has a greater impact on response frequency than exposure duration, which is consistent with Cox et al. (1996) and Placke et al. (1996). In fact, Placke et al. (1996) concludes that "a threshold effect level and strong nonlinearities with respect to concentration appeared to exist…" However, a threshold evaluation may only be conducted when the MOA information supports a threshold evaluation or strong evidence exists that the MOA is not mutagenic. Since the isoprene MOA is not well understood, TCEQ did not apply a threshold carcinogenic approach, consistent with the TCEQ Guidelines (TCEQ 2012).

4.2.5 Key Studies

No reliable human epidemiological or experimental studies were identified for isoprene exposure. Three Russian epidemiological studies (Mitin 1969; Nikul'tseva 1967; Pigolev 1971) were identified in NASA (2000). However, these studies are not reliable; effects are from workers in the rubber industry who were exposed to unknown concentrations of multiple chemicals, including isoprene. Due to the lack of reliable human data, animal studies were considered for the development of a chronic carcinogenic unit risk factor (URF) for isoprene.

4.2.5.1 Melnick et al. (1994a)

An NTP toxicity study of isoprene administered by inhalation to F344/N rats and B6C3F$_1$ mice was identified (Melnick et al. 1994a). Results are also discussed in the following papers: Hong et al. (1997); Melnick and Sills (2001); Melnick et al. (1994b); Melnick et al. (1996). This study was a combination of a dose-finding, subacute study, and two chronic studies. For the chronic stop-exposure study: groups of 40 male rats and 40 male mice were exposed to 70, 220, 700, 2,200, or 7,000 ppm isoprene for 6 h/d 5 d/wk for 6 months. Ten animals per species were

evaluated at the end of the exposure while the rest were allowed to recover for an additional 6 months without isoprene exposure. Only the observations from the 6 month recovery animals are included in the dose-response analysis (i.e., observations from the 10 animals/species evaluated after 6 months were not included). Interstitial cell hyperplasia of the testis was observed in male rats exposed to 7,000 ppm isoprene, and after 6 months of recovery the incidence of benign testicular adenomas was marginally greater than controls. In the mice, the following effects were observed:

- significantly greater than control incidences of hepatocellular neoplasms (adenomas and adenoma or carcinomas) in the 700, 2,200, and 7,000 ppm dose groups (carcinomas in the 7,000 ppm dose group only);
- significantly greater than control incidences of hyperplasia of the alveolar epithelium in the 7,000 ppm dose group;
- significantly greater than control incidences of alveolar/bronchiolar adenomas and adenomas or carcinomas in the 2,200 and 7,000 ppm dose groups;
- significantly greater than control incidences of forestomach neoplasms (squamous cell papillomas or squamous cell carcinomas; site not relevant to humans) in the 7,000 ppm dose group; and
- significantly greater than control incidences of harderian gland adenomas (site not relevant to humans) in the 700, 2,200, and 7,000 ppm dose groups.

4.2.5.2 Melnick et al. (1999)

An NTP toxicology and carcinogenesis study of isoprene administered by inhalation to F344/N rats was identified (Melnick et al. 1999). Results are also discussed in Melnick and Sills (2001). Groups of 50 male and 50 female F344/N rats were exposed to 220, 700, or 7,000 ppm isoprene for 6 h/d 5d/wk for 105 wk (just over 2 years). Findings included:

- significantly greater than control incidences of mammary gland fibroadenoma in males exposed to 7,000 ppm isoprene and all exposed females;
- significantly greater than control incidences of renal tubule adenoma in males exposed to 700 and 7,000 ppm isoprene and renal tubule hyperplasia in males exposed to 7,000 ppm isoprene; and
- significantly greater than control incidences of bilateral interstitial cell adenoma and unilateral and bilateral interstitial cell adenoma (combined) of the testis in males exposed to 700 and 7,000 ppm isoprene;

4.2.5.3 Placke et al. (1996)

A chronic inhalation study in B6C3F$_1$ mice (Placke et al. 1996) was identified for isoprene. Results are also discussed in Cox et al. (1996). The purpose of this study was to investigate the effects of various concentrations and durations of exposure to isoprene in B6C3F$_1$ mice. Twelve groups of mice were dosed.

20-wk Exposure Groups

One group of 50 male B6C3F$_1$ mice was exposed to 280 ppm isoprene for 8 h/d 5d/wk for 20 wk, while another group of 50 male B6C3F$_1$ mice was exposed to 2,200 ppm isoprene for 4 h/d 5d/wk for 20 wk. Findings included the following:

280 ppm exposure for 8h/d 5d/wk

- Significantly greater than controls incidence of hepatocellular adenoma and/or carcinoma;
- significantly greater than controls incidence of harderian gland adenoma and/or carcinoma (not relevant to humans); and
- significantly greater than controls incidence of histiocytic sarcoma.

2,200 ppm exposure for 4h/d 5d/wk

- significantly greater than controls incidence of hepatocellular adenoma and/or carcinoma;
- significantly greater than controls incidence of harderian gland adenoma and/or carcinoma (not relevant to humans); and
- significantly greater than controls incidence of histiocytic sarcoma (not identified in Placke et al. (1996), but identified in Sielken et al. (2012) using a one-sided Fisher exact test).

40-wk Exposure Groups

Three groups of 50 male B6C3F$_1$ mice were exposed to 70, 140, and 2,200 ppm isoprene for 8 h/d 5d/wk for 40 wk. Findings included the following:

- Significantly greater than controls incidence of alveolar/bronchiolar adenoma and/or carcinoma in males exposed to 2,200 ppm;
- significantly greater than controls incidence of hepatocellular adenoma and/or carcinoma in males exposed to 140 and 2,200 ppm;
- significantly greater than controls incidence of harderian gland adenoma and/or carcinoma (site not relevant to humans) in males exposed to 70, 140, and 2,200 ppm (70 was not identified in Placke et al. (1996), but identified in Sielken et al. (2012) using a one-sided Fisher exact test); and
- significantly greater than controls incidence of histiocytic sarcoma in males exposed to 2,200 ppm.

80-wk Exposure Groups

Three groups of 50 male and 50 female B6C3F$_1$ mice were exposed to 0, 10, and 70 ppm isoprene for 8 h/d 5d/wk for 80 wk. Another three groups of 50 male B6C3F$_1$ mice were exposed to 280, 700, and 2,200 ppm isoprene for 8 h/d 5d/wk for 80 wk. One group of 50 male B6C3F$_1$ mice was exposed to 2,200 ppm isoprene for 4 h/d 5d/wk for 80 wk. Findings included the following:

0, 10, and 70 ppm exposure for 8h/d 5d/wk

- Significantly greater than controls incidence of harderian gland adenomas (site not relevant to humans) in females exposed to 70 ppm (no carcinomas were observed); and

- significantly greater than controls incidence of pituitary adenoma in females exposed to 10 and 70 ppm (no carcinomas were observed; 10 was not identified in Placke et al. (1996), but identified in Sielken et al. (2012) using a one-sided Fisher exact test).

280, 700, and 2,200 ppm exposure for 8h/d 5d/wk

- Increased incidence of alveolar/bronchiolar adenoma and/or carcinoma in males exposed to 700 and 2,200 ppm;
- significantly greater than controls incidence of hepatocellular adenoma and/or carcinoma in males exposed to 280, 700, and 2,200 ppm; and
- significantly greater than controls incidence of harderian gland adenoma and/or carcinoma (site not relevant to humans) in males exposed to 280, 700, and 2,200 ppm.

2,200 ppm exposure for 4h/d 5d/wk

- Significantly greater than controls incidence of hepatocellular adenoma and/or carcinoma in males;
- significantly greater than controls incidence of harderian gland adenoma and/or carcinoma (site not relevant to humans) in males; and
- significantly greater than controls incidence of histiocytic sarcoma in males.

4.2.6 Dose-Response Assessment

Due to the complexity of the data, TCEQ hired a statistical expert to review and model the data, Sielken & Associates Consulting, Inc. TCEQ identified the above studies and provided them to Sielken et al. (2012), who reviewed them to make sure they were adequate and contained the necessary data to perform dose-response analysis. Once Sielken et al. (2012) determined the data were adequate, they considered all endpoints consistent with those noted in the National Toxicology Program's 12th Report on Carcinogens (NTP 2011), and corresponding to chronic carcinogenesis, for dose-response modeling. The Sielken et al. (2012) report may be found in Appendix A and B.

4.2.6.1 Adjustments to the data

Sielken et al. (2012) (Appendix A and B) conducted a dose-response assessment of the above key studies. In order to accomplish this, the dose levels and numbers of animals at risk in the data sets were adjusted for differences between the exposure durations and times of response observation, assuming exposure for 24h/d, 7d/wk for a lifetime.

4.2.6.1.1 Adjustment of Study Dose Levels

Since the key studies have variable dosing over time, an adjustment of the dose levels to account for this and to describe the cancer dose-response data was conducted using the Armitage and Doll (2004) mathematical description of carcinogenesis as expressed by Crouch (1983), Crump and Howe (1984), and several others.

Briefly, the multistage theory of carcinogenesis assumes that the transformation of a normal cell to a specified neoplastic stage requires the occurrence of "m" biological events (i.e., cancer stage

1, 2, or 3), which occur in a specific order. Using the calculations described by Sielken et al. (2012) (Appendix A, Section 5.2), the dose can be adjusted for exposure duration and time differences with the following equation:

$$D = d\ x\ \left(\frac{n_{hrs}}{24}\right) x\ \left(\frac{n_{days}}{7}\right) x\ \frac{(T_e - a)^m - (T_e - b)^m}{T^m}$$

where:
D = equivalent lifetime average daily dose
d = experimental dose
n_{hrs} = hours of exposure per day
n_{days} = days of exposure per week
T_e = total study duration, in weeks
T = time, in weeks, corresponding to the end of a normal lifetime; 104 weeks for a 2-year lifetime in mice and rats
a = time when exposure begins, in weeks
b = time when exposure ends, in weeks
m = cancer stage, m = 1, 2, or 3

Adjustments to a continuous exposure duration of 24h/d, 7d/wk were also included. See Section 5.2 in Appendix A for a detailed explanation of this adjustment.

4.2.6.1.2 Adjustment of Number of Study Animals

As described by Sielken et al. (2012) (Appendix A, Section 5.3), when the end of a study does not correspond to the end of a nominal lifetime the number of study subjects at risk of developing the specified response by the end of a nominal lifetime should be adjusted to account for such an inequality. The adjustment is based on the Armitage-Doll theory of multistage carcinogenesis. The adjustment is done to estimate the equivalent number of animals at risk if the time to necropsy were equal to the nominal lifetime.

Using the calculations described by Sielken et al. (2012) (Appendix A, Section 5.3), the number of animals for each endpoint and stage of carcinogenesis (m) can be estimated and adjusted with the following equations:

If $T_{end} \leq T$:

$$Adjusted\ n_{at\ risk,i} = n_{resp,i} + \left(n_{at\ risk,i} - n_{resp,i}\right) x \left(\frac{T_{end}}{T}\right)^m$$

If $T_{end} > T$:

$$Adjusted\ n_{at\ risk,i} = n_{resp,i}\ x\ \left(\frac{T_{end}}{T}\right)^m + \left(n_{at\ risk,i} - n_{resp,i}\right)$$

where:
$n_{at\ risk,\ i}$ = the number of subjects in the i^{th} dose group at the start of the study

$n_{resp, i}$ = the number of subjects that are observed to have the specified response by the end of the study
T_{end} = end of the study
T = end of a nominal lifetime
m = cancer stage, m = 1, 2, or 3

See Section 5.3 in Appendix A for a detailed explanation of this adjustment.

4.2.6.2 Determination of the POD

Following the TCEQ Guidelines (TCEQ 2012), benchmark dose modeling (BMD) was carried out on the adjusted data for endpoints identified by Sielken et al. (2012) (Appendix A, Section 6) for dose-response analysis. The multistage-cancer model was used for these data, with a total of 171 model fits carried out on 57 endpoints (i.e., 57 combinations of study, species, sex, organ, and severity; see Table 4 in Appendix A), with three forms of the dose-response data fitted for each endpoint. See Section 6 in Appendix A for details and Appendix C in Appendix B for BMDS outputs.

In order to determine the POD, and as outlined in the TCEQ Guidelines (TCEQ 2012), all malignant endpoints relevant to humans that were statistically significantly increased (compared to controls) for at least one dose in study animals were included. Only malignant endpoints were considered as the ultimate endpoint for carcinogenic characterization is cancer. Similarly, only statistically significantly increased malignancies were considered to help ensure they were related to isoprene exposure and did not occur by chance. Only three endpoints from Placke et al. (1996) met these criteria and are therefore the only endpoints carried further in the dose-response analysis (Table 4-1 and Figure 4-1).

Table 4-1. Human-relevant Statistically Significant Malignant Endpoints Considered for the Derivation of a URF.

Study	Endpoint	Species	Sex
Placke et al. 1996	Hepatocellular carcinoma	B6C3F$_1$ Mice	Male
Placke et al. 1996	Alveolar/bronchiolar carcinoma	B6C3F$_1$ Mice	Male
Placke et al. 1996	Histiocytic Sarcoma	B6C3F$_1$ Mice	Male

BMD modeling was utilized to determine the exposure concentration at a 10% response level (EC_{10}) for each cancer stage (m = 1, 2, 3) for each malignant endpoint considered (Table 4-2). The EC_{10} is the POD.

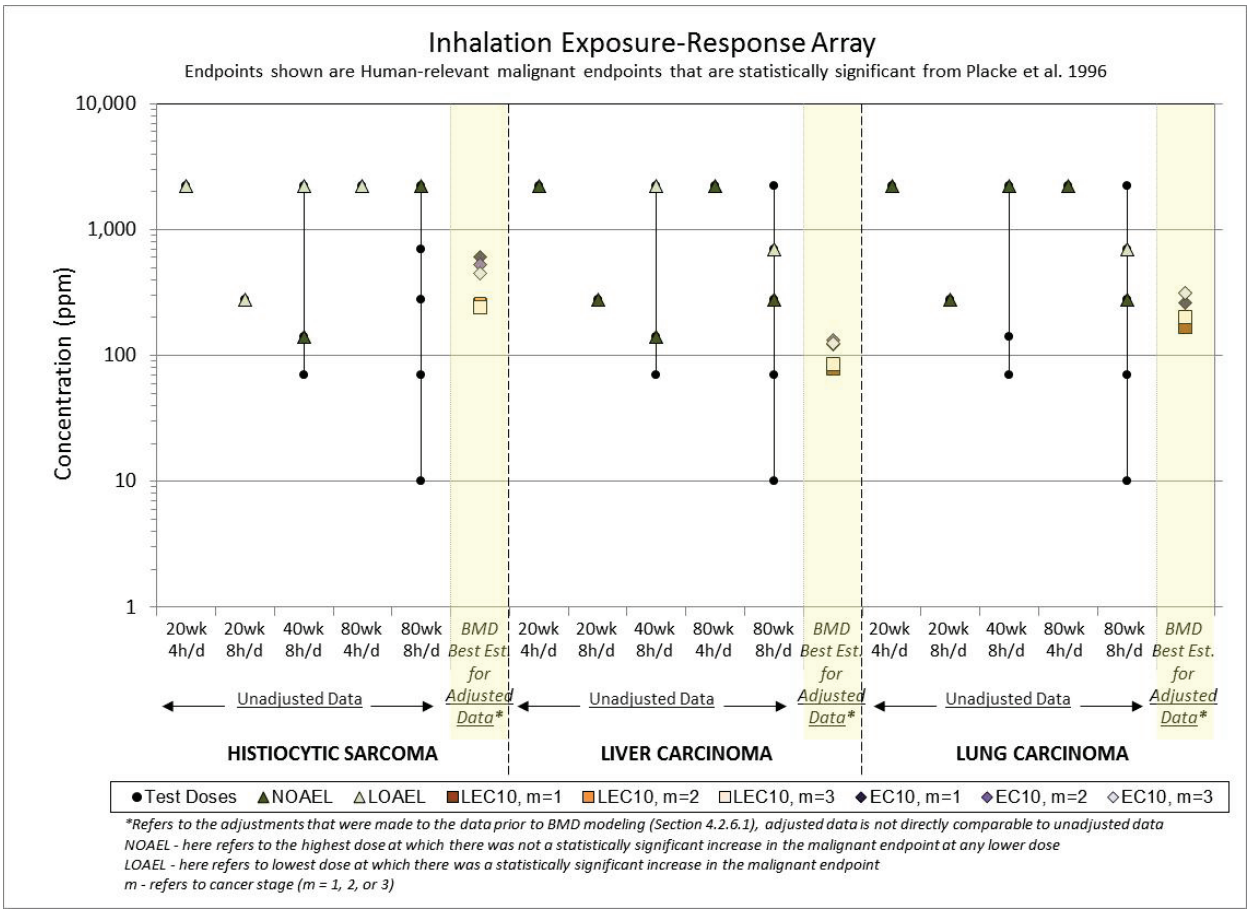

Figure 4-1. Exposure-Response Array Showing the Statistically Significant Human-Relevant Malignant Endpoints.

Table 4-2. Modeled EC_{10} for Malignant Endpoints Considered (ppm).

Study	Endpoint	EC_{10} (ppm)[a]			LEC_{10} (ppm)[a]		
		m=1	m=2	m=3	m=1	m=2	m=3
Placke et al. 1996	Hepatocellular carcinoma	122.96[b]	131.36	125.58	78.50	87.76	86.07
Placke et al. 1996	Alveolar/ bronchiolar carcinoma	263.11	313.39	310.26	168.80	203.23	203.54
Placke et al. 1996	Histiocytic sarcoma	600.67	525.59	446.69	252.67	262.44	242.60

[a]Adjusting for 1, 2, or 3 number of cancer stages
[b]Human-relevant endpoint with the lowest best estimate (EC_{10})

The relevant endpoint with the lowest estimated value was chosen as the critical endpoint. The EC_{10} represents the best estimate lifetime excess cancer risk resulting from continuous exposure to isoprene, whereas the LEC_{10} represents the lower bound of that estimate.

Of the species tested, male mice were the most sensitive and the only species/sex with statistically significant human-relevant cancer endpoints. It is more conservative to take the lowest best estimate rather than using a central tendency across all estimates. Table 4-3 provides a sensitivity analysis comparing the lowest EC_{10} value to all individual EC_{10} and LEC_{10} values, as well as to calculated central tendencies. All values, except the LEC_{10} values associated with the lowest EC_{10}, are larger than the lowest EC_{10}. This pattern would also hold true for the resulting URFs, were the calculations carried further. Therefore, choosing the endpoint associated with the lowest EC_{10} value is more conservative than the other individual estimates and the central tendency of all estimates combined (e.g., mean, midpoint). The difference between the EC_{10} and the LEC_{10} is also very small, less than a factor of 2, reflecting the small variability and uncertainty of the experimental data. The data from Placke et al. (1996) is a very robust dataset, with a total of 600 male mice and 150 female mice exposed. Such a robust dataset provides a level of confidence in the observed data. Since there is some evidence to suggest that isoprene is a threshold chemical, and the endpoint is the most sensitive and associated with the most sensitive species and sex tested, TCEQ chose the best estimate, EC_{10}, as sufficiently conservative rather than the lower bound of the best estimate, LEC_{10}, as the point of departure.

Table 4-3. Comparison of Individual and Central Tendency EC_{10} and LEC_{10} Values to the Lowest EC_{10}.

Study	Endpoint	EC_{10}[a] (Ratio to Lowest EC_{10}[b])			LEC_{10}[a] (Ratio to Lowest EC_{10}[b])		
		m=1	m=2	m=3	m=1	m=2	m=3
Placke et al. 1996	Hepatocellular carcinoma	122.96 (1.00)	131.36 (1.07)	125.58 (1.02)	78.50 (0.64)	87.76 (0.71)	86.07 (0.70)
Placke et al. 1996	Alveolar/ bronchiolar carcinoma	263.11 (2.14)	313.39 (2.55)	310.26 (2.52)	168.80 (1.37)	203.23 (1.65)	203.54 (1.66)
Placke et al. 1996	Histiocytic sarcoma	600.67 (4.89)	525.59 (4.27)	446.69 (3.63)	252.67 (2.05)	262.44 (2.13)	242.60 (1.97)
Geometric Mean[c]		268.85 (2.19)	278.65 (2.27)	259.15 (2.11)	149.60 (1.22)	167.28 (1.36)	161.98 (1.32)
Mean[c]		328.11 (2.67)	323.45 (2.63)	294.18 (2.39)	166.66 (1.36)	184.48 (1.50)	177.40 (1.44)
Midpoint[c]		263.11 (2.14)	313.39 (2.55)	310.26 (2.52)	168.80 (1.37)	203.23 (1.65)	203.54 (1.66)

[a] EC_{10} and LEC_{10} are in ppm
[b] Lowest EC_{10} = 122.96 ppm
[c] Central Tendency was calculated over all endpoints

4.2.6.3 Dosimetric Adjustments

Once the POD was determined for each study, animal concentrations were converted into human equivalent concentrations. Isoprene is not soluble in water; however, it produces both respiratory and remote effects. Isoprene is therefore classified as a Category 2 gas. According to the TCEQ Guidelines (TCEQ 2012), dosimetry for Category 2 gases is under review by USEPA. Until new findings suggest otherwise, the TD will conduct dosimetric adjustments for Category 2 gases

using either Category 1 or 3 dosimetry equations, whichever is most relevant. The most relevant dosimetry classification for isoprene is Category 3 for tumors produced in remote sites. For Category 3 gases:

$$POD_{HEC} = POD_{ADJ} \, x \, \frac{(H_{b/g})_A}{(H_{b/g})_H}$$

where:
$H_{b/g}$ = ratio of the blood:gas partition coefficient
A = animal
H = human

For isoprene, the blood:gas partition coefficients for mice and humans are 2.04 and 0.75, respectively, which is a ratio of 2.7 (Filser et al. 1996). If the animal blood:gas partition coefficient is greater than the human blood:gas partition coefficient, a default value of 1 is used for the regional gas dose ratio (RGDR) (USEPA 1994). Therefore, the modeled EC_{10} becomes the dosimetrically adjusted POD_{HEC} value.

4.2.6.4 Extrapolation to Lower Exposures

4.2.6.4.1 URFs and Air Concentrations at 1 x 10^{-5} Excess Cancer Risk

Unit risk factors (URFs) and isoprene air concentrations at 1 in 100,000 excess cancer risk were calculated from the POD_{HEC} (i.e., the lowest EC_{10}s in Table 4-2). The default approach used by TCEQ when there is not strong evidence available for a threshold MOA is to use a nonthreshold approach. The best estimate lifetime excess cancer risk resulting from continuous exposure to isoprene at 1 ppb in air (i.e., the URF in Table 4-4) was then calculated using the following equation:

$$URF = \frac{0.10}{EC_{10}}$$

Table 4-4. Calculated URFs for Malignant Endpoints Considered (ppb^{-1}).

Study	Endpoint	Species	Sex	URF (ppb^{-1}) adjusting for 1, 2, or 3 number of cancer stages		
				m=1	m=2	m=3
Placke et al. 1996	Hepatocellular carcinoma	$B6C3F_1$ Mice	Male	8.1E-07	7.6E-07	8.0E-07

As identified in the previous section, hepatocellular carcinoma from the Placke et al. (1996) study was chosen as the critical endpoint. The URFs for all three cancer stages (m=1, 2, or 3) for that endpoint were calculated and presented in Table 4-4. Once the URFs were calculated, they were rounded to 2 significant figures.

The URF corresponding to cancer stage m=1 was the largest value (URF = 8.1E-04 per ppm or 8.1E-07 per ppb), which will result in the most conservative (i.e., lowest) calculated 10^{-5} risk air concentration (in ppb or $\mu g/m^3$). The rounded final URF (highlighted value in Table 4-4) was then used to calculate the $^{chronic}ESL_{nonthreshold(c)}$, and the ESL subsequently rounded.

The 10^{-5} risk air concentration (Table 4-5) was calculated based on the URF using the following equation:

$$10^{-5}\ risk\ air\ concentration = \frac{1\ x\ 10^{-5}}{URF}$$

Table 4-5. Air Concentrations Corresponding to 1 in 100,000 Excess Cancer Risk.

Study	Endpoint	URF	10^{-5} Risk Air Concentration
Placke et al. 1996	Hepatocellular carcinoma	8.1E-07 per ppb, or 2.9E-07 per $\mu g/m^3$	12 ppb, or 33 $\mu g/m^3$

4.2.7 Evaluating Susceptibility from Early-Life Exposures

USEPA (2005b) provides default age-dependent adjustment factors (ADAFs) to account for potential increased susceptibility in children due to early-life exposure when a chemical has been identified as acting through a mutagenic MOA for carcinogenesis. Genotoxicity testing of isoprene has included mutagenicity testing in *Salmonella*, demonstration of mutations in the *ras* protooncogene for harderian gland tumors in mice, and cytogenic studies.

Isoprene has been tested using several strains of *S. typhimurium*; however, isoprene, as well as the main and minor monoepoxide metabolites, have not shown to be mutagenic in the presence and absence of Aroclor-induced rat or hamster liver S9 (de Meester et al. 1981; Gervasi et al. 1985; Kushi et al. 1985; Melnick et al. 1994a; Mortelmans et al. 1986). However, the diepoxide for isoprene was found to be mutagenic (Gervasi and Longo 1990; Melnick et al. 1994a; Watson et al. 2001). This is in contrast to 1,3-butadiene, in which the monoepoxide metabolites are mutagenic (Gervasi and Longo 1990).

Hong et al. (1997) used samples from Melnick et al. (1994a) to characterize the genetic alterations in the harderian gland neoplasms observed in both dose groups (2200 and 7000 ppm isoprene). K- and H-*ras* protooncogene mutations were detected at a high frequency in the isoprene-induced tumors, but not detected in control animal tumors. According to the authors, these findings suggest three things: 1) the harderian gland is where isoprene is converted to the reactive intermediate (diepoxide), 2) the diepoxide is significantly distributed systemically from the major formation site, and/or 3) detoxification of the diepoxide in the harderian gland is not sufficient to prevent tumor formation. The conclusion from the authors is that *ras* protooncogene activation contributes to the induction of harderian gland tumors in mice. This study provides some clues to potential mechanism(s) of isoprene carcinogenesis in the harderian gland, but does

not provide conclusive evidence of a mutagenic MOA or mechanism(s) of carcinogenesis in other tissues.

Tice et al. (1988) demonstrated that isoprene induced a significant increase in SCE in bone-marrow cells of $B6C3F_1$ male mice exposed to 438, 1750, and 7000 ppm isoprene for 6 h/d for 12 days. However, there was not a significant increase in the frequency of chromosomal aberrations and the mitotic index was not altered. The authors concluded that isoprene would likely induce tumors at multiple sites in $B6C3F_1$ mice, but that isoprene would likely not be as potent a carcinogen as 1,3-butadiene. It is unusual for a compound to be positive in a micronucleus test yet negative in a chromosomal aberration test (Shelby and Witt 1995). It has been postulated that isoprene is more of an aneugen rather than a clastogen, in which carcinogenic activity could have a low-concentration threshold for cancer induction (NASA 2000; Tice et al. 1988).

Isoprene has not been demonstrated to have a mutagenic MOA for liver carcinogenicity considering the scientifically-rigorous standard set under the TCEQ Guidelines (Section 5.7.5 of TCEQ (2012)). Demonstrating plausibility is not tantamount to an adequately robust demonstration that mutagenicity is in fact *the* initiating event in target tissues. The data are not sufficient to definitively determine the specific carcinogenic MOA(s). Since the MOA for isoprene-induced liver cancer has not been sufficiently demonstrated to be mutagenic, consistent with the TCEQ Guidelines (TCEQ 2012), ADAFs will not be applied to the final URF at this time. This issue will be reevaluated periodically as new scientific data become available.

4.2.8 Uncertainty Analysis

Underlying uncertainties are an inherent part of any analysis. Although conservative choices have been made in the derivation of a URF for isoprene (e.g., selecting the smallest EC_{10} as a POD, assuming a dose adjustment factor of 1, etc.) to account for uncertainties in the derivation of the estimates, there are sources of uncertainty that cannot explicitly be included. In this case, there are four main areas of uncertainty relating to the development of a carcinogenic toxicity factor for isoprene: interspecies differences, dose-response assessment, site concordance, and linear low-dose extrapolation.

4.2.8.1 Interspecies Differences

There are significant species differences in the metabolism of isoprene, with mice showing more sensitivity to isoprene than other animals. Mice have a larger maximal metabolic velocity for isoprene, at 3 times that of rats (Peter et al. 1987), and especially for the diepoxide formation, which is 6 times greater than rats and rabbits (Longo et al. 1985). Bond et al. (1991) demonstrate that mice appear to metabolize isoprene at a lower rate than rats since rats metabolized a greater fraction of the inhaled dose. During high concentration exposures, the mouse minute volume decreases about 20% (exposures to 2000 ppm), while rat respiration does not change much (Bond et al. 1991; Dahl et al. 1987). Finally, mouse hemoglobin adduct formation was 2 times higher in mice than rats (Sun et al. 1989). TCEQ did not identify any studies on the metabolism

of isoprene in humans, so how these species differences relate to human metabolism of isoprene is unknown.

4.2.8.2 Site Concordance

Increased incidences of the following neoplasms have been observed in mice and rats:

Mice (m = male, f = female)

- circulatory system (m, f)
- hematopoietic system (m)
- pituitary gland (f)
- liver (m)
- lung (m)
- forestomach (m)
- harderian gland (m, f)

Rats (m = male, f = female)

- kidney (m)
- mammary gland (m, f)
- testis (m)

As indicated in Figure 1, cytochrome P450 is responsible for the metabolism of isoprene to the metabolites EPOX-I and EPOX-II, and subsequently to the diepoxide. The amount of diepoxide formed is a function of the balance between oxidation to the diepoxide and subsequent detoxification by epoxide hydrolase and glutathione S-transferase. It is possible for species differences to exist between these enzyme systems, which could then account for the differences in sensitivity to isoprene exposure (Bogaards et al. 2001). Since there are no reliable epidemiological data available to help inform whether or not humans would develop tumors at the same sites as mice, this is an area of uncertainty.

4.2.8.3 Dose-Response Assessment

In dose-response assessment, if a toxicodynamic model is not available for use, the observed range of data may be fitted empirically to models to extend the dose-response analysis of tumor incidence to lower doses and response levels. The use of empirical models on the range of observed data introduces model uncertainty into the assessment. There are several different curve-fitting models that are available. Models used in the dose-response assessment that fit the observed data reasonably well may lead to several-fold differences in estimated risk at the lower end of the observed range (USEPA 2005a). For this dose-response assessment, the multistage quantal dose-response model was used with adjustments to the data. Even though model uncertainty is introduced by using an empirical model, the resulting EC_{10} and LEC_{10} values are very close together, showing a tight data fit.

4.2.8.4 Linear Low-Dose Extrapolation

Isoprene is an endogenously produced chemical. The rates of endogenous production in humans, rats, and mice are reported to be 0.15, 1.9, and 0.4 µmol/kg/h, respectively (Hartmann and Kessler 1990; Peter et al. 1987; Taalman 1996). Breath concentrations were estimated to be 50 – 400 µg/m^3 for nonsmokers, as reported in NTP (1999). Gelmont et al. (1981) reported isoprene to be the major hydrocarbon exhaled in human breath (up to 70%) in all but one of 30 volunteers. Filser et al. (1996) found that the rate of metabolism for mice and rats are about 14 and 8 times faster than in humans, respectively. This metabolic rate represents only the endogenously produced isoprene that is metabolized; 90% of endogenously produced isoprene in humans undergoes metabolism, while 10% is exhaled. Given that isoprene is an endogenously produced chemical that is present within the body, which has a significant ability to detoxify and eliminate it, as well as exhaled, this, along with other evidence, suggests that isoprene may be a threshold chemical. Therefore, there are large uncertainties in the use of a linear low-dose extrapolation method for the determination of carcinogenic potential.

Other than the endogenous production of isoprene, the genotoxicity and carcinogenicity data seem to suggest that isoprene is a threshold chemical. As discussed in NASA (2000), the cytogenic data are suggestive that isoprene exhibits aneugenic activity rather than clastogenic activity; isoprene was positive in a micronucleus test, yet negative in a chromosomal aberration test (Shelby and Witt 1995; Tice et al. 1988). Mutagenicity assays have demonstrated that isoprene does not appear to be mutagenic in *Salmonella*, with or without metabolic activation (de Meester et al. 1981; Gervasi et al. 1985; Kushi et al. 1985; Mortelmans et al. 1986). Isoprene metabolites were also not mutagenic, except for the diepoxide metabolite (Gervasi and Longo 1990). It has also been postulated that the equivalent dose metric hypothesis is not applicable for isoprene based on experimental data; carcinogenic data does not appear to follow a linear trend (Cox et al. 1996; Placke et al. 1996).

Isoprene metabolism is complex; there is stereoselectivity in the oxidation of isoprene to the mono- and di-epoxides (Watson et al. 2001). Even with the suggestive evidence of a threshold, the MOA has not been clearly defined and is not completely understood at this time. Therefore, TCEQ choses to use the default linear low-dose extrapolation approach.

Chapter 5 References

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Appendix A: Dose-Response Modeling and Inhalation Toxicity Factors for Isoprene Report

Dose-Response Modeling and Inhalation Toxicity Factors for Isoprene

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Executive Summary

At the request of the Texas Commission on Environmental Quality (TCEQ), under Work Order No. 582-9-80187-09 Amendment 1, Sielken & Associates Consulting Inc. (Sielken & Associates or S&A) has (1) reviewed the literature relevant to isoprene and cancer dose-response modeling datasets and (2) performed cancer dose response modeling and determined inhalation toxicity factors [specifically, unit risk factors (URFs)] for the carcinogenic section of TCEQ's isoprene development support document (DSD).

TCEQ has identified three study reports for dose-response modeling and the estimation of a unit risk factor for isoprene. The three studies are NTP (1994, 1999) and Placke et al. (1996). Sielken & Associates has reviewed these three studies and determined that they are adequate studies and that they contain data necessary to perform dose-response modeling.

Using the dose-response data from Placke et al. (1996) which includes response data for different inhalation ppm levels (exposure intensity) and different exposure durations (either 4 or 8 hours per day, 5 days per week, and either 20, 40, or 80 weeks), Sielken & Associates concludes that, for isoprene inhalation exposure, exposure intensity has a greater impact on response frequency than exposure duration. This conclusion is consistent with several publications in the literature.

Sielken & Associates' objective with respect to determining URFs is to determine URFs per lifetime average daily ppm concentration of isoprene assuming that exposure is 24 hours per day, 7 days per week, for a lifetime. To meet this objective, Sielken & Associates has adjusted the dose levels and numbers of animals at risk in the data sets corresponding to the three animal studies for differences between the exposure durations and times of response observation and the objective of characterizing exposure for 24 hours per day, 7 days per week, for a lifetime (assumed environmental exposure). Our calculations suggest that a reasonable characterization of the highest URF is approximately 0.010 per environmental ppm based on all endpoints or approximately 0.001 per environmental ppm based on malignant responses (i.e., carcinoma, sarcoma, and lymphoma) in rats and male mice (i.e., all animals except the female mice in Placke et al. 1996). In characterizing human URFs, Sielken & Associates has assumed that animals and humans have equivalent response frequencies when exposed to the same ppm levels. Alternative dosimetric adjustment factors of approximately 1.7 have been considered by OEHHA and USEPA for compounds frequently considered to be similar to isoprene. This would mean dividing our calculated URFs by a factor of 1.7.

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1. Introduction

At the request of the Texas Commission on Environmental Quality (TCEQ), under Work Order No. 582-9-80187-09 Amendment 1, Sielken & Associates Consulting Inc. (Sielken & Associates or S&A) has (1) reviewed the literature relevant to isoprene and cancer dose-response modeling datasets and (2) performed cancer dose response modeling and determined inhalation toxicity factors [specifically, unit risk factors (URFs)] for the carcinogenic section of TCEQ's isoprene development support document (DSD).

2. Datasets

TCEQ has identified three study reports for dose-response modeling and the estimation of a unit risk factor for isoprene. The three studies are NTP (1994, 1999) and Placke et al. (1996) which TCEQ described as follows:

Melnick et al. 1994a

Melnick et al. (1994a) is an NTP toxicity study of isoprene administered by inhalation to F344/N rats and B6C3F$_1$ mice. This study was a combination of a dose-finding, subacute study, and two chronic studies. For the chronic stop-exposure study: groups of 40 male rats and 40 male mice were exposed to 0, 70, 220, 700, 2,200, or 7,000 ppm isoprene for 6 h/d 5 d/wk for 6 months. Ten animals per species were evaluated at the end of the exposure while the rest were allowed to recover for an additional 6 months without isoprene exposure.

Melnick et al. 1999

Melnick et al. (1999) is an NTP toxicology and carcinogenesis study of isoprene administered by inhalation to F344/N rats. Groups of 50 male and 50 female F344/N rats were exposed to 220, 700, or 7,000 ppm isoprene for 6 h/d 5 d/wk for 105 wk (just over 2 years).

Placke et al. 1996

Placke et al. (1996) is a chronic inhalation study of isoprene in B6C3F$_1$ mice. Results are also discussed in Cox et al. (1996). The purpose of this study was to investigate the effects of various concentrations and durations of exposure to isoprene in B6C3F$_1$ mice. Twelve groups total were dosed.

20-wk Exposure Group

One group of 50 male B6C3F$_1$ mice was exposed to 280 ppm isoprene for 8 h/d 5d/wk for 20 wk, while another group of 50 male B6C3F$_1$ mice was exposed to 2,200 ppm isoprene for 4 h/d 5d/wk for 20 wk.

40-wk Exposure Group

Three groups of 50 male B6C3F$_1$ mice were exposed to 70, 140, and 2,200 ppm isoprene for 8 h/d 5d/wk for 40 wk.

80-wk Exposure Group

Three groups of 50 male and 50 female B6C3F$_1$ mice were exposed to 0, 10, and 70 ppm isoprene for 8 h/d 5d/wk for 80 wk. Another three groups of 50 male B6C3F$_1$ mice were exposed to 280, 700, and 2,200 ppm isoprene for 8 h/d 5d/wk for 80 wk. One group of 50 male B6C3F$_1$ mice was exposed to 2,200 ppm isoprene for 4 h/d 5d/wk for 80 wk.

The NTP (1994) study reports the findings of two-week and 13-week inhalation experiments in male and female F344/N rats and $B6C3F_1$ mice. Target concentrations of isoprene in the 2-week inhalation experiments were 0, 438, 875, 1750, 3500 and 7000 ppm. In the 13-week stop-exposure experiments the exposure concentrations were 0, 70, 220, 700, 2200, and 7000 ppm.

The NTP (1994) study also reports the finding of experiments on male rats and male mice exposed to isoprene vapors for 6 months (26 weeks) followed by a 6-month recovery period (stop-exposure protocol). The 6-month exposures were for 6 hours/day and 5 days/week. Responses were observed at the end of 1 year (53 weeks). In this study the exposure concentrations were 0, 70, 220, 700, 2200, and 7000 ppm.

The NTP (1999) study reports the finding of experiments on male rats exposed to isoprene vapors for 105 weeks. The 2 years (105 weeks) exposures were for 6 hours/day and 5 days/week. Responses were observed at the end of 2 years (106 weeks). In this study the exposure concentrations were 0, 220, 700, and 7000 ppm.

The Placke et al. (1996) study reports the findings of a 104-week experiment on male and female $B6C3F_1$ mice. The animals were exposed to different exposure patterns of isoprene. Ten groups were exposed to isoprene 8 hrs/day, 5 days/week for different periods of time. The ten exposure-duration (ppm-weeks) groups were 0-80, 10-80, 70-40, 70-80, 140-40, 280-20, 280-80, 700-80, 2200-40, and 2200-80. Two groups were exposed to isoprene 4 hrs/day, 5 days/week (2200-20 and 2200-80). The experimental groups were kept until 96 or 105 weeks on study. Placke et al. found "exposure-related increased incidence of liver, lung, Harderian gland and forestomach tumors, and hemangiosarcomas and histiocytic sarcomas."

Table 1 shows an overview of the experimental designs for the portions of NTP(1994), Placke et al. (1996), and NTP(1999) used for dose-response modeling herein. The exposure groups in Placke et al. (1996) are shown in Table 2, and some of the comparisons related to the time pattern of exposure are suggested in Table 3.

Table 1. Overview of experimental designs for the portions of NTP(1994), Placke et al. (1996), and NTP(1999) used for dose-response modeling herein

Study	Species	Gender	ppm levels	Exposure Duration			Time of Response Observation / Necropsy
				hours/day	days/week	weeks	weeks
NTP (1994)	F344/N Rats	Males	0, 70, 220, 700, 2200, 7000	6	5	26	53
NTP (1994)	$B6C3F_1$ Mice	Males	0, 70, 220, 700, 2200, 7000	6	5	26	53
Placke et al. (1996)	$B6C3F_1$ Mice	Males	0, 10, 70, 140, 280, 700, 2200	4 or 8	5	20 or 40 or 80	96 or 105
Placke et al. (1996)	$B6C3F_1$ Mice	Females	0, 10, 70	8	5	80	105
NTP (1999)	F344/N Rats	Males	0, 220, 700, 7000	6	5	105	106
NTP (1999)	F344/N Rats	Females	0, 220, 700, 7000	6	5	105	106

Table 2. Experimental design for the Placke et al. (1996) study on B6C3F$_1$ mice

Group #	Sex	Time of response observation / necropsy: Weeks	ppm	hrs/day	days/wk	weeks	duration (hours)	ppm×duration
1	Male	105	280	8	5	20	800	224,000
2	Male	105	2,200	4	5	20	400	880,000
3	Male	105	70	8	5	40	1,600	112,000
4	Male	105	140	8	5	40	1,600	224,000
5	Male	105	2,200	8	5	40	1,600	3,520,000
6	Male	105	0	8	5	80	3,200	0
7	Male	96	10	8	5	80	3,200	32,000
8	Male	96	70	8	5	80	3,200	224,000
9	Male	96	280	8	5	80	3,200	896,000
10	Male	96	700	8	5	80	3,200	2,240,000
11	Male	96	2,200	8	5	80	3,200	7,040,000
12	Male	96	2,200	4	5	80	1,600	3,520,000
13	Female	105	0	8	5	80	3,200	0
14	Female	105	10	8	5	80	3,200	32,000
15	Female	105	70	8	5	80	3,200	224,000

Table 3. Experimental design for the Placke et al. (1996) study on B6C3F$_1$ mice: Comparisons related to the time pattern of exposure

Group #	Sex	ppm	hrs/day	days/wk	weeks	duration (hours)	ppm×duration	Comparisons		
13	Female	0	8	5	80	3,200	0	Gender		
14	Female	10	8	5	80	3,200	32,000	Gender		
15	Female	70	8	5	80	3,200	224,000	Gender		
6	Male	0	8	5	80	3,200	0	Gender		
7	Male	10	8	5	80	3,200	32,000	Gender		
3	Male	70	8	5	40	1,600	112,000	Gender		
1	Male	280	8	5	20	800	224,000		Weeks	
4	Male	140	8	5	40	1,600	224,000		Weeks	
8	Male	70	8	5	80	3,200	224,000		Weeks	
2	Male	2,200	4	5	20	400	880,000			Hrs & Wks
9	Male	280	8	5	80	3,200	896,000			Hrs & Wks
10	Male	700	8	5	80	3,200	2,240,000			
5	Male	2,200	8	5	40	1,600	3,520,000			Hrs & Wks
12	Male	2,200	4	5	80	1,600	3,520,000			Hrs & Wks
11	Male	2,200	8	5	80	3,200	7,040,000			

3. Response Endpoints

According to the National Toxicology Program, Report on Carcinogens (Twelfth Edition, 2011, pages 247-249), exposure to isoprene by inhalation caused tumors at several different tissue sites in mice and rats. Specifically, the following responses were noted:

In mice of both sexes, isoprene caused

*blood-vessel cancer (**hemangiosarcoma**) and*

*benign or malignant tumors of the **Harderian gland** (adenoma or carcinoma) and*

*the **lung** (alveolar/bronchiolar adenoma or carcinoma).*

In male mice, it also caused cancer of the

***hematopoietic system** (histiocytic sarcoma) and*

*benign or malignant tumors of the **liver** (hepatocellular adenoma or carcinoma)*

*and **forestomach** (squamous-cell papilloma or carcinoma).*

In rats of both sexes, isoprene caused

*benign or malignant tumors of the **mammary gland** (fibroadenoma or carcinoma)*

*and **kidney** (renal-cell adenoma or carcinoma).*

In male rats, it also caused

*benign tumors of the **testis** (adenoma)*

These observations were said to be based on "NTP 1995" (i.e., NTP (1994)), Placke *et al.* 1996, and Melnick and Sills 2001 (i.e., NTP (1999)).

Placke et al. (1996) found "exposure-related increased incidence of liver, lung, Harderian gland and forestomach tumors, and hemangiosarcomas and histiocytic sarcomas." These responses were not necessarily associated with "statistically significant" increases.

Only tumor responses are considered. For example, hyperplasia is not considered to be a cancer tumor.

Because the observation time for responses in the two-week study and the 13-week study in NTP 1994 correspond to observations made at the end of two weeks and

thirteen weeks, respectively, and our objective is to characterize chronic carcinogenesis, these studies are not included in our analyses herein. Also, because our objective is to characterize chronic carcinogenesis, the observations made at twelve months after the beginning of the NTP 1994 stop-exposure study are included in our analyses herein, but not the observations made after only six months.

Although the narrative in the NTP Report on Carcinogens suggests that lung tumors (alveolar/bronchiolar adenoma or carcinoma) in both male and female mice were increased, the data themselves only suggest that lung tumors are increased for males but not females (in female mice the response rates were 5/50, 6/50, and 5/50 at 0, 800, and 5600 ppm×weeks, respectively, in Placke et al. (1996)).

Similarly, although the narrative in the NTP Report on Carcinogens suggests that kidney tumors in both male and female mice were increased, the data themselves only suggest that kidney tumors are increased for males but not females (Table 9 in NTP 1999).

Although the abstract in Placke et al. (1996) states that "There was an exposure-related increased incidence of liver, lung, Harderian gland and forestomach tumors, and hemangiosarcomas and histiocytic sarcomas." This observation does not necessarily apply to both male and female mice. For example, in female mice the response rates for hemangiosarcoma in the heart were 0/50, 0/50, and 0/50 at 0, 800, and 5600 ppm×weeks, respectively. Hence, hemangiosarcoma in the heart is not included as a response in our analyses herein.

Table 4 shows a combined indication of potential responses for cancer dose-response modeling.

Table 5 (NTP 1994), Table 6 (Placke et al. 1996), and Table 7 (NTP 1999) show the response data for the responses with at least one dose for which the study report indicated a statistically significant increased response rate compared to controls or a statistically significant trend. If the adenoma or fibroadenoma or papilloma response was "statistically significant", we also included the corresponding responses of "carcinoma" and "adenoma/carcinoma" (i.e., adenoma and/or carcinoma) whenever there were reported data for at least "carcinoma". Table 5 also includes the response data in NTP 1994 for "malignant lymphoma" because "any lymphoma" was a response in Placke et al. 1996 that had at least one dose for which the study report indicated a statistically significant increased response rate compared to controls. In what follows we refer to these responses as "candidate" responses.

The dose levels (ppm) in Tables 5, 6, and 7 do not reflect any adjustments for exposure duration (hours/day, days/week, or weeks) or time of response observation / necropsy (53, 96, 104, 105, or 106 weeks). The "Number at Risk" do not reflect any adjustments for time of response observation / necropsy (53, 96, 104, 105, or 106 weeks).

Table 4. List of potential responses for cancer dose-response modeling based on NTP's Report on Carcinogens (2011) and Placke et al. (1996)

Species	Gender	Target Organ	Response	Study
Rats (F344/N)	Male	Kidney	Adenoma	NTP 1999
			Carcinoma	NTP 1999
			Adenoma and/or Carcinoma	NTP 1999
Rats (F344/N)	Male	Mammary Gland	Fibroadenoma	NTP 1999
			Carcinoma	
			Fibroadenoma and/or Carcinoma	
Rats (F344/N)	Male	Testis	Adenoma	NTP 1994
				NTP 1999
				NTP 1994, 1999 Combined
Rats (F344/N)	Female	Mammary Gland	Fibroadenoma	NTP 1999
			Carcinoma	
			Fibroadenoma and/or Carcinoma	
$B6C3F_1$	Male	Circulatory System: Heart	Hemangiosarcoma	Placke et al. 1996
$B6C3F_1$	Male	Circulatory System: Spleen	Hemangiosarcoma	Placke et al. 1996
$B6C3F_1$	Male	Forestomach	Papilloma	NTP 1994
				Placke et al. 1996
				Placke et al. 1996, NTP 1994 Combined
			Carcinoma	NTP 1994
				Placke et al. 1996
				Placke et al. 1996, NTP 1994 Combined
			Papilloma and/or Carcinoma	NTP 1994
				Placke et al. 1996

Species	Gender	Target Organ	Response	Study
				Placke et al. 1996, NTP 1994 Combined
$B6C3F_1$	Male	Harderian Gland	Adenoma	NTP 1994
				Placke et al. 1996
				Placke et al. 1996, NTP 1994 Combined
			Carcinoma	NTP 1994
				Placke et al. 1996
				Placke et al. 1996, NTP 1994 Combined
			Adenoma and/or Carcinoma	NTP 1994
				Placke et al. 1996
				Placke et al. 1996, NTP 1994 Combined
$B6C3F_1$	Male	Hematopoietic System	Any Lymphoma	NTP 1994
				Placke et al. 1996
				Placke et al. 1996, NTP 1994 Combined
$B6C3F_1$	Male	Hematopoietic System	Any histiocytic sarcoma	Placke et al. 1996
$B6C3F_1$	Male	Liver	Adenoma	NTP 1994
				Placke et al. 1996
				Placke et al. 1996, NTP 1994 Combined
			Carcinoma	NTP 1994
				Placke et al. 1996
				Placke et al. 1996, NTP 1994 Combined
			Adenoma and/or Carcinoma	NTP 1994

Species	Gender	Target Organ	Response	Study
				Placke et al. 1996
				Placke et al. 1996, NTP 1994 Combined
$B6C3F_1$	Male	Lung	Adenoma	NTP 1994
				Placke et al. 1996
				Placke et al. 1996, NTP 1994 Combined
			Carcinoma	NTP 1994
				Placke et al. 1996
				Placke et al. 1996, NTP 1994 Combined
			Adenoma and/or Carcinoma	NTP 1994
				Placke et al. 1996
				Placke et al. 1996, NTP 1994 Combined
$B6C3F_1$	Female	Circulatory System: Spleen	Hemangiosarcoma	Placke et al. 1996
$B6C3F_1$	Female	Harderian Gland	Adenoma	Placke et al. 1996
$B6C3F_1$	Female	Pituitary Gland	Adenoma	Placke et al. 1996

Table 5. Response data for the responses with at least one dose for which the NTP 1994 study report indicated a statistically significant increased response rate compared to controls: Also includes the response data for "malignant lymphoma" because "any lymphoma" was a response in Placke et al. 1996 that had at least one dose for which the study report indicated a statistically significant increased response rate compared to controls: **The dose levels (ppm) do not reflect any adjustments for exposure duration (hours/day, days/week, or weeks) or time of response observation / necropsy (53 weeks): The "Number at Risk" does not reflect any adjustments for time of response observation / necropsy (53 weeks)**

Isoprene Exposure (ppm)		0[1]	70[2]	220	700	2200	7000
Target Organ	Response						
Male F344/N Rats							
Testis	Number at Risk	30	30	30	30	29	30
	Adenoma	3*	3	4	7	8	9
Male B6C3F$_1$ Mice							
Forestomach	Number at Risk	30	30	30	30	30	30
	Papilloma	0**	0	0	1	2	5*
	Carcinoma	0	0	0	0	2	1
	Papilloma/Carcinoma	0**	0	0	1	4	6**
Harderian Gland	Number at Risk	30	30	30	30	30	30
	Adenoma	2**	6	4	14**	13**	12**
	Carcinoma	0	0	0	0	1	0
	Adenoma/Carcinoma	2**	6	4	14**	13**	12**
Liver	Number at Risk	30	30	29	30	30	28
	Adenoma	4**	2	6	15*	18*	16*
	Carcinoma	4**	1	3	5	4	9
	Adenoma/Carcinoma	7**	3	7	15**	18**	17**
Lung	Number at Risk	30	30	29	30	30	28
	Adenoma	2**	2	1	4	10*	8*
	Carcinoma	0**	0	0	1	1	3
	Adenoma/Carcinoma	2**	2	1	5	10*	9*
Hematopoietic System	Number at Risk	30	30	30	30	30	30
	Malignant Lymphoma	1	0	0	2	1	2

*statistically significant at the 5% significance level
**statistically significant at the 1% significance level
[1]statistically significant results in the control group are for the Cochran-Armitage trend test for an increasing trend
[2]statistically significant results in the exposed groups are for the Fisher exact test for an increase in the incidence versus the incidence in the control group

Table 6. Response data for the responses with at least one dose for which the Placke et al. (1996) study report indicated a statistically significant increased response rate compared to controls: **The dose levels (ppm) do not reflect any adjustments for exposure duration (hours/day, days/week, or weeks) or time of response observation / necropsy (96 or 105 weeks): The "Number at Risk" does not reflect any adjustments for time of response observation / necropsy (96 or 105 weeks)**

Isoprene Exposure (ppm)		0	10	70	70	140	280	280	700	2,200	2,200	2,200	2,200
Duration of Exposure (weeks)		80	80	40	80	40	20	80	80	20	80	40	80
Period of Exposure (hrs/day)		8	8	8	8	8	8	8	8	4	4	8	8
Cumulative Exposure (ppm-weeks)		0	800	2,800	5,600	5,600	5,600	22,400	56,000	22,000	88,000	88,000	176,000
Target Organ	Response												
Male B6C3F$_1$ Mice													
Circulatory System: Heart	Number at Risk	49	50	49	50	50	50	50	50	50	50	49	50
	Hemangiosarcoma	0**	0	0	0	0	0	2	1	4	1	1	1
Circulatory System: Spleen	Number at Risk	49	48	47	50	50	47	50	48	48	50	47	49
	Hemangiosarcoma	1	3	1	2	3	2	1	2	2	2	0	1
Forestomach[3]	Number at Risk	50	48	47	50	49	46	50	47	48	50	47	50
	Papilloma	0**	0	0	0	0	0	0	1	0	1	2	1
	Carcinoma	0**	0	0	0	0	0	1	0	1	1	0	3
	Papilloma/Carcinoma	0**	0	0	0	0	0	1	1	1	2	2	4
Harderian Gland[3]	Number at Risk	47	49	48	50	50	49	50	49	49	50	49	50
	Adenoma	4**	4	13*	9	12*	16**	17**	26**	19**	28**	31**	35**
	Carcinoma	0	0	0	0	2	3	1	3	1	2	0	2
	Adenoma/Carcinoma	4**	4	13*	9	14*	19**	18**	29**	20**	30**	31**	37**
Hematopoietic System	Number at Risk	50	50	50	50	50	50	50	50	50	50	50	50
	Any Lymphoma	2**	1	2	4	1	7	5	4	4	4	5	6
Hematopoietic System	Number at Risk	50	50	50	50	50	50	50	50	50	50	50	50
	Histiocytic Sarcoma	0**	2	2	2	1	8**	4	2	5**	7**	7**	2
Liver[3]	Number at Risk	50	50	49	50	50	49	50	48	50	50	47	50
	Adenoma	11**	12	14	15	22*	18	24**	27**	22*	21*	28**	30**
	Carcinoma	9**	6	11	9	10	12	16	17*	12	15	18*	16
	Adenoma/Carcinoma	20**	18	25	24	32*	30*	40**	44**	34**	36**	46**	46**

Isoprene Exposure (ppm)		0	10	70	70	140	280	280	700	2,200	2,200	2,200	2,200
Duration of Exposure (weeks)		80	80	40	80	40	20	80	80	20	80	40	80
Period of Exposure (hrs/day)		8	8	8	8	8	8	8	8	4	4	8	8
Cumulative Exposure (ppm-weeks)		0	800	2,800	5,600	5,600	5,600	22,400	56,000	22,000	88,000	88,000	176,000
Target Organ	Response												
Lung[3]	Number at Risk	50	50	50	50	50	50	50	50	50	50	49	50
	Adenoma	11**	16	8	4	10	16	13	23**	14	15	29**	30**
	Carcinoma	0**	1	0	2	1	3	1	7**	2	3	3	7**
	Adenoma/Carcinoma	11**	17	8	6	11	19	14	30**	16	18	32**	37**
Female B6C3F$_1$ Mice													
Circulatory System: Spleen	Number at Risk	50	49		50								
	Hemangiosarcoma	1*	1		4								
Harderian Gland	Number at Risk	49	49		49								
	Adenoma	2*	3		8*								
	Carcinoma	0	0		0								
	Adenoma/Carcinoma	2*	3		8*								
Pituitary Gland	Number at Risk	49	46		49								
	Adenoma	1*	6*		9**								

*statistically significant at the 5% significance level
**statistically significant at the 1% significance level
[1]statistically significant results in the control group are for the Cochran-Armitage trend test for an increasing trend
[2]statistically significant results in the exposed groups are for the Fisher exact test for an increase in the incidence versus the incidence in the control group
[3]the combined responses papilloma/carcinoma and adenoma/carcinoma is the sum of the two individual responses because Placke et al. did not report these responses combined

Table 7. Response data for the responses with at least one dose for which the NTP 1999 study report indicated a statistically significant increased response rate compared to controls: **The dose levels (ppm) do not reflect any adjustments for exposure duration (hours/day, days/week, or weeks) or time of response observation / necropsy (106 weeks): The "Number at Risk" does not reflect any adjustments for time of response observation / necropsy (106 weeks)**

Isoprene Exposure (ppm)		0[1]	220[2]	700	7000
Number of Animals at Risk		50	50	50	50
Target Organ	Response				
Male F344/N Rats					
Kidney	Adenoma	2**	4	8*	15**
	Carcinoma	0	0	1	0
	Adenoma/Carcinoma	2**	4	8*	15**
Mammary Gland	Fibroadenoma	2**	4	6	21**
	Carcinoma	0	1	1	2
	Fibroadenoma/Carcinoma	2**	5	7	21**
Testis	Adenoma	33**	37	44**	48**
Female F344/N Rats					
Mammary Gland	Fibroadenoma	19	35**	32**	32**
	Carcinoma	4	2	1	3
	Fibroadenoma/Carcinoma	20	35**	32*	32*

*statistically significant at the 5% significance level
**statistically significant at the 1% significance level
[1]statistically significant results in the control group are for the Cochran-Armitage trend test for an increasing trend
[2]statistically significant results in the exposed groups are for the Fisher exact test for an increase in the incidence versus the incidence in the control group

4. Analyses Comparing Impacts of Exposure Duration and Intensity

Using as an example the dose-response data on liver adenoma and/or carcinoma from Placke et al. (1996) which includes response data for different inhalation ppm levels (exposure intensity) and different exposure durations (either 4 or 8 hours per day, 5 days per week, and either 20, 40, or 80 weeks), Sielken & Associates concludes that, for isoprene inhalation exposure, exposure intensity has a greater impact on response frequency than exposure duration. This conclusion is consistent with several publications in the literature. For example, Placke et al. (1996) states that

> *Statistical analyses indicated that the product of isoprene concentration, and length/duration of exposure was not a sufficient basis for predicting tumor risk at any site. Extrapolation of tumor probability between the high and low doses based on cumulative exposure was not appropriate and could not be justified by statistical models. A threshold effect level and strong nonlinearities with respect to concentration appeared to exist for tumor development in this study.*

Cox et al. (1996) states that

> *Most statistical risk assessment models assume that equal doses, measured on a scale such as mg/kg/day, create equal tumor risks. This equivalent dose metric (EDM) hypothesis allows risks to be extrapolated from high concentrations to low· concentrations and from one species, sex, and strain to another, since it implies that all administered dose histories corresponding to the same total dose create the same risk. This paper tests the EDM hypothesis using data on tumor rates in B6C3F$_1$ mice administered isoprene via inhalation. Its major conclusion is that the EDM hypothesis does not hold for isoprene. For example, it appears that exposure concentration has a greater impact on tumor rates than weeks of exposure.*

> On a slightly different note, Placke et al. (1996) also states that

> *Tumors at different anatomic sites did not occur statistically independently of each other. For example, liver adenomas and lung adenomas were significantly positively associated, especially at higher concentrations. This means that, when other factors (e.g. exposure concentration and duration) were the same, then a randomly selected mouse was more likely to have one of these tumors if it also had the other.*

In order to evaluate the relative impacts of exposure intensity and duration, Sielken & Associates did some dose-response modeling on three different subsets of the dose-response data for liver adenoma and/or carcinoma in B6C3F$_1$ mice from Placke et al. (1996) which includes response data for different inhalation ppm levels (exposure intensity) and different exposure durations (either 4 or 8 hours per day, 5 days per week, and either 20, 40, or 80 weeks). The subsets were chosen so that within the same subset each group has the same exposure duration (both the same

number of weeks of exposure and the same number of hours of exposure per day (for 5 days per week)). The subsets and the corresponding dose-response data are shown in Table 8.

For each subset, the dose-response modeling was done using three different dose metrics. The three dose metrics are (1) ppm ignoring exposure weeks and hours per day, (2) ppm×weeks ignoring exposure hours per day, and (3) ppm×weeks×hours. Table 9 shows the estimated effective concentration (EC) corresponding to a 10% extra risk, i.e., the EC_{10}, for each dose metric and each of the three subsets. The only dose metric for which the EC_{10}'s for the three subsets generally agree is (1) ppm ignoring exposure weeks and hours per day. When exposure duration is incorporated into the dose metric (either as ppm×weeks or ppm×weeks×hours), then the EC_{10}'s for the three subsets generally disagree. This suggests that exposure intensity is more important than exposure duration as an exposure characteristic.

This suggestion that exposure intensity is more important than exposure duration as an exposure characteristic is important in comparing the potential severity of different exposure scenarios. However, the suggestion does not resolve the issue of how to combine the results for different experimental designs for the purpose of determining a URF for isoprene. This latter issue is addressed in Section 5.

The U.S. Environmental Protection Agency (USEPA) default dose-response modeling methodology and associated BenchMark Dose Software (BMDS Version 2.2) is used to estimate the EC_{10}s herein. In this modeling, the response data are treated as quantal response data. That is, only the presence or absence of the specified response is counted, and any time-to-response data are ignored. The dose-response model is the multistage quantal response model with the degree of the polynomial in this model set equal to the number of distinct doses minus one (but no greater than 5). Later in this report, the URF is characterized in terms of the maximum likelihood estimate (MLE) of the effective concentration (EC_{10}) corresponding to a lifetime extra risk of 0.10, and the URF = $0.10/EC_{10}$.

Table 8. Subsets of the dose-response data for liver adenoma and/or carcinoma in male B6C3F$_1$ mice from Placke et al. (1996)

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9	Group 10	Group 11	Group 12
Isoprene Exposure (ppm)	0	10	70	70	140	280	280	700	2,200	2,200	2,200	2,200
Duration of Exposure (weeks)	80	80	40	80	40	20	80	80	20	80	40	80
Period of Exposure (hrs/day)	8	8	8	8	8	8	8	8	4	4	8	8
ppm	0	10	70	70	140	280	280	700	2,200	2,200	2,200	2,200
ppm*weeks	0	800	2,800	5,600	5,600	5,600	22,400	56,000	44,000	176,000	88,000	176,000
ppm*weeks*hrs	0	6,400	22,400	44,800	44,800	44,800	179,200	448,000	176,000	704,000	704,000	1,408,000
# at Risk	50	50	49	50	50	49	50	48	50	50	47	50
# Responses	20	18	25	24	32	30	40	44	34	36	46	46
	Subset 1: Groups with the same exposure duration (80 weeks and 8 hours/day)											
Included in Subset	X	X		X			X	X				X
	Subset 2: Groups with the same exposure duration (40 weeks and 8 hours/day)											
Included in Subset	X		X		X						X	
	Subset 3: Groups with the same exposure duration (20 weeks and 8 hours/day)											
Included in Subset	X					X						

Table 9. The estimated effective concentration (EC) corresponding to a 10% extra risk, i.e., the EC_{10}, for each dose metric and each of three different subsets of the dose-response data for liver adenoma and/or carcinoma in $B6C3F_1$ mice from Placke et al. (1996)

Dose Metric used for Dose-Response Modeling	Subset	Estimated EC_{10}
ppm	Duration = 80 weeks, 8 hours per day	64.34 ppm
ppm	Duration = 40 weeks, 8 hours per day	61.88 ppm
ppm	Duration = 20 weeks, 8 hours per day	67.58 ppm
ppm×weeks	Duration = 80 weeks, 8 hours per day	5,146.81 ppm×weeks
ppm×weeks	Duration = 40 weeks, 8 hours per day	2,475.22 ppm×weeks
ppm×weeks	Duration = 20 weeks, 8 hours per day	1,351.53 ppm×weeks
ppm×weeks ×hours	Duration = 80 weeks, 8 hours per day	41,174.50 ppm×weeks ×hours
ppm×weeks ×hours	Duration = 40 weeks, 8 hours per day	19,801.80 ppm×weeks ×hours
ppm×weeks ×hours	Duration = 20 weeks, 8 hours per day	10,812.30 ppm×weeks ×hours

5. Adjusting Dose Scales for Exposure Duration and Time of Response Observation / Necropsy

5.1 Introduction

The multistage quantal dose-response model with

P(d) = Probability of a "specified response" occurring by the end of a "specified time period" at "dose d"

and

$$P(d) = 1 - \exp[- (q_0 + q_1 \times d + q_2 \times d^2 + ... + q_k \times d^k)]$$

where q_0, q_1, q_2, ... q_k are parameters to be estimated is a default dose-response model frequently used by regulatory agencies (e.g., TCEQ, USEPA, OEHHA).

The model is "quantal" or "dichotomous" when the specified response either "occurs" or "doesn't occur" or, equivalently, the specified response is either "present" or "absent" or "yes" or "no" and is counted as either 1 or 0.

The "specified response" is usually death with a specified type of "tumor" observed (e.g., detected during an external examination or found during a necropsy). (Note that "death with" does not necessarily imply "death caused by".)

The end of a "specified time period" is usually the end of a "lifetime."

The "dose d" is flexible. It can refer to an administered dose, a delivered dose, or a biologically effective dose (BED). The dose d is often the "lifetime average daily dose (LADD)", but the dose d does not have to be the LADD. If the dose d is based on the multistage theory of carcinogenesis, then the dose d corresponding to a discontinuous exposure (e.g., an intermittent exposure or an exposure at different magnitudes (levels) at different times) will not be an LADD.

The multistage quantal dose-response model is not a time-to-response model because the specified response is either recorded as 1 or 0 and the time of the response is either unknown or ignored (except that the specified response either occurred or did not occur during the specified time period).

5.2 Adjusted Doses Based on the Multistage Theory of Carcinogenesis

The Armitage and Doll (1954) mathematical description of carcinogenesis as expressed by Crouch (1983), Crump and Howe (1984), and several others allows for the analysis of data sets with variable dosing over time. The model assumes that cancer derives from a single cell after it has undergone a series of transformations. The model has been used to describe cancer dose response data in animal bioassays as well as in the general population.

Assumptions are required for the application of the Armitage-Doll model regarding: 1) the mathematical relationship between applied dose and the probability that a "stage transition" has occurred, 2) the stage affected by the carcinogen, and 3) the number of "stages."

The multistage theory of carcinogenesis assumes that the transformation of a normal cell to a specified neoplastic stage requires the occurrence of m biological events (transitions) and that these events occur in one specific order. Mathematically, if λ_i is the transition rate for a cell from the i-th stage to the i+1-th stage in an m-stage carcinogenic process (i=0, 1, 2, ..., m-1 and i=0 corresponds to the normal or background stage), then **the hazard rate $H(T_e)$** corresponding to a single cell leading to the specified response (tumor) occurring by a specified time T_e under Armitage and Doll (1954) becomes

$$H(T_e) = {}_0\int^{T_e} \lambda_{m-1} \times {}_0\int^{t_{m-1}} \lambda_{m-2} \times {}_0\int^{t_{m-2}} \lambda_{m-3} \times \ldots \times {}_0\int^{t_1} \lambda_0 \, dt_0 \, dt_1 \ldots dt_{m-2} \, dt_{m-1}$$

which corresponds to an (m-1)-stage cell having to make the final transition to the m-th stage at some time $t_{(m-1)}$ between time 0 and time T_e, preceded by an (m-2)-th stage cell having to make a transition to the (m-1)-th stage at some time $t_{(m-2)}$ between time 0 and time $t_{(m-1)}$, and so forth back to a normal (0-th stage) stage cell having to make a transition to the 1-th stage at some time t_0 between time 0 and time t_1 (see also Crump and Howe, 1984, Kodell et al., 1987, or Holland and Sielken, 1993). Therefore, if there are N independent normal cell lines, the **probability of developing cancer by age T_e** is the probability of at least one of these cell lines reaching the m-th stage, that is,

$$P(T_e) = 1 - \exp[\, - N \times H(T_e) \,].$$

In the special case where λ_i is independent of time and linearly dependent on dose, then

$$\lambda_i = \alpha_i + \beta_i \times d$$

and

$$P(T_e) = 1 - \exp\{-N \times [(\alpha_0+\beta_0 \times d) \times (\alpha_1+\beta_1 \times d) \times \ldots \times (\alpha_{m-1}+\beta_{m-1} \times d)] \times (T_e)^m / m! \,]$$

or, equivalently,

$$P(T_e) = 1–\exp \{ - [q_0 + q_1 \times d + q_2 \times d^2 + ... + q_m \times d^m \}$$

which is commonly referred to as the multistage model or the Armitage-Doll multistage model.

If λ_i is linearly dependent on dose and dose is dependent on time, say d(t), but λ_i is otherwise independent of time, then

$$\lambda_i = \lambda_i(t) = \alpha_i + \beta_i \times d(t)$$

and

$$H(T_e) = {}_0\int^{T_e} \lambda_{m-1} \times {}_0\int^{t}_{m-1} \lambda_{m-2} \times {}_0\int^{t}_{m-2} \lambda_{m-3} \times ... \times {}_0\int^{t1} \lambda_0 \, dt_0 \, dt_1 \, ... \, dt_{m-2} \, dt_{m-1}$$

depends on which specific λ_i are time dependent and the functional form of d(t). In particular, if only λ_0 is dose dependent and

$$\begin{aligned} d(t) &= 0 \text{ for } t<a \\ &= d \text{ for } a \le t \le b \\ &= 0 \text{ for } t>b \end{aligned}$$

then, as shown in mathematical detail in Appendix A, the extra risk at time T_e for this situation is equal to the extra risk at time T corresponding to the end of a normal lifetime at a constant dose D from time 0 to time T when

$$D= d \times \{ [T_e – a]^m - [T_e – b]^m \} / T^m.$$

That is, the extra risk at time T_e with intermittent dose d(t), namely

$$\{ P[T_e, d(t)] - P[T_e, 0] \} / P[T_e, 0]$$

equals the extra risk at time T with a constant dose D, namely

$$\{ P[T, D] - P[T, 0] \} / P[T, 0].$$

In this sense, the constant dose D is equivalent to the time-dependent dose d(t). In the dose-response modeling we transform the intermittent experimental doses d(t) to this equivalent doses D and then estimate EC_{10}s in units of D (i.e., constant environmental ppm). This same equivalence is alluded to and used in both OEHHA (2004) and OEHHA (2010) and both reference Crouch (1983). Although the basis of this equivalence is only alluded to in these references, it is more clearly stated above and mathematically demonstrated in detail in Appendix A.

In the dose-response modeling that follows, the dose is this D for specified values of m, j=1 (i.e., the only transition rate that is dose-dependent is the first transition

rate which is from the normal stage to the first stage), T_e=104, T=104, a=0, b, n_{hrs}, and n_{days}. Note that T_e=104 and T=104 corresponds to a two-year mouse or rat lifetime.

The above formula for D assumes that the "d" has been adjusted to the dose value for 24 hours per day and 7 days per week. In order to adjust for a d that is for n_{hrs} hours per day and n_{days} days per week, the formula for D becomes

$$D = d \times (n_{hrs} / 24) \times (n_{days}/ 7) \times [(T_e - a)^m – (T_e – b)^m] / T^m.$$

For example, for the male mice in Placke et al. (1996) exposed to 10 ppm for 8 hours per day, 5 days per week, T_e=105 weeks, T=104 weeks, a=0, and b=80 weeks, the equivalent constant dose D (24 hours per day, 7 days per week, for a nominal lifetime (104 weeks)) is

$$D = d \times (n_{hrs} / 24) \times (n_{days}/ 7) \times [(T_e - a)^m – (T_e – b)^m] / T^m$$

$$= 10 \times (8 / 24) \times (5 / 7) \times [(104 - 0)^m – (104 – 80)^m] / 104^m$$

= 1.83 for m=1, = 2.25 for m=2, and = 2.35 for m = 3.

which are the values for D in Table 12.

5.3 Adjusted Numbers of Subjects at Risk Based on the Multistage Theory of Carcinogenesis

If the end of a study (T_{end}) is not equal to the end of a nominal lifetime (T), then the number of subjects at risk of developing the specified response by the end of a nominal lifetime in the dose-response modeling needs to be adjusted for this inequality. If

m = the number of stages in the multistage carcinogenic process,

$n_{at\ risk}(i)$ = the number of subjects in the i-th dose group at the start of the study,

$n_{resp}(i)$ = the number of subjects that are observed to have the specified response by the end of the study (T_{end}) , and

T = end of a nominal lifetime,

then, if $T_{end} \leq T$, the adjusted number of subjects at risk in the i-th dose group in the dose-response modeling is

$$\text{Adjusted } n_{at\ risk}(i) = n_{resp}(i) + [n_{at\ risk}(i) - n_{resp}(i)] \times (T_{end} / T)^m.$$

This adjusted number of subjects at risk in the i-th dose group equals the number of subjects who were observed to have the specified response by the end of the study (T_{end}) – these subjects obviously had sufficient time to develop the specified response -- plus a proportional change in the number of subjects [$n_{at\ risk}(i) - n_{resp}(i)$] in the i-th dose group that did not develop the specified response by the end of the study but who might have developed the specified response if they had been at risk for a little longer period of time (i.e, from T_{end} to T). The adjustment factor $(T_{end} / T)^m$ follows from the mathematics of the multistage model of carcinogenesis.

For example, if there were 50 animals put on test in the i-th dose group, 20 animals developed the specified response by the end of the experiment at T_{end} = 78 weeks, and the nominal lifetime is 104 weeks, then the adjusted number of subjects at risk would be

$$20 + (50\text{-}20) \times (78 / 104)^m$$

= 42.5 for m=1, and

= 36.875 for m=2.

If $T_{end} > T$, the adjusted number of subjects at risk in the i-th dose group in the dose-response modeling is

$$\text{Adjusted } n_{at\ risk}(i) = n_{resp}(i) \times (T_{end} / T)^m + [n_{at\ risk}(i) - n_{resp}(i)] .$$

This adjusted number of subjects at risk in the i-th dose group equals (a) the number of animals who did not develop the specified response by $T_{end} > T$ – who obviously did not develop the specified response by $T<T_{end}$ plus (b) the adjusted number of subjects who were observed to have the specified response by the end of the study (T_{end}) – these subjects were at risk for more than a nominal lifetime response and each effectively represented slightly more than 1 lifetime at risk. The adjustment factor $(T_{end} / T)^m$ follows from the mathematics of the multistage model of carcinogenesis.

For example, if T_{end} is greater than T so that the number of responses is greater than it might have been if the end of the study was shortened to T, then the adjusted number of subjects at risk would be increased. For example, if there were 50 animals put on test in the i-th dose group, 20 animals developed the specified response by the end of the experiment at T_{end} = 130 weeks, and the nominal lifetime is 104 weeks, then the adjusted number of subjects at risk would be

$20 \times (130 / 104)^m + (50-20)$

= 55 for m=1, and

= 61.25 for m=2.

5.4 Adjusted Doses

For the NTP (1994) study for male rats, the adjusted doses corresponding to m=1 or 2 or 3, j=1, T_e=104 weeks, T=104 weeks, a=0, and b=26 weeks are shown in Table 10.

Table 10. Adjusted doses for male rats in NTP(1994): m=1 or 2 or 3, j=1, T_e=104 weeks, T=104 weeks, 5 days per week, a=0, and b=26 weeks

Isoprene Exposure (ppm)	weeks	hr/day	Observation Time (Necropsy Time) (weeks)	m=1	m=2	m=3
				Armitage-Doll ppm level (Adjusted to Specified hrs/day and days/week)	Armitage-Doll ppm level (Adjusted to Specified hrs/day and days/week)	Armitage-Doll ppm level (Adjusted to Specified hrs/day and days/week)
0	26	6	53	0.00	0.00	0.00
70	26	6	53	3.13	5.47	7.23
220	26	6	53	9.82	17.19	22.71
700	26	6	53	31.25	54.69	72.27
2200	26	6	53	98.21	171.88	227.12
7000	26	6	53	312.50	546.88	722.66

For the NTP (1994) study for male mice, the adjusted doses corresponding to m=1 or 2 or 3, j=1, T_e=104 weeks, T=104 weeks, a=0, and b=26 weeks are shown in Table 11.

Table 11. Adjusted doses for male mice in NTP(1994): m=1 or 2 or 3, j=1, T_e=104 weeks, T=104 weeks, 5 days per week, a=0, and b=26 weeks

Isoprene Exposure (ppm)	weeks	hr/day	Observation Time (Necropsy Time) (weeks)	m=1	m=2	m=3
				Armitage-Doll ppm level (Adjusted to Specified hrs/day and days/week)	Armitage-Doll ppm level (Adjusted to Specified hrs/day and days/week)	Armitage-Doll ppm level (Adjusted to Specified hrs/day and days/week)
0	26	6	53	0.00	0.00	0.00
70	26	6	53	3.13	5.47	7.23
220	26	6	53	9.82	17.19	22.71
700	26	6	53	31.25	54.69	72.27
2200	26	6	53	98.21	171.88	227.12
7000	26	6	53	312.50	546.88	722.66

For the Placke et al. (1996) study for male mice, the adjusted doses corresponding to

m=1 or 2 or 3, j=1, T_e=104 weeks, T=104 weeks, a=0, and b=20, 40, or 80 weeks

are shown in Table 12.

Table 12. Adjusted doses for male mice in Placke et al. (1996): m=1 or 2 or 3, j=1, T_e=104 weeks, T=104 weeks, 5 days per week, a=0, and b=variable number of weeks

Isoprene Exposure (ppm)	weeks	hr/day	Observation Time (Necropsy Time) (weeks)	m=1	m=2	m=3
				Armitage-Doll ppm level (Adjusted to Specified hrs/day and days/week)	Armitage-Doll ppm level (Adjusted to Specified hrs/day and days/week)	Armitage-Doll ppm level (Adjusted to Specified hrs/day and days/week)
0	80	8	105	0.00	0.00	0.00
10	80	8	105	1.83	2.25	2.35
70	40	8	105	6.41	10.36	12.78
70	80	8	105	12.82	15.78	16.46
140	40	8	105	12.82	20.71	25.57
280	20	8	105	12.82	23.18	31.54
280	80	8	96	51.28	63.12	65.85
700	80	8	96	128.21	157.79	164.62
2200	20	4	96	50.37	91.05	123.90
2200	80	4	96	201.47	247.96	258.69
2200	40	8	96	201.47	325.44	401.74
2200	80	8	96	402.93	495.91	517.37

For the Placke et al. (1996) study for female mice, the adjusted doses corresponding to

m=1 or 2 or 3, j=1, T_e=104 weeks, T=104 weeks, a=0, and b= 80 weeks

are shown in Table 13.

Table 13. Adjusted doses for female mice in Placke et al. (1996): m=1 or 2 or 3, j=1, T_e=104 weeks, T=104 weeks, 5 days per week, a=0, and b=80 weeks

Isoprene Exposure (ppm)	weeks	hr/day	Observation Time (Necropsy Time) (weeks)	m=1	m=2	m=3
				Armitage-Doll ppm level (Adjusted to Specified hrs/day and days/week)	Armitage-Doll ppm level (Adjusted to Specified hrs/day and days/week)	Armitage-Doll ppm level (Adjusted to Specified hrs/day and days/week)
0	80	8	105	0.00	0.00	0.00
10	80	8	105	1.83	2.25	2.35
70	80	8	105	12.82	15.78	16.46

For the NTP (1999) study for male rats, the adjusted doses corresponding to m=1 or 2 or 3, j=1, T_e=104 weeks, T=104 weeks, a=0, and b=104 weeks are shown in Table 14.

Table 14. Adjusted doses for male rats in NTP (1999): m=1 or 2 or 3, j=1, T_e=104 weeks, T=104 weeks, 5 days per week, a=0, and b=104 weeks

Isoprene Exposure (ppm)	weeks	hr/day	Observation Time (Necropsy Time) (weeks)	m=1	m=2	m=3
				Armitage-Doll ppm level (Adjusted to Specified hrs/day and days/week)	Armitage-Doll ppm level (Adjusted to Specified hrs/day and days/week)	Armitage-Doll ppm level (Adjusted to Specified hrs/day and days/week)
0	105	6	106	0.00	0.00	0.00
220	105	6	106	39.29	39.29	39.29
700	105	6	106	125.00	125.00	125.00
7000	105	6	106	1250.00	1250.00	1250.00

For the NTP (1999) study for female rats, the adjusted doses corresponding to m=1 or 2 or 3, j=1, T_e=104 weeks, T=104 weeks, a=0, and b=104 weeks are shown in Table 15.

Table 15. Adjusted doses for female rats in NTP (1999): m=1 or 2 or 3, j=1, T_e=104 weeks, T=104 weeks, 5 days per week, a=0, and b=104 weeks

Isoprene Exposure (ppm)	weeks	hr/day	Observation Time (Necropsy Time) (weeks)	m=1	m=2	m=3
				Armitage-Doll ppm level (Adjusted to Specified hrs/day and days/week)	Armitage-Doll ppm level (Adjusted to Specified hrs/day and days/week)	Armitage-Doll ppm level (Adjusted to Specified hrs/day and days/week)
0	105	6	106	0.00	0.00	0.00
220	105	6	106	39.29	39.29	39.29
700	105	6	106	125.00	125.00	125.00
7000	105	6	106	1250.00	1250.00	1250.00

5.5 Adjusted Numbers of Subjects at Risk

As described in Section 5.3, if the end of a study (T_{end}) is not equal to the end of a nominal lifetime (T), then the number of subjects at risk of developing the specified risk by the end of a nominal lifetime in the dose-response modeling needs to be adjusted for this inequality. If $T_{end} \leq T$, then the adjusted number of subjects at risk in the i-th dose group in the dose-response modeling is

$$\text{Adjusted } n_{at\ risk}(i) = n_{resp}(i) + [n_{at\ risk}(i) - n_{resp}(i)] \times (T_{end} / T)^m.$$

If $T_{end} > T$, then the adjusted number of subjects at risk in the i-th dose group in the dose-response modeling is

$$\text{Adjusted } n_{at\ risk}(i) = n_{resp}(i) \times (T_{end} / T)^m + [n_{at\ risk}(i) - n_{resp}(i)] .$$

Table 16. Dose- response data from the stop-exposure NTP 1994 study on male F344/N rats and male B6C3F$_1$ mice with adjusted doses and adjusted numbers of animals at risk

Isoprene Exposure	**Experimental ppm**	**0**[1]	**70**[2]	**220**	**700**	**2200**	**7000**
	1 Adjusted dose[3]	**0.00**	**3.13**	**9.82**	**31.25**	**98.21**	**312.50**
	2 Adjusted dose	**0.00**	**5.47**	**17.19**	**54.69**	**171.88**	**546.88**
	3 Adjusted dose	**0.00**	**7.23**	**22.71**	**72.27**	**227.12**	**722.66**
Target Organ	**Response**						
Male F344/N Rats							
Testis	Number at Risk	30	30	30	30	29	30
	1 Adjusted # at Risk[4]	16.8	16.8	17.3	18.7	18.7	19.7
	2 Adjusted # at Risk	10.0	10.0	10.8	13.0	13.5	14.5
	3 Adjusted # at Risk	6.6	6.6	7.4	10.0	10.8	11.8
	Adenoma	3*	3	4	7	8	9
Male B6C3F$_1$ Mice							
Forestomach	Number at Risk	30	30	30	30	30	30
	1 Adjusted # at Risk	15.3	15.3	15.3	15.8	16.3	17.7
	2 Adjusted # at Risk	7.8	7.8	7.8	8.5	9.3	11.5
	3 Adjusted # at Risk	4.0	4.0	4.0	4.8	5.7	8.3
	Papilloma	0**	0	0	1	2	5*
	1 Adjusted # at Risk	15.3	15.3	15.3	15.3	16.3	15.8
	2 Adjusted # at Risk	7.8	7.8	7.8	7.8	9.3	8.5
	3 Adjusted # at Risk	4.0	4.0	4.0	4.0	5.7	4.8
	Carcinoma	0	0	0	0	2	1
	1 Adjusted # at Risk	15.3	15.3	15.3	15.8	17.3	18.2
	2 Adjusted # at Risk	7.8	7.8	7.8	8.5	10.8	12.2
	3 Adjusted # at Risk	4.0	4.0	4.0	4.8	7.4	9.2
	Papilloma/Carcinoma	0**	0	0	1	4	6**
Harderian Gland	Number at Risk	30	30	30	30	30	30
	1 Adjusted # at Risk	16.3	18.2	17.3	22.2	21.7	21.2
	2 Adjusted # at Risk	9.3	12.2	10.8	18.2	17.4	16.7
	3 Adjusted # at Risk	5.7	9.2	7.4	16.1	15.2	14.4
	Adenoma	2**	6	4	14**	13**	12**
	1 Adjusted # at Risk	15.3	15.3	15.3	15.3	15.8	15.3
	2 Adjusted # at Risk	7.8	7.8	7.8	7.8	8.5	7.8
	3 Adjusted # at Risk	4.0	4.0	4.0	4.0	4.8	4.0
	Carcinoma	0	0	0	0	1	0
	1 Adjusted # at Risk	16.3	18.2	17.3	22.2	21.7	21.2
	2 Adjusted # at Risk	9.3	12.2	10.8	18.2	17.4	16.7
	3 Adjusted # at Risk	5.7	9.2	7.4	16.1	15.2	14.4
	Adenoma/Carcinoma	2**	6	4	14**	13**	12**

Isoprene Exposure	**Experimental ppm**	**0[1]**	**70[2]**	**220**	**700**	**2200**	**7000**
	1 Adjusted dose[3]	**0.00**	**3.13**	**9.82**	**31.25**	**98.21**	**312.50**
	2 Adjusted dose	**0.00**	**5.47**	**17.19**	**54.69**	**171.88**	**546.88**
	3 Adjusted dose	**0.00**	**7.23**	**22.71**	**72.27**	**227.12**	**722.66**
Target Organ	**Response**						
Liver	Number at Risk	30	30	29	30	30	28
	1 Adjusted # at Risk	17.3	16.3	17.7	22.6	24.1	22.1
	2 Adjusted # at Risk	10.8	9.3	12.0	18.9	21.1	19.1
	3 Adjusted # at Risk	7.4	5.7	9.0	17.0	19.6	17.6
	Adenoma	4**	2	6	15*	18*	16*
	1 Adjusted # at Risk	17.3	15.8	16.3	17.7	17.3	18.7
	2 Adjusted # at Risk	10.8	8.5	9.8	11.5	10.8	13.9
	3 Adjusted # at Risk	7.4	4.8	6.4	8.3	7.4	11.5
	Carcinoma	4**	1	3	5	4	9
	1 Adjusted # at Risk	18.7	16.8	18.2	22.6	24.1	22.6
	2 Adjusted # at Risk	13.0	10.0	12.7	18.9	21.1	19.9
	3 Adjusted # at Risk	10.0	6.6	9.9	17.0	19.6	18.5
	Adenoma/Carcinoma	7**	3	7	15**	18**	17**
Lung	Number at Risk	30	30	29	30	30	28
	1 Adjusted # at Risk	16.3	16.3	15.3	17.3	20.2	18.2
	2 Adjusted # at Risk	9.3	9.3	8.3	10.8	15.2	13.2
	3 Adjusted # at Risk	5.7	5.7	4.7	7.4	12.6	10.6
	Adenoma	2**	2	1	4	10*	8*
	1 Adjusted # at Risk	15.3	15.3	14.8	15.8	15.8	15.7
	2 Adjusted # at Risk	7.8	7.8	7.5	8.5	8.5	9.5
	3 Adjusted # at Risk	4.0	4.0	3.8	4.8	4.8	6.3
	Carcinoma	0**	0	0	1	1	3
	1 Adjusted # at Risk	16.3	16.3	15.3	17.7	20.2	18.7
	2 Adjusted # at Risk	9.3	9.3	8.3	11.5	15.2	13.9
	3 Adjusted # at Risk	5.7	5.7	4.7	8.3	12.6	11.5
	Adenoma/Carcinoma	2**	2	1	5	10*	9*
Any Lymphoma	Number at Risk	30	30	30	30	30	30
	1 Adjusted # at Risk	15.8	15.3	15.3	16.3	15.8	16.3
	2 Adjusted # at Risk	8.5	7.8	7.8	9.3	8.5	9.3
	3 Adjusted # at Risk	4.8	4.0	4.0	5.7	4.8	5.7
	Malignant	1	0	0	2	1	2

*statistically significant at the 5% significance level

**statistically significant at the 1% significance level

[1]statistically significant results in the control group are for the Cochran-Armitage trend test for an increasing trend using the unadjusted experimental concentration (ppm) and the unadjusted number of animals at risk

[2]statistically significant results in the exposed groups are for the Fisher exact test for an increase in the incidence versus the incidence in the control group using the unadjusted number of animals at risk

[3]Adjusted ppm for the duration of the experimental exposure (26 weeks), the days per week (5) and hours per day (6) to calculate an equivalent continuous exposure for an entire lifetime (104 weeks), 7 days a

week and 24 hours a day using the Armitage-Doll adjustment for a 1-, 2- and 3-stage multistage carcinogen affecting the first stage

[4]Adjusted number of animals at risk for the observation/necropsy time (53 weeks) to calculate the number at risk corresponding to a lifetime observation period (104 weeks) using the Armitage-Doll adjustment for a 1-, 2- and 3-stage multistage carcinogen affecting the first stage

Table 17. Dose response data from the Placke et al. 1996 study on male and female B6C3F$_1$ mice with adjusted exposure concentrations and adjusted numbers of animals at risk

Isoprene Exposure (ppm)		**0**	**10**	**70**	**70**	**140**	**280**	**280**	**700**	**2,200**	**2,200**	**2,200**	**2,200**
Duration of Exposure (weeks)		**80**	**80**	**40**	**80**	**40**	**20**	**80**	**80**	**20**	**80**	**40**	**80**
Period of Exposure (hrs/day)		**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**4**	**4**	**8**	**8**
Cumulative Exposure (ppm-weeks)		**0**	**800**	**2,800**	**5,600**	**5,600**	**5,600**	**22,400**	**56,000**	**22,000**	**88,000**	**88,000**	**176,000**
1 Adjusted ppm[3]		**0.00**	**1.83**	**6.41**	**12.82**	**12.82**	**12.82**	**51.28**	**128.21**	**50.37**	**201.47**	**201.47**	**402.93**
2 Adjusted ppm		**0.00**	**2.25**	**10.36**	**15.78**	**20.71**	**23.18**	**63.12**	**157.79**	**91.05**	**247.96**	**325.44**	**495.91**
3 Adjusted ppm		**0.00**	**2.35**	**12.78**	**16.46**	**25.57**	**31.54**	**65.85**	**164.62**	**123.90**	**258.69**	**401.74**	**517.37**
Target Organ	**Response**												
Male B6C3F$_1$ Mice													
Observation Time (weeks)		**105**	**105**	**105**	**105**	**105**	**105**	**96**	**96**	**96**	**96**	**96**	**96**
Circulatory System: Heart	Number at Risk	49	50	49	50	50	50	50	50	50	50	49	50
	1 Adjusted # at Risk[4]	49.0	50.0	49.0	50.0	50.0	50.0	46.3	46.2	46.5	46.2	45.3	46.2
	2 Adjusted # at Risk	49.0	50.0	49.0	50.0	50.0	50.0	42.9	42.8	43.2	42.8	41.9	42.8
	3 Adjusted # at Risk	49.0	50.0	49.0	50.0	50.0	50.0	39.8	39.5	40.2	39.5	38.8	39.5
	Hemangiosarcoma	0**	0	0	0	0	0	2	1	4	1	1	1
Circulatory System: Spleen	Number at Risk	49	48	47	50	50	47	50	48	48	50	47	49
	1 Adjusted # at Risk	49.0	48.0	47.0	50.0	50.0	47.0	46.2	44.5	44.5	46.3	43.4	45.3
	2 Adjusted # at Risk	49.0	48.1	47.0	50.0	50.1	47.0	42.8	41.2	41.2	42.9	40.0	41.9
	3 Adjusted # at Risk	49.0	48.1	47.0	50.1	50.1	47.1	39.5	38.2	38.2	39.8	37.0	38.8
	Hemangiosarcoma	1	3	1	2	3	2	1	2	2	2	0	1
Forestomach[5]	Number at Risk	50	48	47	50	49	46	50	47	48	50	47	50
	1 Adjusted # at Risk	50.0	48.0	47.0	50.0	49.0	46.0	46.2	43.5	44.3	46.2	43.5	46.2
	2 Adjusted # at Risk	50.0	48.0	47.0	50.0	49.0	46.0	42.6	40.2	40.9	42.8	40.3	42.8
	3 Adjusted # at Risk	50.0	48.0	47.0	50.0	49.0	46.0	39.3	37.2	37.8	39.5	37.4	39.5
	Papilloma	0**	0	0	0	0	0	0	1	0	1	2	1
	1 Adjusted # at Risk	50.0	48.0	47.0	50.0	49.0	46.0	46.2	43.4	44.4	46.2	43.4	46.4
	2 Adjusted # at Risk	50.0	48.0	47.0	50.0	49.0	46.0	42.8	40.0	41.0	42.8	40.0	43.0
	3 Adjusted # at Risk	50.0	48.0	47.0	50.0	49.0	46.0	39.5	37.0	38.0	39.5	37.0	40.0
	Carcinoma	0**	0	0	0	0	0	1	0	1	1	0	3

Isoprene Exposure (ppm)		**0**	**10**	**70**	**70**	**140**	**280**	**280**	**700**	**2,200**	**2,200**	**2,200**	**2,200**
Duration of Exposure (weeks)		**80**	**80**	**40**	**80**	**40**	**20**	**80**	**80**	**20**	**80**	**40**	**80**
Period of Exposure (hrs/day)		**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**4**	**4**	**8**	**8**
Cumulative Exposure (ppm-weeks)		**0**	**800**	**2,800**	**5,600**	**5,600**	**5,600**	**22,400**	**56,000**	**22,000**	**88,000**	**88,000**	**176,000**
1 Adjusted ppm[3]		**0.00**	**1.83**	**6.41**	**12.82**	**12.82**	**12.82**	**51.28**	**128.21**	**50.37**	**201.47**	**201.47**	**402.93**
2 Adjusted ppm		**0.00**	**2.25**	**10.36**	**15.78**	**20.71**	**23.18**	**63.12**	**157.79**	**91.05**	**247.96**	**325.44**	**495.91**
3 Adjusted ppm		**0.00**	**2.35**	**12.78**	**16.46**	**25.57**	**31.54**	**65.85**	**164.62**	**123.90**	**258.69**	**401.74**	**517.37**
Target Organ	**Response**												
	1 Adjusted # at Risk	50.0	48.0	47.0	50.0	49.0	46.0	46.2	43.5	44.4	46.3	43.5	46.5
	2 Adjusted # at Risk	50.0	48.0	47.0	50.0	49.0	46.0	42.8	40.2	41.0	42.9	40.3	43.2
	3 Adjusted # at Risk	50.0	48.0	47.0	50.0	49.0	46.0	39.5	37.2	38.0	39.8	37.4	40.2
	Papilloma/Carcinoma	0**	0	0	0	0	0	1	1	1	2	2	4
Harderian Gland[5]	Number at Risk	47	49	48	50	50	49	50	49	49	50	49	50
	1 Adjusted # at Risk	47.0	49.0	48.1	50.1	50.1	49.2	47.5	47.2	46.7	48.3	47.6	48.8
	2 Adjusted # at Risk	47.1	49.1	48.3	50.2	50.2	49.3	45.1	45.6	44.6	46.7	46.3	47.8
	3 Adjusted # at Risk	47.1	49.1	48.4	50.3	50.3	49.5	43.0	44.1	42.6	45.3	45.2	46.8
	Adenoma	4**	4	13*	9	12*	16**	17**	26**	19**	28**	31**	35**
	1 Adjusted # at Risk	47.0	49.0	48.0	50.0	50.0	49.0	46.2	45.5	45.3	46.3	45.2	46.3
	2 Adjusted # at Risk	47.0	49.0	48.0	50.0	50.0	49.1	42.8	42.2	41.9	42.9	41.8	42.9
	3 Adjusted # at Risk	47.0	49.0	48.0	50.0	50.1	49.1	39.5	39.2	38.8	39.8	38.5	39.8
	Carcinoma	0	0	0	0	2	3	1	3	1	2	0	2
	1 Adjusted # at Risk	47.0	49.0	48.1	50.1	50.1	49.2	47.5	47.5	46.8	48.5	47.6	49.0
	2 Adjusted # at Risk	47.1	49.1	48.3	50.2	50.3	49.4	45.3	46.0	44.7	47.0	46.3	48.1
	3 Adjusted # at Risk	47.1	49.1	48.4	50.3	50.4	49.6	43.2	44.7	42.8	45.7	45.2	47.2
	Adenoma/Carcinoma	4**	4	13*	9	14*	19**	18**	29**	20**	30**	31**	37**
Hematopoietic System:	Number at Risk	50	50	50	50	50	50	50	50	50	50	50	50
	1 Adjusted # at Risk	50.0	50.0	50.0	50.0	50.0	50.1	46.5	46.5	46.5	46.5	46.5	46.6
	2 Adjusted # at Risk	50.0	50.0	50.0	50.1	50.0	50.1	43.3	43.2	43.2	43.2	43.3	43.5
	3 Adjusted # at Risk	50.1	50.0	50.1	50.1	50.0	50.2	40.4	40.2	40.2	40.2	40.4	40.6
	Any Lymphoma	2**	1	2	4	1	7	5	4	4	4	5	6
Hematopoietic System	Number at Risk	50	50	50	50	50	50	50	50	50	50	50	50
	1 Adjusted # at Risk	50.0	50.0	50.0	50.0	50.0	50.1	46.5	46.3	46.5	46.7	46.7	46.3
	2 Adjusted # at Risk	50.0	50.0	50.0	50.0	50.0	50.2	43.2	42.9	43.3	43.6	43.6	42.9
	3 Adjusted # at Risk	50.0	50.1	50.1	50.1	50.0	50.2	40.2	39.8	40.4	40.8	40.8	39.8

Isoprene Exposure (ppm)		**0**	**10**	**70**	**70**	**140**	**280**	**280**	**700**	**2,200**	**2,200**	**2,200**	**2,200**
Duration of Exposure (weeks)		**80**	**80**	**40**	**80**	**40**	**20**	**80**	**80**	**20**	**80**	**40**	**80**
Period of Exposure (hrs/day)		**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**4**	**4**	**8**	**8**
Cumulative Exposure (ppm-weeks)		**0**	**800**	**2,800**	**5,600**	**5,600**	**5,600**	**22,400**	**56,000**	**22,000**	**88,000**	**88,000**	**176,000**
1 Adjusted ppm[3]		**0.00**	**1.83**	**6.41**	**12.82**	**12.82**	**12.82**	**51.28**	**128.21**	**50.37**	**201.47**	**201.47**	**402.93**
2 Adjusted ppm		**0.00**	**2.25**	**10.36**	**15.78**	**20.71**	**23.18**	**63.12**	**157.79**	**91.05**	**247.96**	**325.44**	**495.91**
3 Adjusted ppm		**0.00**	**2.35**	**12.78**	**16.46**	**25.57**	**31.54**	**65.85**	**164.62**	**123.90**	**258.69**	**401.74**	**517.37**
Target Organ	**Response**												
	Histiocytic Sarcoma	0**	2	2	2	1	8**	4	2	5**	7**	7**	2
Liver[5]	Number at Risk	50	50	49	50	50	49	50	48	50	50	47	50
	1 Adjusted # at Risk	50.1	50.1	49.1	50.1	50.2	49.2	48.0	46.4	47.8	47.8	45.5	48.5
	2 Adjusted # at Risk	50.2	50.2	49.3	50.3	50.4	49.3	46.2	44.9	45.9	45.7	44.2	47.0
	3 Adjusted # at Risk	50.3	50.3	49.4	50.4	50.6	49.5	44.4	43.5	44.0	43.8	42.9	45.7
	Adenoma	11**	12	14	15	22*	18	24**	27**	22*	21*	28**	30**
	1 Adjusted # at Risk	50.1	50.1	49.1	50.1	50.1	49.1	47.4	45.6	47.1	47.3	44.8	47.4
	2 Adjusted # at Risk	50.2	50.1	49.2	50.2	50.2	49.2	45.0	43.4	44.4	44.8	42.7	45.0
	3 Adjusted # at Risk	50.3	50.2	49.3	50.3	50.3	49.3	42.7	41.4	41.9	42.5	40.8	42.7
	Carcinoma	9**	6	11	9	10	12	16	17*	12	15	18*	16
	1 Adjusted # at Risk	50.2	50.2	49.2	50.2	50.3	49.3	49.2	47.7	48.8	48.9	46.9	49.7
	2 Adjusted # at Risk	50.4	50.3	49.5	50.5	50.6	49.6	48.5	47.4	47.6	47.9	46.9	49.4
	3 Adjusted # at Risk	50.6	50.5	49.7	50.7	50.9	49.9	47.9	47.1	46.6	47.0	46.8	49.1
	Adenoma/Carcinoma	20**	18	25	24	32*	30*	40**	44**	34**	36**	46**	46**
Lung[5]	Number at Risk	50	50	50	50	50	50	50	50	50	50	49	50
	1 Adjusted # at Risk	50.1	50.2	50.1	50.0	50.1	50.2	47.2	47.9	47.2	47.3	47.5	48.5
	2 Adjusted # at Risk	50.2	50.3	50.2	50.1	50.2	50.3	44.5	46.0	44.7	44.8	46.0	47.0
	3 Adjusted # at Risk	50.3	50.5	50.2	50.1	50.3	50.5	42.1	44.2	42.3	42.5	44.7	45.7
	Adenoma	11**	16	8	4	10	16	13	23**	14	15	29**	30**
	1 Adjusted # at Risk	50.0	50.0	50.0	50.0	50.0	50.0	46.2	46.7	46.3	46.4	45.5	46.7
	2 Adjusted # at Risk	50.0	50.0	50.0	50.0	50.0	50.1	42.8	43.6	42.9	43.0	42.2	43.6
	3 Adjusted # at Risk	50.0	50.0	50.0	50.1	50.0	50.1	39.5	40.8	39.8	40.0	39.2	40.8
	Carcinoma	0**	1	0	2	1	3	1	7**	2	3	3	7**
	1 Adjusted # at Risk	50.1	50.2	50.1	50.1	50.1	50.2	47.2	48.5	47.4	47.5	47.7	49.0
	2 Adjusted # at Risk	50.2	50.3	50.2	50.1	50.2	50.4	44.7	47.0	45.0	45.3	46.5	48.1
	3 Adjusted # at Risk	50.3	50.5	50.2	50.2	50.3	50.6	42.3	45.7	42.7	43.2	45.4	47.2

Isoprene Exposure (ppm)		**0**	**10**	**70**	**70**	**140**	**280**	**280**	**700**	**2,200**	**2,200**	**2,200**	**2,200**
Duration of Exposure (weeks)		**80**	**80**	**40**	**80**	**40**	**20**	**80**	**80**	**20**	**80**	**40**	**80**
Period of Exposure (hrs/day)		**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**4**	**4**	**8**	**8**
Cumulative Exposure (ppm-weeks)		**0**	**800**	**2,800**	**5,600**	**5,600**	**5,600**	**22,400**	**56,000**	**22,000**	**88,000**	**88,000**	**176,000**
1 Adjusted ppm[3]		**0.00**	**1.83**	**6.41**	**12.82**	**12.82**	**12.82**	**51.28**	**128.21**	**50.37**	**201.47**	**201.47**	**402.93**
2 Adjusted ppm		**0.00**	**2.25**	**10.36**	**15.78**	**20.71**	**23.18**	**63.12**	**157.79**	**91.05**	**247.96**	**325.44**	**495.91**
3 Adjusted ppm		**0.00**	**2.35**	**12.78**	**16.46**	**25.57**	**31.54**	**65.85**	**164.62**	**123.90**	**258.69**	**401.74**	**517.37**
Target Organ	**Response**												
	Adenoma/Carcinoma	11**	17	8	6	11	19	14	30**	16	18	32**	37**
Female B6C3F$_1$ Mice													
Observation Time (weeks)		**105**	**105**		**105**								
Circulatory System: Spleen	Number at Risk	50	49		50								
	1 Adjusted # at Risk	50.0	49.0		50.0								
	2 Adjusted # at Risk	50.0	49.0		50.1								
	3 Adjusted # at Risk	50.0	49.0		50.1								
	Hemangiosarcoma	1*	1		4								
Harderian Gland	Number at Risk	49	49		49								
	1 Adjusted # at Risk	49.0	49.0		49.1								
	2 Adjusted # at Risk	49.0	49.1		49.2								
	3 Adjusted # at Risk	49.1	49.1		49.2								
	Adenoma	2*	3		8*								
Pituitary Gland	Number at Risk	49	46		49								
	1 Adjusted # at Risk	49.0	46.1		49.1								
	2 Adjusted # at Risk	49.0	46.1		49.2								
	3 Adjusted # at Risk	49.0	46.2		49.3								
	Adenoma	1*	6*		9**								

*statistically significant at the 5% significance level

**statistically significant at the 1% significance level

[1]statistically significant results in the control group are for the Cochran-Armitage trend test for an increasing trend

[2]statistically significant results in the exposed groups are for the Fisher exact test for an increase in the incidence versus the incidence in the control group

[3]Adjusted ppm for the duration of the experimental exposure (20, 40 or 80 weeks), the days per week (5) and hours per day (4 or 8) to calculate an equivalent continuous exposure for an entire lifetime (104 weeks), 7 days a week and 24 hours a day using the Armitage-Doll adjustment for a 1-, 2- and 3-stage multistage carcinogen affecting the first stage

[4]Adjusted number of animals at risk for the observation/necropsy time (105 or 96 weeks) to calculate the number at risk corresponding to a lifetime observation period (104 weeks) using the Armitage-Doll adjustment for a 1-, 2- and 3-stage multistage carcinogen affecting the first stage
[5]the combined responses papilloma/carcinoma and adenoma/carcinoma is the sum of the two individual responses as Placke et al. did not report these responses combined

Table 18. Dose response data from the two-year NTP 1999 study on male and female F344/N rats with adjusted exposure concentrations and adjusted numbers of animals at risk

Isoprene Exposure	**Experimental ppm**	**0[1]**	**220[2]**	**700**	**7000**
	Adjusted ppm[3]	**0.00**	**39.29**	**125.00**	**1250.00**
Number of Animals at Risk		**50**	**50**	**50**	**50**
Target Organ	**Response**				
Male F344/N Rats					
Kidney	1 Adjusted # at Risk[4]	50.0	50.1	50.2	50.3
	2 Adjusted # at Risk	50.1	50.2	50.3	50.6
	3 Adjusted # at Risk	50.1	50.2	50.5	50.9
	Adenoma	2**	4	8*	15**
	1 Adjusted # at Risk	50.0	50.0	50.0	50.0
	2 Adjusted # at Risk	50.0	50.0	50.0	50.0
	3 Adjusted # at Risk	50.0	50.0	50.1	50.0
	Carcinoma	0	0	1	0
	1 Adjusted # at Risk	50.0	50.1	50.2	50.3
	2 Adjusted # at Risk	50.1	50.2	50.3	50.6
	3 Adjusted # at Risk	50.1	50.2	50.5	50.9
	Adenoma/Carcinoma	2**	4	8*	15**
Mammary Gland	1 Adjusted # at Risk	50.0	50.1	50.1	50.4
	2 Adjusted # at Risk	50.1	50.2	50.2	50.8
	3 Adjusted # at Risk	50.1	50.2	50.4	51.2
	Fibroadenoma	2**	4	6	21**
	1 Adjusted # at Risk	50.0	50.0	50.0	50.0
	2 Adjusted # at Risk	50.0	50.0	50.0	50.1
	3 Adjusted # at Risk	50.0	50.1	50.1	50.1
	Carcinoma	0	1	1	2
	1 Adjusted # at Risk	50.0	50.1	50.1	50.4
	2 Adjusted # at Risk	50.1	50.2	50.3	50.8
	3 Adjusted # at Risk	50.1	50.3	50.4	51.2
	Fibroadenoma/Carcinoma	2**	5	7	21**
Testis	1 Adjusted # at Risk	50.6	50.7	50.8	50.9
	2 Adjusted # at Risk	51.3	51.4	51.7	51.9
	3 Adjusted # at Risk	51.9	52.2	52.6	52.8
	Adenoma	33**	37	44**	48**
Female F344/N Rats					
Mammary Gland	1 Adjusted # at Risk	50.4	50.7	50.6	50.6
	2 Adjusted # at Risk	50.7	51.4	51.2	51.2
	3 Adjusted # at Risk	51.1	52.1	51.9	51.9
	Fibroadenoma	19	35**	32**	32**

Isoprene Exposure	**Experimental ppm**	**0[1]**	**220[2]**	**700**	**7000**
	Adjusted ppm[3]	**0.00**	**39.29**	**125.00**	**1250.00**
Number of Animals at Risk		**50**	**50**	**50**	**50**
Target Organ	**Response**				
	1 Adjusted # at Risk	50.1	50.0	50.0	50.1
	2 Adjusted # at Risk	50.2	50.1	50.0	50.1
	3 Adjusted # at Risk	50.2	50.1	50.1	50.2
	Carcinoma	4	2	1	3
	1 Adjusted # at Risk	50.4	50.7	50.6	50.6
	2 Adjusted # at Risk	50.8	51.4	51.2	51.2
	3 Adjusted # at Risk	51.2	52.1	51.9	51.9
	Fibroadenoma/Carcinoma	20	35**	32*	32*

*statistically significant at the 5% significance level
**statistically significant at the 1% significance level
[1]statistically significant results in the control group are for the Cochran-Armitage trend test for an increasing trend using the unadjusted experimental concentration (ppm)
[2]statistically significant results in the exposed groups are for the Fisher exact test for an increase in the incidence versus the incidence in the control group
[3]Adjusted ppm for the days per week (5) and hours per day (6) to calculate an equivalent continuous exposure 24 hours a day, 7 days a week for an entire lifetime
[4]Adjusted number of animals at risk for the observation/necropsy time (106 weeks) to calculate the number at risk corresponding to a lifetime observation period (104 weeks) using the Armitage-Doll adjustment for a 1-, 2- and 3-stage multistage carcinogen affecting the first stage

6. Dose-Response Modeling Results

A total of 171 dose-response model fits were carried out. There were 57 endpoints (57 combinations of study, species, gender, organ, and severity). For each of the endpoints analyzed, three forms of the response data were fit by the multistage model. The three forms of the response data correspond to the three forms of the adjusted doses and the three forms of the adjusted numbers of animals at risk. The three forms correspond to (1) m=1 with one transition rate from a normal cell to a first-stage (tumor) cell, (2) m=2 with one transition rate from a normal cell to a first stage cell and a second transition rate from a first-stage cell to a second-stage (tumor) cell, and (3) m=3 with one transition rate from a normal cell to a first stage cell, a second transition rate from a first-stage cell to a second-stage cell, and a third transition rate from a second-stage cell to a third-stage (tumor) cell.

The dose-response modeling is done with the experimental doses adjusted to the constant lifetime environmental dose D in ppm that is equivalent to the time-dependent dose d(t) as described in Section 5.2. The numerical values of the adjusted doses are given In Section 5.4. The dose-response modeling is also done with the experimental numbers of animals at risk adjusted to the equivalent number of animals at risk if the time to necropsy (T_e) were equal to the nominal animal lifetime (T) as described in Section 5.3. The numerical values of the adjusted numbers of animals at risk are given in Section 5.5.

The figures from BMDS showing the fits of the multistage models are reproduced in Appendix C. These figures show that the fitted multistage models are nearly all linear models. (That is, the estimated coefficients for the higher order terms in the fitted multistage models are generally all zero.)

The estimated (fitted) multistage models are used to identify the EC_{10}. This EC_{10} is in units of a constant environmental ppm. That is, a constant ppm level 24 hours per day and 7 days per week for a lifetime. The best estimates of these EC_{10}s are shown in Table 19. At the same time that the EC_{10} is calculated, a lower bound (a so-called 95% lower confidence limit) denoted by LEC_{10} on the EC_{10} is calculated. The LEC_{10} is calculated in BMDS using the “standard default” procedure that determines the fit of the multistage model with the largest slope that is not statistically detectable as a bad fit. Then, this largest slope is used to calculate the LEC_{10}. Table 19 includes both the LEC_{10} and the EC_{10} values.

Table 20 is the same as Table 19 except that the results have been first grouped by species and gender and then the results for the same organ and severity are grouped.

Table 21 contains the unit risk factor (URF) corresponding to the EC_{10} and the upper bound (URF_UB, upper 95% confidence limit) on the URF corresponding to the LEC_{10}.

Table 19. Estimated EC_{10} and LEC_{10} for the endpoints analyzed for three alternative adjustments to the doses and numbers of animals at risk (i.e., m=1, 2, or 3): ppm is environmental ppm, that is 24 hours per day, 7 days per week for a lifetime

Organ	Severity	EC_{10} (ppm) adjusting for 1, 2, or 3 number of stages			LEC_{10} (ppm) adjusting for 1, 2, or 3 number of stages		
		m=1	m=2	m=3	m=1	m=2	m=3
NTP 1994: Male F344/N Rats							
Testis	Adenoma	69.33	82.82	69.78	34.06	37.63	28.73
NTP 1999: Male F344/N Rats							
Kidney	Adenoma	432.94	432.94	432.94	262.64	262.64	262.64
	Carcinoma	>1250[1]	>1250	>1250	>1250	>1250	>1250
	Adenoma/Carcinoma	432.94	432.94	432.94	262.64	262.64	262.64
Mammary Gland	Fibroadenoma	261.68	261.68	269.65	178.70	178.70	183.80
	Carcinoma	>1250	>1250	>1250	>1250	>1250	>1250
	Fibroadenoma/Carcinoma	265.14	265.14	273.63	178.96	178.96	184.27
Testis	Adenoma	58.77	73.92	89.70	32.98	42.69	51.97
NTP 1999: Female F344/N Rats							
Mammary Gland	Fibroadenoma	588.76	653.03	593.24	209.90	223.04	215.79
	Carcinoma	>1250	>1250	>1250	>1250	>1250	>1250
	Fibroadenoma/Carcinoma	648.87	724.86	650.52	217.26	231.04	223.12
NTP 1994 and NTP 1999 Combined: Male F344/N Rats							
Testis	Adenoma	52.55	95.63	86.99	34.87	48.29	53.30
NTP 1994: Male $B6C3F_1$ Mice							
Forestomach	Papilloma	86.93	84.71	64.02	51.02	49.41	36.69
	Carcinoma	234.01	210.83	130.03	102.99	92.68	56.84
	Papilloma/Carcinoma	64.40	63.25	50.29	40.58	39.54	30.82
Harderian Gland	Adenoma	47.34	55.79	65.94	24.73	26.87	26.21
	Carcinoma	713.76	596.15	344.34	195.73	163.47	94.38
	Adenoma/Carcinoma	47.34	55.79	65.94	24.73	26.87	26.21
Liver	Adenoma	21.91	32.18	25.49	13.65	17.62	12.08
	Carcinoma	98.24	95.41	99.68	36.48	36.68	33.12
	Adenoma/Carcinoma	22.67	28.34	32.41	13.74	15.37	14.21
Lung	Adenoma	47.07	54.88	47.32	27.61	30.21	23.30
	Carcinoma	131.92	124.20	89.17	68.39	64.15	45.50
	Adenoma/Carcinoma	40.45	43.80	46.02	24.42	25.22	22.86
Hematopoietic System	Any Lymphoma	314.61	307.85	211.41	100.34	94.02	60.74
Placke et al. 1996: Male $B6C3F_1$ Mice							
Heart	Hemangiosarcoma	976.69	664.91	662.78	349.80	395.19	410.64
Spleen	Hemangiosarcoma	>403	>496	>517	>403	>496	>517
Forestomach	Papilloma	1016.32	1174.77	843.00	400.19	466.42	483.16
	Carcinoma	476.25	586.66	625.44	365.59	453.23	475.05
	Papilloma/Carcinoma	454.60	530.69	535.69	283.38	349.99	363.93

Organ	Severity	EC_{10} (ppm) adjusting for 1, 2, or 3 number of stages			LEC_{10} (ppm) adjusting for 1, 2, or 3 number of stages		
		m=1	m=2	m=3	m=1	m=2	m=3
Harderian Gland	Adenoma	28.27	33.90	34.94	23.06	27.86	28.82
	Carcinoma	>403	1412.21	1342.28	>403	494.24	504.45
	Adenoma/Carcinoma	25.88	31.49	31.82	21.18	25.92	26.32
Hematopoietic System	Any Lymphoma	472.10	510.34	470.01	229.51	263.04	253.65
Hematopoietic System	Histiocytic Sarcoma	600.67	525.59	446.69	252.67	262.44	242.60
Liver	Adenoma	52.84	60.80	57.60	38.43	45.12	43.56
	Carcinoma	122.96	131.36	125.58	78.50	87.76	86.07
	Adenoma/Carcinoma	12.53	15.29	16.39	9.95	12.20	13.11
Lung	Adenoma	51.75	61.43	61.28	39.70	47.73	47.42
	Carcinoma	263.11	313.39	310.26	168.80	203.23	203.54
	Adenoma/Carcinoma	35.70	43.56	45.75	28.45	34.66	35.59
NTP 1994 and Placke et al. 1996 Combined: Male $B6C3F_1$ Mice							
Forestomach	Papilloma	444.70	460.52	485.55	290.78	358.37	406.30
	Carcinoma	608.62	594.01	585.37	355.17	437.44	443.29
	Papilloma/Carcinoma	259.74	347.53	379.96	186.45	246.35	282.44
Harderian Gland	Adenoma	30.01	34.77	34.84	24.61	28.70	28.88
	Carcinoma	>403	1351.33	1185.85	>403	514.93	480.56
	Adenoma/Carcinoma	27.83	32.68	32.16	22.88	27.02	26.70
Liver	Adenoma	45.57	53.10	49.67	34.60	40.88	38.44
	Carcinoma	109.85	118.53	129.86	74.68	83.15	83.17
	Adenoma/Carcinoma	14.24	16.73	17.10	11.52	13.50	13.78
Lung	Adenoma	51.22	60.14	57.00	40.13	47.53	45.54
	Carcinoma	235.00	278.11	299.60	158.90	186.74	188.98
	Adenoma/Carcinoma	36.47	43.09	42.97	29.53	35.07	35.11
Hematopoietic System	Any Lymphoma	440.84	470.84	448.07	229.87	257.36	243.43
Placke et al. 1996: Female $B6C3F_1$ Mice							
Spleen	Hemangiosarcoma	16.51	20.32	21.19	9.03	11.11	11.59
Harderian Gland	Adenoma	9.90	12.19	12.72	5.05	6.22	6.48
Pituitary Gland	Adenoma	7.68	9.46	9.87	3.98	4.90	5.11

[1]>#, where # is the highest dose, implies that the EC_{10} or LEC_{10} is at least three times higher than the highest dose

Table 20. Estimated EC_{10} and LEC_{10} for the endpoints analyzed for three alternative adjustments to the doses and numbers of animals at risk (i.e., m=1, 2, or 3): ppm is environmental ppm, that is 24 hours per day, 7 days per week for a lifetime: Grouped by species and gender and then the results for the same organ and severity are grouped together

Organ	**Severity / Study**	**EC_{10} (ppm) adjusting for 1, 2, or 3 number of stages**			**LEC_{10} (ppm) adjusting for 1, 2, or 3 number of stages**		
		m=1	**m=2**	**m=3**	**m=1**	**m=2**	**m=3**
Male F344/N Rats							
Testis	Adenoma						
	NTP1994	69.33	82.82	69.78	34.06	37.63	28.73
	NTP1999	58.77	73.92	89.70	32.98	42.69	51.97
	NTP1994 & NTP1999	52.55	95.63	86.99	34.87	48.29	53.30
NTP 1999: Male F344/N Rats							
Kidney	Adenoma	432.94	432.94	432.94	262.64	262.64	262.64
	Carcinoma	>1250[1]	>1250	>1250	>1250	>1250	>1250
	Adenoma/Carcinoma	432.94	432.94	432.94	262.64	262.64	262.64
Mammary Gland	Fibroadenoma	261.68	261.68	269.65	178.70	178.70	183.80
	Carcinoma	>1250	>1250	>1250	>1250	>1250	>1250
	Fibroadenoma/Carcinoma	265.14	265.14	273.63	178.96	178.96	184.27
NTP 1999: Female F344/N Rats							
Mammary Gland	Fibroadenoma	588.76	653.03	593.24	209.90	223.04	215.79
	Carcinoma	>1250	>1250	>1250	>1250	>1250	>1250
	Fibroadenoma/Carcinoma	648.87	724.86	650.52	217.26	231.04	223.12
Male $B6C3F_1$ Mice							
Forestomach	Papilloma						
	NTP1994	86.93	84.71	64.02	51.02	49.41	36.69
	Placke1996	1016.32	1174.77	843.00	400.19	466.42	483.16
	NTP1994 & Placke1996	444.70	460.52	485.55	290.78	358.37	406.30
	Carcinoma						
	NTP1994	234.01	210.83	130.03	102.99	92.68	56.84
	Placke1996	476.25	586.66	625.44	365.59	453.23	475.05
	NTP1994 & Placke1996	608.62	594.01	585.37	355.17	437.44	443.29
	Papilloma/Carcinoma						
	NTP1994	64.40	63.25	50.29	40.58	39.54	30.82
	Placke1996	454.60	530.69	535.69	283.38	349.99	363.93
	NTP1994 & Placke1996	259.74	347.53	379.96	186.45	246.35	282.44
Harderian Gland	Adenoma	47.34	55.79	65.94	24.73	26.87	26.21
	NTP1994	28.27	33.90	34.94	23.06	27.86	28.82
	Placke1996	30.01	34.77	34.84	24.61	28.70	28.88
	NTP1994 & Placke1996						

Organ	Severity / Study	EC_{10} (ppm) adjusting for 1, 2, or 3 number of stages			LEC_{10} (ppm) adjusting for 1, 2, or 3 number of stages		
		m=1	m=2	m=3	m=1	m=2	m=3
Harderian Gland	Carcinoma						
	NTP1994	713.76	596.15	344.34	195.73	163.47	94.38
	Placke1996	>403	1412.21	1342.28	>403	494.24	504.45
	NTP1994 & Placke1996	>403	1351.33	1185.85	>403	514.93	480.56
	Adenoma/Carcinoma						
	NTP1994	47.34	55.79	65.94	24.73	26.87	26.21
	Placke1996	25.88	31.49	31.82	21.18	25.92	26.32
	NTP1994 & Placke1996	27.83	32.68	32.16	22.88	27.02	26.70
Liver	Adenoma						
	NTP1994	21.91	32.18	25.49	13.65	17.62	12.08
	Placke1996	52.84	60.80	57.60	38.43	45.12	43.56
	NTP1994 & Placke1996	45.57	53.10	49.67	34.60	40.88	38.44
	Carcinoma						
	NTP1994	98.24	95.41	99.68	36.48	36.68	33.12
	Placke1996	122.96	131.36	125.58	78.50	87.76	86.07
	NTP1994 & Placke1996	109.85	118.53	129.86	74.68	83.15	83.17
	Adenoma/Carcinoma						
	NTP1994	22.67	28.34	32.41	13.74	15.37	14.21
	Placke1996	12.53	15.29	16.39	9.95	12.20	13.11
	NTP1994 & Placke1996	14.24	16.73	17.10	11.52	13.50	13.78
Lung	Adenoma						
	NTP1994	47.07	54.88	47.32	27.61	30.21	23.30
	Placke1996	51.75	61.43	61.28	39.70	47.73	47.42
	NTP1994 & Placke1996	51.22	60.14	57.00	40.13	47.53	45.54
	Carcinoma						
	NTP1994	131.92	124.20	89.17	68.39	64.15	45.50
	Placke1996	263.11	313.39	310.26	168.80	203.23	203.54
	NTP1994 & Placke1996	235.00	278.11	299.60	158.90	186.74	188.98
	Adenoma/Carcinoma						
	NTP1994	40.45	43.80	46.02	24.42	25.22	22.86
	Placke1996	35.70	43.56	45.75	28.45	34.66	35.59
	NTP1994 & Placke1996	36.47	43.09	42.97	29.53	35.07	35.11
Hematopoietic System	Any Lymphoma						
	NTP1994	314.61	307.85	211.41	100.34	94.02	60.74
	Placke1996	472.10	510.34	470.01	229.51	263.04	253.65
	NTP1994 & Placke1996	440.84	470.84	448.07	229.87	257.36	243.43
Placke et al. 1996: Male $B6C3F_1$ Mice							
Heart	Hemangiosarcoma	976.69	664.91	662.78	349.80	395.19	410.64
Spleen	Hemangiosarcoma	>403	>496	>517	>403	>496	>517
Hematopoietic System	Histiocytic Sarcoma	600.67	525.59	446.69	252.67	262.44	242.60

Organ	**Severity / Study**	**EC_{10} (ppm) adjusting for 1, 2, or 3 number of stages**			**LEC_{10} (ppm) adjusting for 1, 2, or 3 number of stages**		
		m=1	**m=2**	**m=3**	**m=1**	**m=2**	**m=3**
Placke et al. 1996: Female $B6C3F_1$ Mice							
Spleen	Hemangiosarcoma	16.51	20.32	21.19	9.03	11.11	11.59
Harderian Gland	Adenoma	9.90	12.19	12.72	5.05	6.22	6.48
Pituitary Gland	Adenoma	7.68	9.46	9.87	3.98	4.90	5.11

Table 21. Unit risk factor (URF) corresponding to the EC_{10} and the upper bound (URF_UB, upper 95% confidence limit) on the URF corresponding to the LEC_{10}: Estimated EC_{10} and LEC_{10} for the endpoints analyzed for three alternative adjustments to the doses and numbers of animals at risk (i.e., m=1, 2, or 3): ppm is environmental ppm, that is 24 hours per day, 7 days per week for a lifetime: Grouped by species and gender and then the results for the same organ and severity are grouped together

Organ	Severity / Study	URF (ppm^{-1}) adjusting for 1, 2, or 3 number of stages			95% UCL on URF (ppm^{-1}) adjusting for 1, 2, or 3 number of stages		
		m=1	m=2	m=3	m=1	m=2	m=3
Male F344/N Rats							
Testis	Adenoma						
	NTP1994	1.4×10^{-3}	1.2×10^{-3}	1.4×10^{-3}	2.9×10^{-3}	2.7×10^{-3}	3.5×10^{-3}
	NTP1999	1.7×10^{-3}	1.4×10^{-3}	1.1×10^{-3}	3.0×10^{-3}	2.3×10^{-3}	1.9×10^{-3}
	NTP1994 & NTP1999	1.9×10^{-3}	1.0×10^{-3}	1.1×10^{-3}	2.9×10^{-3}	2.1×10^{-3}	1.9×10^{-3}
Summary Statistics for Testis in Male F344/N Rats							
	minimum	0.0014	0.0010	0.0011	0.0029	0.0021	0.0019
	maximum	0.0019	0.0014	0.0014	0.0030	0.0027	0.0035
	average	0.0017	0.0012	0.0012	0.0029	0.0024	0.0024
NTP 1999: Male F344/N Rats							
Kidney	Adenoma	2.3×10^{-4}	2.3×10^{-4}	2.3×10^{-4}	3.8×10^{-4}	3.8×10^{-4}	3.8×10^{-4}
	Carcinoma	$<8.0\times10^{-5}$	$<8.0\times10^{-5}$	$<8.0\times10^{-5}$	$<8.0\times10^{-5}$	$<8.0\times10^{-5}$	$<8.0\times10^{-5}$
	Adenoma/Carcinoma	2.3×10^{-4}	2.3×10^{-4}	2.3×10^{-4}	3.8×10^{-4}	3.8×10^{-4}	3.8×10^{-4}
Mammary Gland	Fibroadenoma	3.8×10^{-4}	3.8×10^{-4}	3.7×10^{-4}	5.6×10^{-4}	5.6×10^{-4}	5.4×10^{-4}
	Carcinoma	$<8.0\times10^{-5}$	$<8.0\times10^{-5}$	$<8.0\times10^{-5}$	$<8.0\times10^{-5}$	$<8.0\times10^{-5}$	$<8.0\times10^{-5}$
	Fibroadenoma/Carcinoma	3.8×10^{-4}	3.8×10^{-4}	3.7×10^{-4}	5.6×10^{-4}	5.6×10^{-4}	5.4×10^{-4}
Summary Statistics for Kidney and Mammary Glands in Male F344/N Rats in NTP 1999							
	minimum	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	maximum	0.0004	0.0004	0.0004	0.0006	0.0006	0.0005
	average	0.0002	0.0002	0.0002	0.0003	0.0003	0.0003
Summary Statistics for All Endpoints for Male F344/N Rats							
	minimum	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	maximum	0.0019	0.0014	0.0014	0.0030	0.0027	0.0035
	average	0.0007	0.0005	0.0005	0.0012	0.0010	0.0010
NTP 1999: Female F344/N Rats							
Mammary Gland	Fibroadenoma	1.7×10^{-4}	1.5×10^{-4}	1.7×10^{-4}	4.8×10^{-4}	4.5×10^{-4}	4.6×10^{-4}
	Carcinoma	$<8.0\times10^{-5}$	$<8.0\times10^{-5}$	$<8.0\times10^{-5}$	$<8.0\times10^{-5}$	$<8.0\times10^{-5}$	$<8.0\times10^{-5}$
	Fibroadenoma/Carcinoma	1.5×10^{-4}	1.4×10^{-4}	1.5×10^{-4}	4.6×10^{-4}	4.3×10^{-4}	4.5×10^{-4}

Organ	Severity / Study	URF (ppm^{-1}) adjusting for 1, 2, or 3 number of stages			95% UCL on URF (ppm^{-1}) adjusting for 1, 2, or 3 number of stages		
		m=1	m=2	m=3	m=1	m=2	m=3
Summary Statistics for All Endpoints for Female F344/N Rats							
	minimum	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	maximum	0.0002	0.0002	0.0002	0.0005	0.0005	0.0005
	average	0.0001	0.0001	0.0001	0.0003	0.0003	0.0003
Summary Statistics for All Endpoints for F344/N Rats							
	minimum	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	maximum	0.0019	0.0014	0.0014	0.0030	0.0027	0.0035
	average	0.0007	0.0005	0.0005	0.0012	0.0010	0.0010
Male $B6C3F_1$ Mice							
Forestomach	Papilloma						
	NTP1994	1.2×10^{-3}	1.2×10^{-3}	1.6×10^{-3}	2.0×10^{-3}	2.0×10^{-3}	2.7×10^{-3}
	Placke1996	9.8×10^{-5}	8.5×10^{-5}	1.2×10^{-4}	2.5×10^{-4}	2.1×10^{-4}	2.1×10^{-4}
	NTP1994 & Placke1996	2.2×10^{-4}	2.2×10^{-4}	2.1×10^{-4}	3.4×10^{-4}	2.8×10^{-4}	2.5×10^{-4}
	Carcinoma						
	NTP1994	4.3×10^{-4}	4.7×10^{-4}	7.7×10^{-4}	9.7×10^{-4}	1.1×10^{-3}	1.8×10^{-3}
	Placke1996	2.1×10^{-4}	1.7×10^{-4}	1.6×10^{-4}	2.7×10^{-4}	2.2×10^{-4}	2.1×10^{-4}
	NTP1994 & Placke1996	1.6×10^{-4}	1.7×10^{-4}	1.7×10^{-4}	2.8×10^{-4}	2.3×10^{-4}	2.3×10^{-4}
	Papilloma/Carcinoma						
	NTP1994	1.6×10^{-3}	1.6×10^{-3}	2.0×10^{-3}	2.5×10^{-3}	2.5×10^{-3}	3.2×10^{-3}
	Placke1996	2.2×10^{-4}	1.9×10^{-4}	1.9×10^{-4}	3.5×10^{-4}	2.9×10^{-4}	2.7×10^{-4}
	NTP1994 & Placke1996	3.9×10^{-4}	2.9×10^{-4}	2.6×10^{-4}	5.4×10^{-4}	4.1×10^{-4}	3.5×10^{-4}
Harderian Gland	Adenoma						
	NTP1994	2.1×10^{-3}	1.8×10^{-3}	1.5×10^{-3}	4.0×10^{-3}	3.7×10^{-3}	3.8×10^{-3}
	Placke1996	3.5×10^{-3}	2.9×10^{-3}	2.9×10^{-3}	4.3×10^{-3}	3.6×10^{-3}	3.5×10^{-3}
	NTP1994 & Placke1996	3.3×10^{-3}	2.9×10^{-3}	2.9×10^{-3}	4.1×10^{-3}	3.5×10^{-3}	3.5×10^{-3}
	Carcinoma						
	NTP1994	1.4×10^{-4}	1.7×10^{-4}	2.9×10^{-4}	5.1×10^{-4}	6.1×10^{-4}	1.1×10^{-3}
	Placke1996	$<2.5\times10^{-4}$	7.1×10^{-5}	7.5×10^{-5}	$<2.5\times10^{-4}$	2.0×10^{-4}	2.0×10^{-4}
	NTP1994 & Placke1996	$<2.5\times10^{-4}$	7.4×10^{-5}	8.4×10^{-5}	$<2.5\times10^{-4}$	1.9×10^{-4}	2.1×10^{-4}
	Adenoma/Carcinoma						
	NTP1994	2.1×10^{-3}	1.8×10^{-3}	1.5×10^{-3}	4.0×10^{-3}	3.7×10^{-3}	3.8×10^{-3}
	Placke1996	3.9×10^{-3}	3.2×10^{-3}	3.1×10^{-3}	4.7×10^{-3}	3.9×10^{-3}	3.8×10^{-3}
	NTP1994 & Placke1996	3.6×10^{-3}	3.1×10^{-3}	3.1×10^{-3}	4.4×10^{-3}	3.7×10^{-3}	3.7×10^{-3}
Liver	Adenoma						
	NTP1994	4.6×10^{-3}	3.1×10^{-3}	3.9×10^{-3}	7.3×10^{-3}	5.7×10^{-3}	8.3×10^{-3}
	Placke1996	1.9×10^{-3}	1.6×10^{-3}	1.7×10^{-3}	2.6×10^{-3}	2.2×10^{-3}	2.3×10^{-3}
	NTP1994 & Placke1996	2.2×10^{-3}	1.9×10^{-3}	2.0×10^{-3}	2.9×10^{-3}	2.4×10^{-3}	2.6×10^{-3}
	Carcinoma						
	NTP1994	1.0×10^{-3}	1.0×10^{-3}	1.0×10^{-3}	2.7×10^{-3}	2.7×10^{-3}	3.0×10^{-3}
	Placke1996	8.1×10^{-4}	7.6×10^{-4}	8.0×10^{-4}	1.3×10^{-3}	1.1×10^{-3}	1.2×10^{-3}
	NTP1994 & Placke1996	9.1×10^{-4}	8.4×10^{-4}	7.7×10^{-4}	1.3×10^{-3}	1.2×10^{-3}	1.2×10^{-3}

Organ	Severity / Study	URF (ppm^{-1}) adjusting for 1, 2, or 3 number of stages			95% UCL on URF (ppm^{-1}) adjusting for 1, 2, or 3 number of stages		
		m=1	m=2	m=3	m=1	m=2	m=3
Liver	Adenoma/Carcinoma						
	NTP1994	4.4×10^{-3}	3.5×10^{-3}	3.1×10^{-3}	7.3×10^{-3}	6.5×10^{-3}	7.0×10^{-3}
	Placke1996	8.0×10^{-3}	6.5×10^{-3}	6.1×10^{-3}	1.0×10^{-2}	8.2×10^{-3}	7.6×10^{-3}
	NTP1994 & Placke1996	7.0×10^{-3}	6.0×10^{-3}	5.8×10^{-3}	8.7×10^{-3}	7.4×10^{-3}	7.3×10^{-3}
Lung	Adenoma						
	NTP1994	2.1×10^{-3}	1.8×10^{-3}	2.1×10^{-3}	3.6×10^{-3}	3.3×10^{-3}	4.3×10^{-3}
	Placke1996	1.9×10^{-3}	1.6×10^{-3}	1.6×10^{-3}	2.5×10^{-3}	2.1×10^{-3}	2.1×10^{-3}
	NTP1994 & Placke1996	2.0×10^{-3}	1.7×10^{-3}	1.8×10^{-3}	2.5×10^{-3}	2.1×10^{-3}	2.2×10^{-3}
	Carcinoma						
	NTP1994	7.6×10^{-4}	8.1×10^{-4}	1.1×10^{-3}	1.5×10^{-3}	1.6×10^{-3}	2.2×10^{-3}
	Placke1996	3.8×10^{-4}	3.2×10^{-4}	3.2×10^{-4}	5.9×10^{-4}	4.9×10^{-4}	4.9×10^{-4}
	NTP1994 & Placke1996	4.3×10^{-4}	3.6×10^{-4}	3.3×10^{-4}	6.3×10^{-4}	5.4×10^{-4}	5.3×10^{-4}
	Adenoma/Carcinoma						
	NTP1994	2.5×10^{-3}	2.3×10^{-3}	2.2×10^{-3}	4.1×10^{-3}	4.0×10^{-3}	4.4×10^{-3}
	Placke1996	2.8×10^{-3}	2.3×10^{-3}	2.2×10^{-3}	3.5×10^{-3}	2.9×10^{-3}	2.8×10^{-3}
	NTP1994 & Placke1996	2.7×10^{-3}	2.3×10^{-3}	2.3×10^{-3}	3.4×10^{-3}	2.9×10^{-3}	2.8×10^{-3}
Hematopoietic System	Any Lymphoma						
	NTP1994	3.2×10^{-4}	3.2×10^{-4}	4.7×10^{-4}	1.0×10^{-3}	1.1×10^{-3}	1.6×10^{-3}
	Placke1996	2.1×10^{-4}	2.0×10^{-4}	2.1×10^{-4}	4.4×10^{-4}	3.8×10^{-4}	3.9×10^{-4}
	NTP1994 & Placke1996	2.3×10^{-4}	2.1×10^{-4}	2.2×10^{-4}	4.4×10^{-4}	3.9×10^{-4}	4.1×10^{-4}
Summary Statistics for the Endpoints for Male Mice in both NTP 1994 and Placke et al. 1996							
	minimum	0.0001	0.0001	0.0001	0.0003	0.0002	0.0002
	maximum	0.0080	0.0065	0.0061	0.0100	0.0082	0.0083
	average	0.0018	0.0015	0.0016	0.0026	0.0023	0.0025
Placke et al. 1996: Male B6C3F$_1$ Mice							
Heart	Hemangiosarcoma	1.0×10^{-4}	1.5×10^{-4}	1.5×10^{-4}	2.9×10^{-4}	2.5×10^{-4}	2.4×10^{-4}
Spleen	Hemangiosarcoma	$<2.5\times10^{-4}$	$<2.0\times10^{-4}$	$<1.9\times10^{-4}$	$<2.5\times10^{-4}$	$<2.0\times10^{-4}$	$<1.9\times10^{-4}$
Hematopoietic System	Histiocytic Sarcoma	1.7×10^{-4}	1.9×10^{-4}	2.2×10^{-4}	4.0×10^{-4}	3.8×10^{-4}	4.1×10^{-4}
Summary Statistics for the Endpoints for Male Mice only in Placke et al. 1996							
	minimum	0.0001	0.0002	0.0002	0.0003	0.0002	0.0002
	maximum	0.0003	0.0002	0.0002	0.0004	0.0004	0.0004
	average	0.0002	0.0002	0.0002	0.0003	0.0003	0.0003
Summary Statistics for All Endpoints for Male Mice							
	minimum	0.0001	0.0001	0.0001	0.0003	0.0002	0.0002
	maximum	0.0080	0.0065	0.0061	0.0100	0.0082	0.0083
	average	0.0017	0.0014	0.0015	0.0025	0.0022	0.0023

Organ	Severity / Study	URF (ppm^{-1}) adjusting for 1, 2, or 3 number of stages			95% UCL on URF (ppm^{-1}) adjusting for 1, 2, or 3 number of stages		
		m=1	m=2	m=3	m=1	m=2	m=3
Placke et al. 1996: Female $B6C3F_1$ Mice							
Spleen	Hemangiosarcoma	6.1×10^{-3}	4.9×10^{-3}	4.7×10^{-3}	1.1×10^{-2}	9.0×10^{-3}	8.6×10^{-3}
Harderian Gland	Adenoma	1.0×10^{-2}	8.2×10^{-3}	7.9×10^{-3}	2.0×10^{-2}	1.6×10^{-2}	1.5×10^{-2}
Pituitary Gland	Adenoma	1.3×10^{-2}	1.1×10^{-2}	1.0×10^{-2}	2.5×10^{-2}	2.0×10^{-2}	2.0×10^{-2}
Summary Statistics for All Endpoints for Female Mice							
minimum		0.0061	0.0049	0.0047	0.0110	0.0090	0.0086
maximum		0.0130	0.0110	0.0100	0.0250	0.0200	0.0200
average		0.0097	0.0080	0.0075	0.0187	0.0150	0.0145
Summary Statistics for All Endpoints for Mice							
minimum		0.0001	0.0001	0.0001	0.0003	0.0002	0.0002
maximum		0.0130	0.0110	0.0100	0.0250	0.0200	0.0200
average		0.0022	0.0019	0.0019	0.0035	0.0030	0.0032
Summary Statistics for All Endpoints for Rats and Mice							
minimum		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
maximum		0.0130	0.0110	0.0100	0.0250	0.0200	0.0200
average		0.0019	0.0016	0.0016	0.0030	0.0026	0.0027
Summary Statistics for All Endpoints for Mice Except Pituitary Gland Adenoma in Female Mice							
minimum		0.0001	0.0001	0.0001	0.0003	0.0002	0.0002
maximum		0.0100	0.0082	0.0079	0.0200	0.0160	0.0150
average		0.0020	0.0017	0.0017	0.0031	0.0026	0.0028
Summary Statistics for All Endpoints for Rats and Mice Except Pituitary Gland Adenoma in Female Mice							
minimum		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
maximum		0.0100	0.0082	0.0079	0.0200	0.0160	0.0150
average		0.0017	0.0014	0.0014	0.0026	0.0022	0.0024
Summary Statistics for Carcinoma, Sarcoma, and Lymphoma Endpoints for Rats and Mice							
minimum		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
maximum		0.0061	0.0049	0.0047	0.0110	0.0090	0.0086
average		0.0006	0.0005	0.0006	0.0011	0.0010	0.0011

Organ	Severity / Study	URF (ppm^{-1}) adjusting for 1, 2, or 3 number of stages			95% UCL on URF (ppm^{-1}) adjusting for 1, 2, or 3 number of stages		
		m=1	m=2	m=3	m=1	m=2	m=3
Summary Statistics for All Carcinoma and Adenoma/Carcinoma Endpoints for Rats and Mice (Excludes Only Adenoma, only Fibroadenoma, and only Papilloma)							
	minimum	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
	maximum	0.0080	0.0065	0.0061	0.0110	0.0090	0.0086
	average	0.0014	0.0012	0.0012	0.0021	0.0019	0.0020
Summary Statistics for All Endpoints for Rats and Male Mice (i.e., without the Female Mice in Placke et al. 1996)							
	minimum	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
	maximum	0.0080	0.0065	0.0061	0.0100	0.0082	0.0083
	average	0.0014	0.0012	0.0012	0.0021	0.0019	0.0020
Summary Statistics for Carcinoma, Sarcoma, and Lymphoma Endpoints for Rats and Male Mice (i.e., without the Female Mice in Placke et al. 1996)							
	minimum	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
	maximum	0.0010	0.0010	0.0011	0.0027	0.0027	0.0030
	average	0.0003	0.0003	0.0004	0.0006	0.0006	0.0008
Summary Statistics for All Carcinoma and Adenoma/Carcinoma Endpoints for Rats and Male Mice (Excludes Only Adenoma, only Fibroadenoma, and only Papilloma) (Excludes the Female Mice in Placke et al. 1996)							
	minimum	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
	maximum	0.0080	0.0065	0.0061	0.0100	0.0082	0.0076
	average	0.0013	0.0011	0.0011	0.0019	0.0017	0.0018

Figures 1 to 6 are a supplement to Table 21 which contains the unit risk factor (URF) corresponding to the EC_{10} and the upper bound on the URF (URF_UB, upper 95% confidence limit on the URF) corresponding to the LEC_{10}. These figures show the average URFs and URF_UBs by organ and response (including severity). The "average" is the average over the combinations of study, species, and gender for which this response was analyzed. The specific combinations are shown in Table 22. It should be noted that the endpoints for rats are distinct from the endpoints for mice.

Figures 7 to 12 are similar to Figures 1 to 6 except that Figures 7 to 12 are for **rats** only and show the individual URFs and URF_UBs by study and gender as well as by organ and response (including severity) instead of averages. Figures 13 to 18 are analogous to Figures 7 to 12 except that Figures 13 to 18 are for **mice** only and show the individual URFs and URF_UBs by study and gender as well as by organ and response (including severity) instead of averages. Although Figures 7 to 12 and Figures 13 to 18 both indicate the complete set of endpoints (organ and response), the figures clearly indicate that the endpoints for rats are distinct from the endpoints for mice.

Table 22. Combinations of study, species, and gender for which a response was analyzed

Organ	Response/Severity	**NTP 1994: Male F344/N Rats**	**NTP 1999: Male F344/N Rats**	**NTP 1999: Female F344/N Rats**	**NTP 1994 and NTP 1999 Combined: Male F344/N Rats**	**NTP 1994: Male B6C3F$_1$ Mice**	**Placke et al. 1996: Male B6C3F$_1$ Mice**	**NTP 1994 and Placke et al. 1996 Combined: Male B6C3F$_1$ Mice**	**Placke et al. 1996: Female B6C3F$_1$ Mice**	# in Avg.
Rats										
Kidney	Adenoma		Yes							1
	Carcinoma		Yes							1
	Adenoma/Carcinoma		Yes							1
Mammary Gland	Fibroadenoma		Yes	Yes						2
	Carcinoma		Yes	Yes						2
	Fibroadenoma/Carcinoma		Yes	Yes						2
Testis	Adenoma	Yes	Yes		Yes					3
Mice										
Heart	Hemangiosarcoma						Yes			1
Spleen	Hemangiosarcoma						Yes		Yes	2
Forestomach	Papilloma					Yes	Yes	Yes		3
	Carcinoma					Yes	Yes	Yes		3
	Papilloma/Carcinoma					Yes	Yes	Yes		3
Harderian Gland	Adenoma					Yes	Yes	Yes	Yes	4
	Carcinoma					Yes	Yes	Yes		3
	Adenoma/Carcinoma					Yes	Yes	Yes		3
Hematopoietic System	Any Lymphoma					Yes	Yes	Yes		3
	Histiocytic Sarcoma					Yes				1
Liver	Adenoma					Yes	Yes	Yes		3
	Carcinoma					Yes	Yes	Yes		3
	Adenoma/Carcinoma					Yes	Yes	Yes		3
Lung	Adenoma					Yes	Yes	Yes		3
	Carcinoma					Yes	Yes	Yes		3
	Adenoma/Carcinoma					Yes	Yes	Yes		3
Pituitary Gland	Adenoma								Yes	1
Total										57

Figure 1. Average URFs ($0.10/EC_{10}$) by organ and response (including severity): The "average" is the average over the combinations of study, species, and gender for which this response was analyzed: m=1

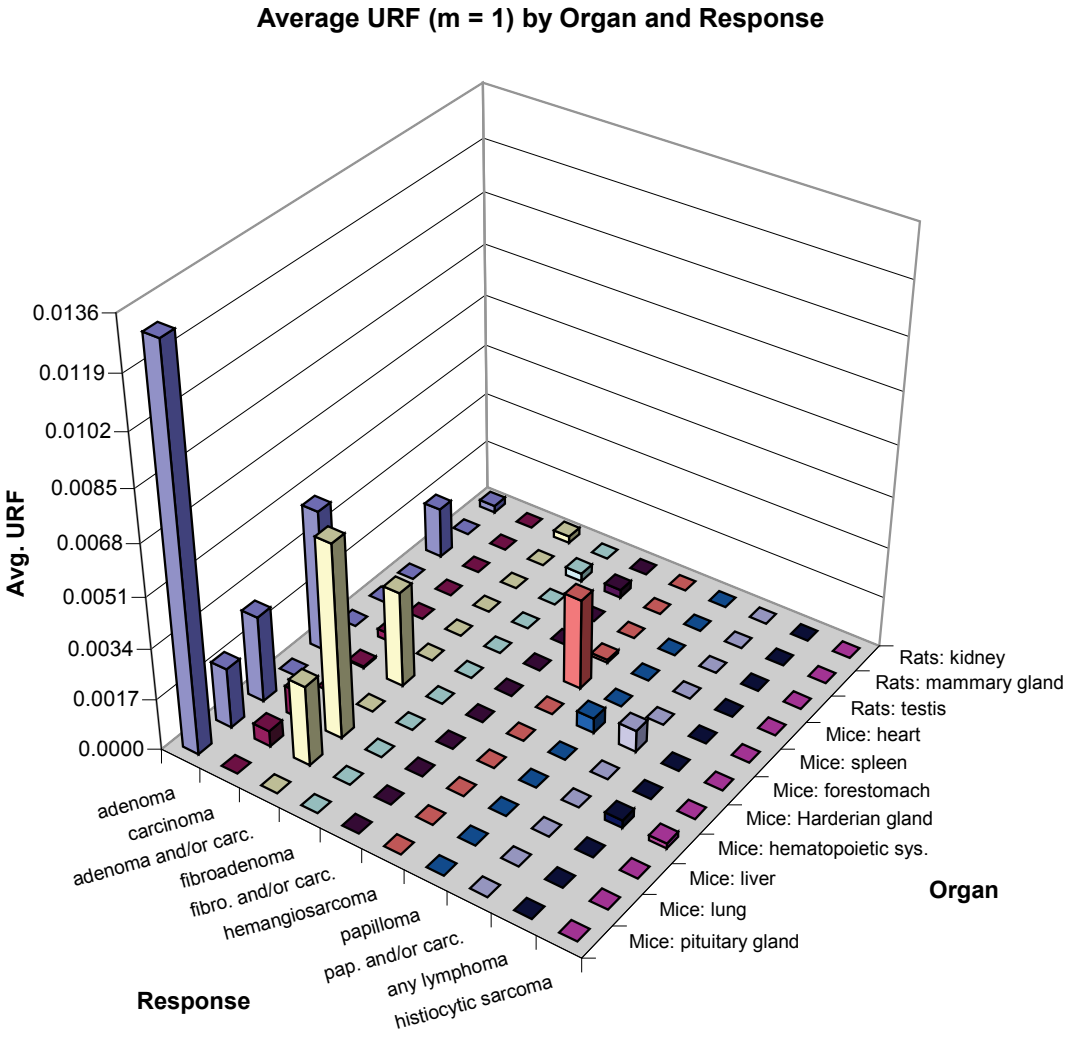


Figure 2. Average URFs ($0.10/EC_{10}$) by organ and response (including severity): The "average" is the average over the combinations of study, species, and gender for which this response was analyzed: m=2

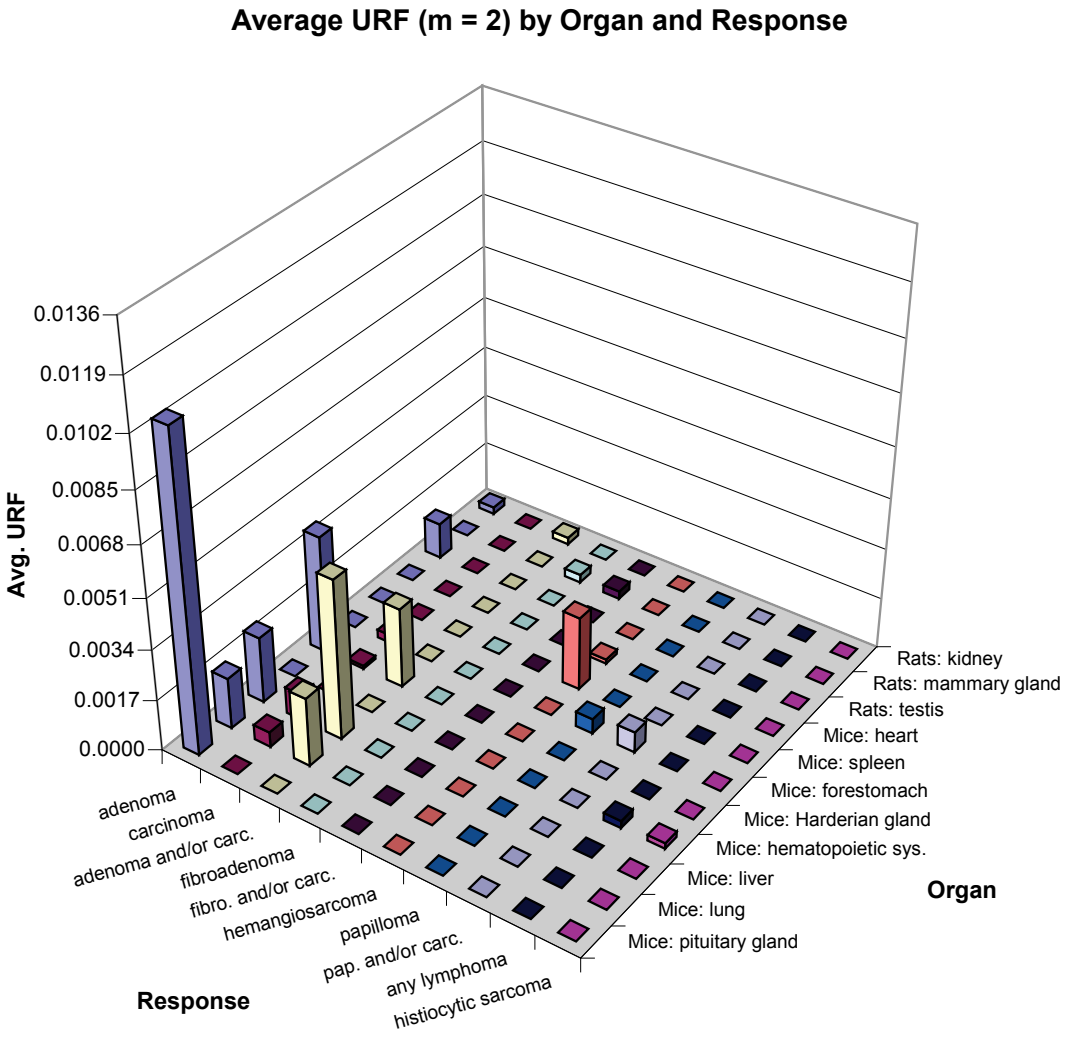


Figure 3. Average URFs ($0.10/EC_{10}$) by organ and response (including severity): The "average" is the average over the combinations of study, species, and gender for which this response was analyzed: m=3

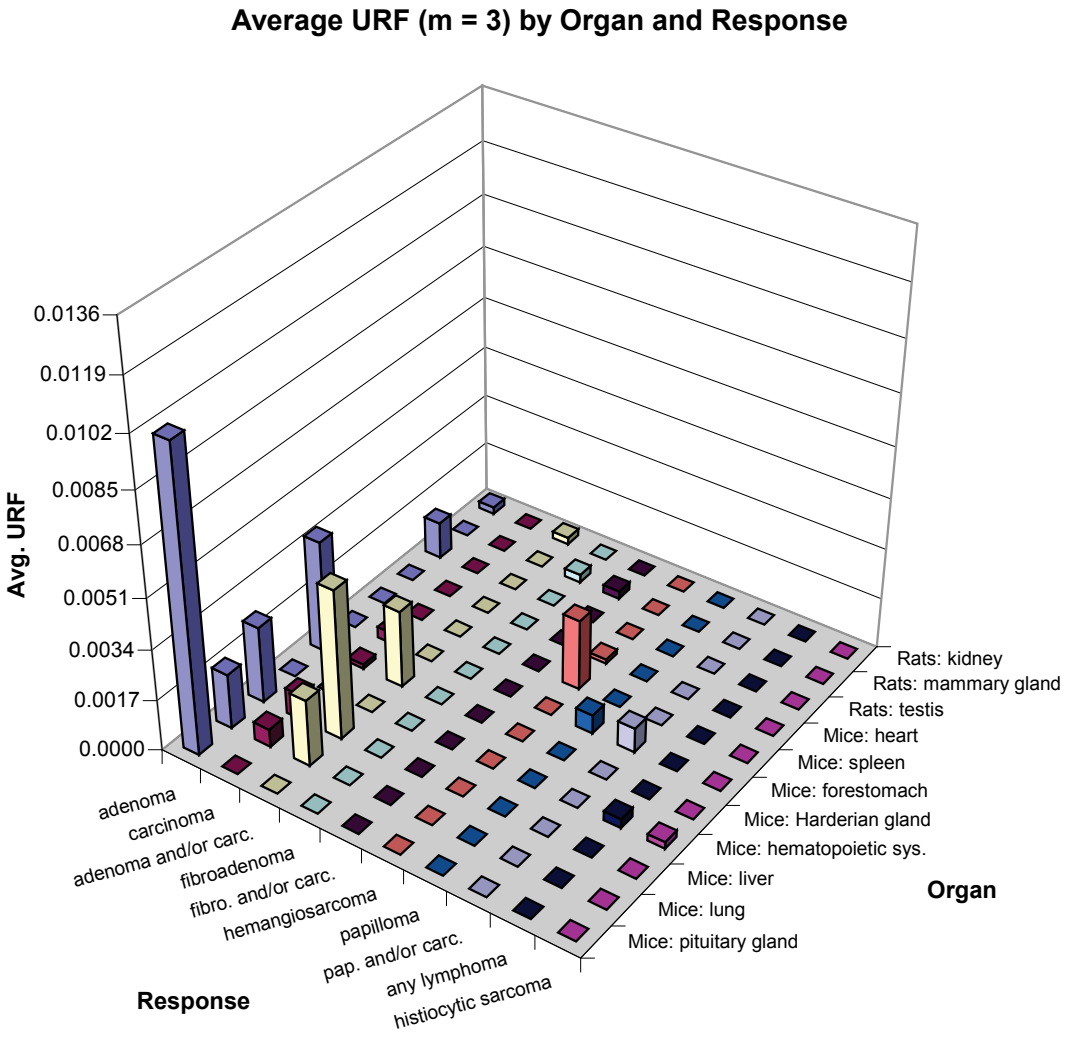


Figure 4. Average URF_UBs (0.10/LEC_{10}) by organ and response (including severity): The "average" is the average over the combinations of study, species, and gender for which this response was analyzed: m=1

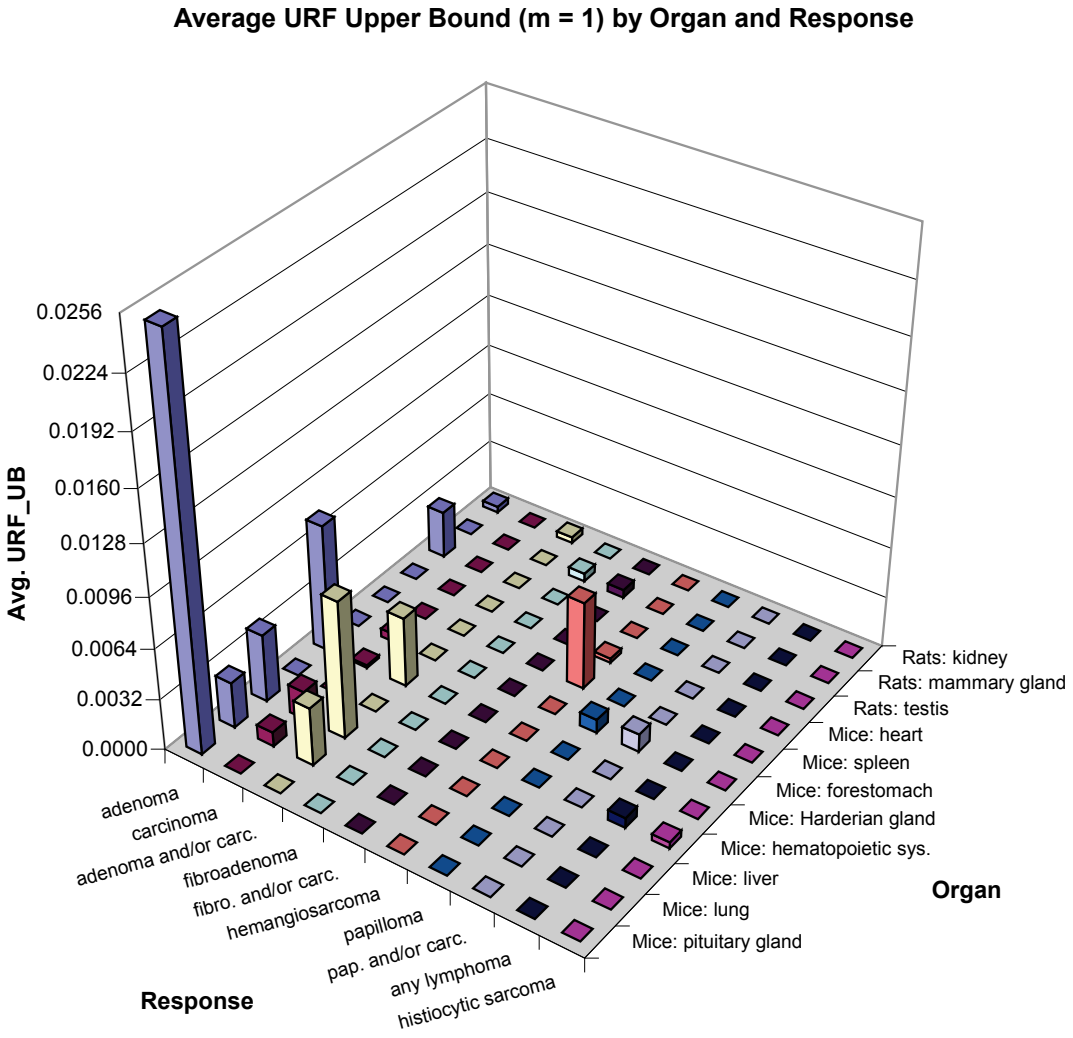


Figure 5. Average URF_UBs (0.10/LEC_{10}) by organ and response (including severity): The "average" is the average over the combinations of study, species, and gender for which this response was analyzed: m=2

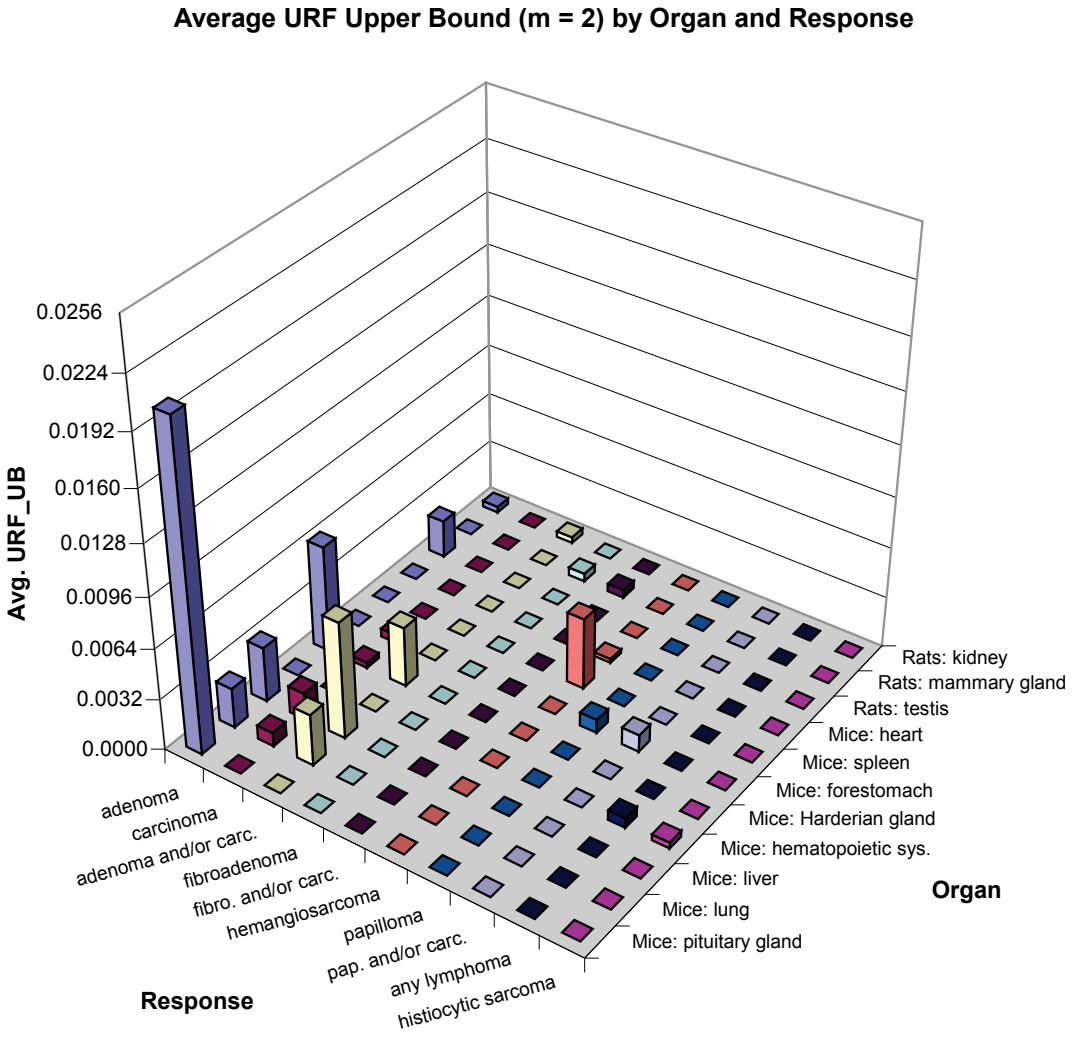


Figure 6. Average URF_UBs ($0.10/LEC_{10}$) by organ and response (including severity): The "average" is the average over the combinations of study, species, and gender for which this response was analyzed: m=3

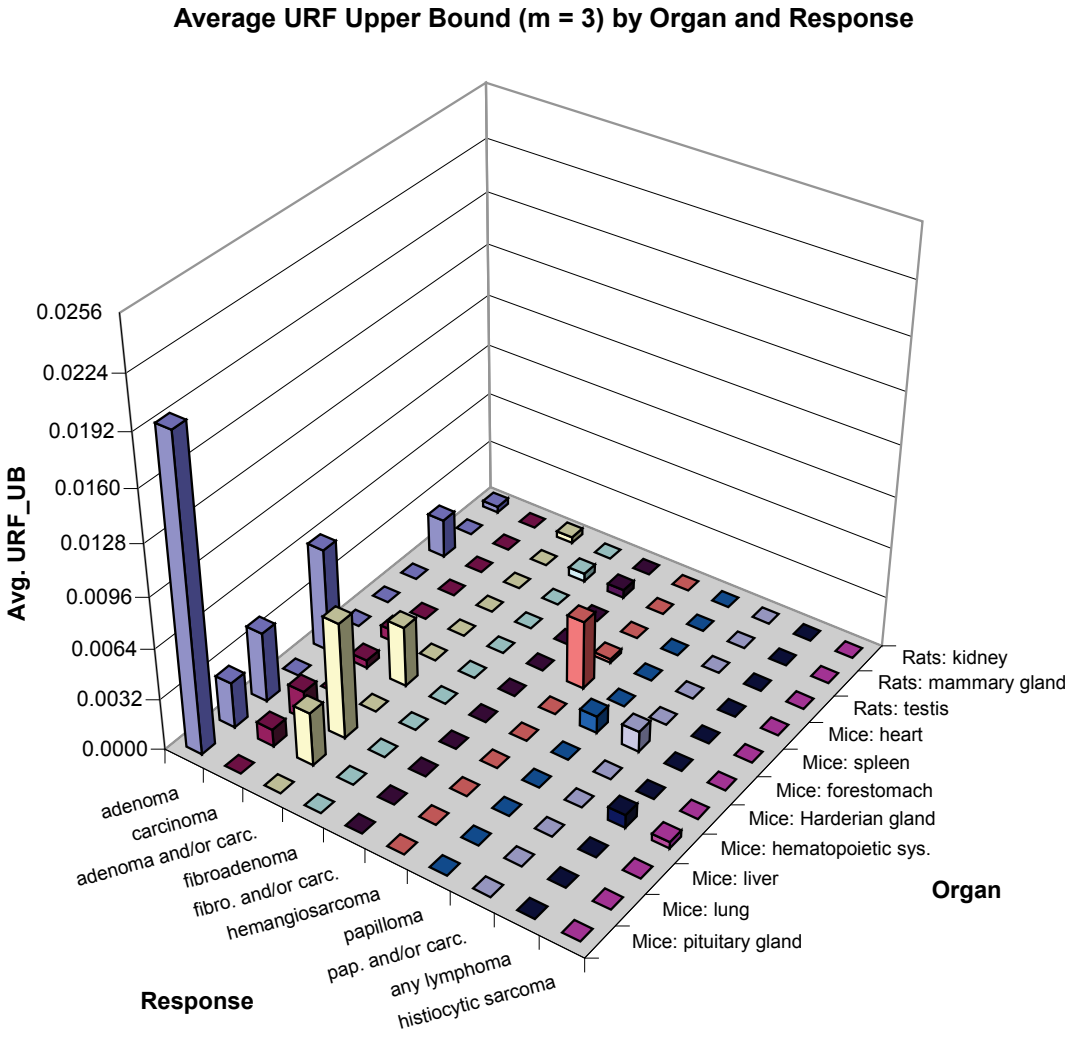


Figure 7. Rat based URFs (URF=0.10/EC_{10}) by organ and response (including severity): m=1

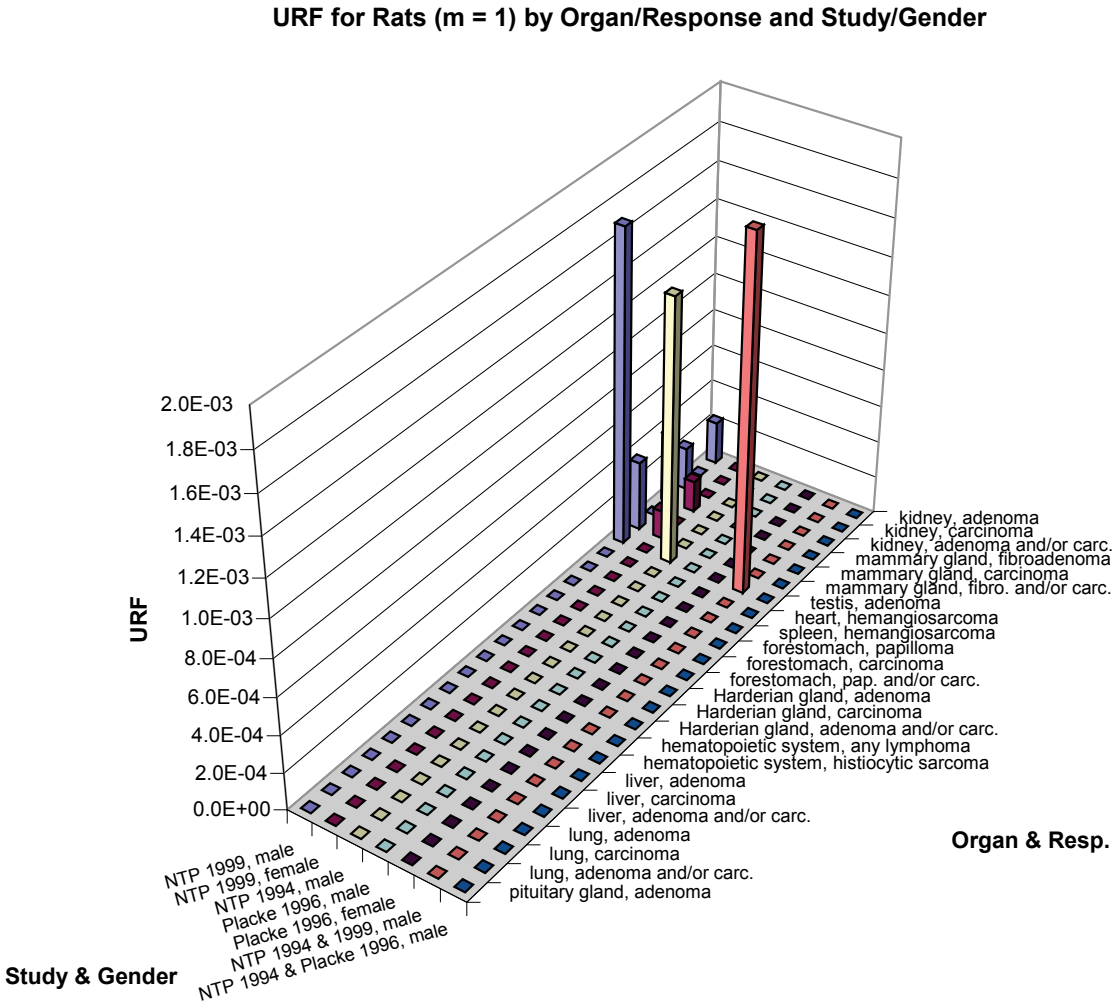


Figure 8. Rat based URFs (URF=0.10/EC_{10}) by organ and response (including severity): m=2

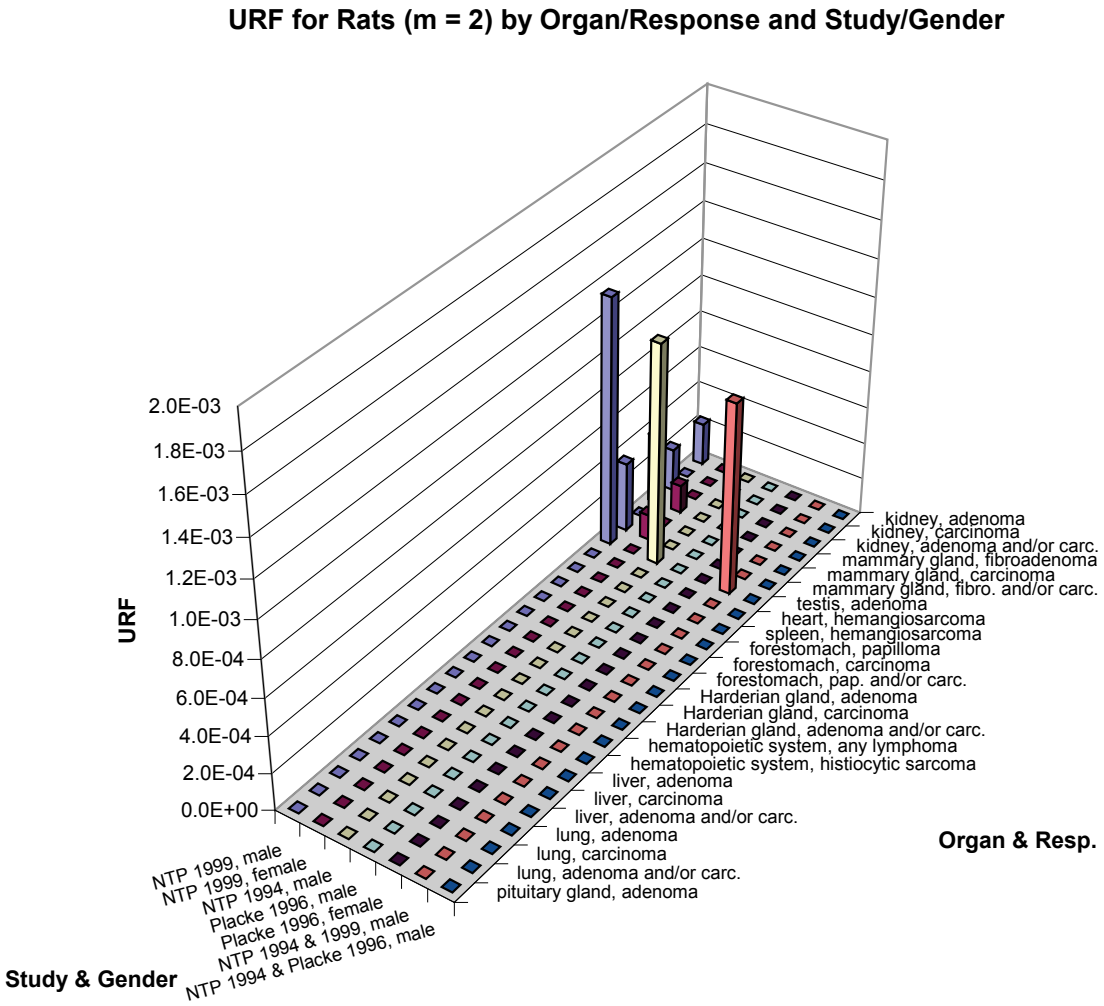


Figure 9. Rat based URFs (URF=0.10/EC_{10}) by organ and response (including severity: m=3

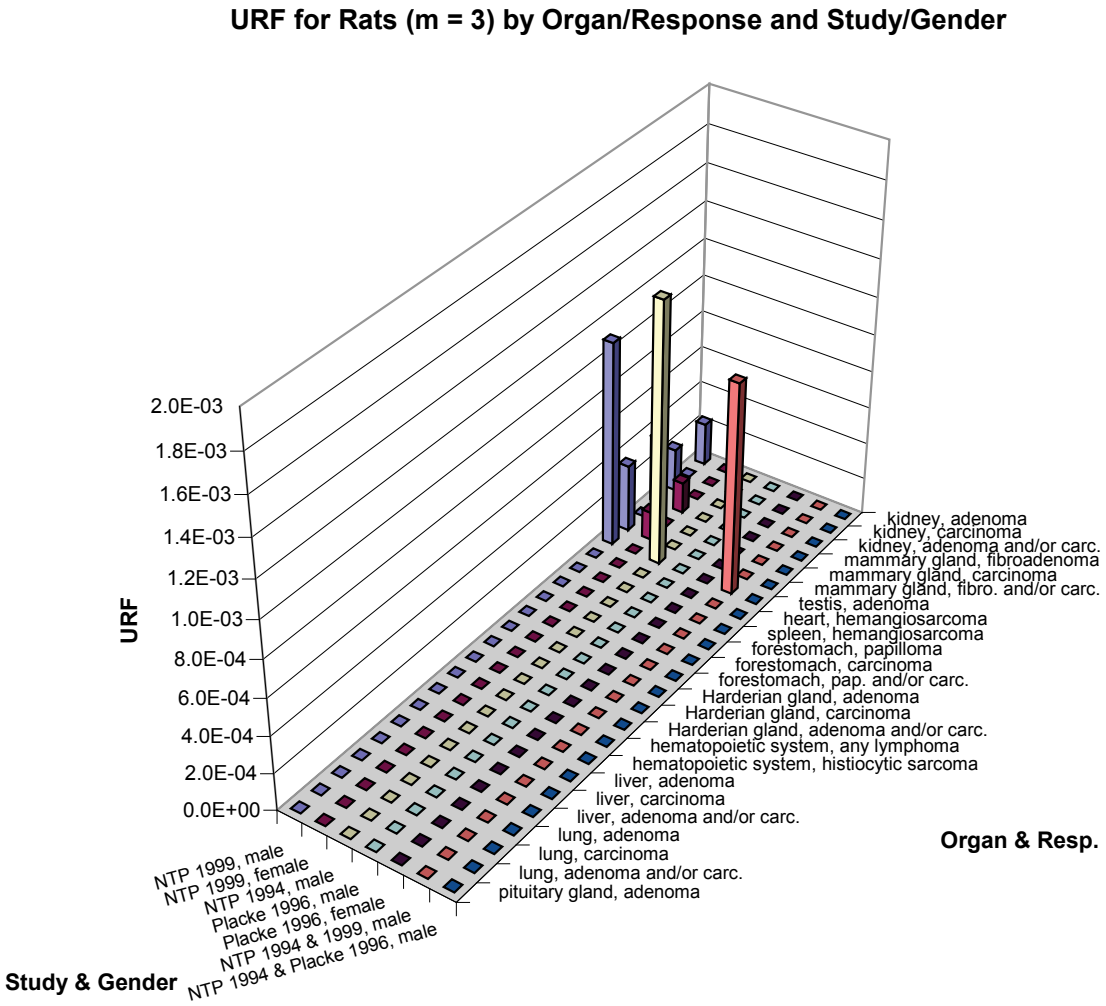


Figure 10. Rat based upper bound URFs (URF_UB=0.10/LEC_{10}) by organ and response (including severity): m=1

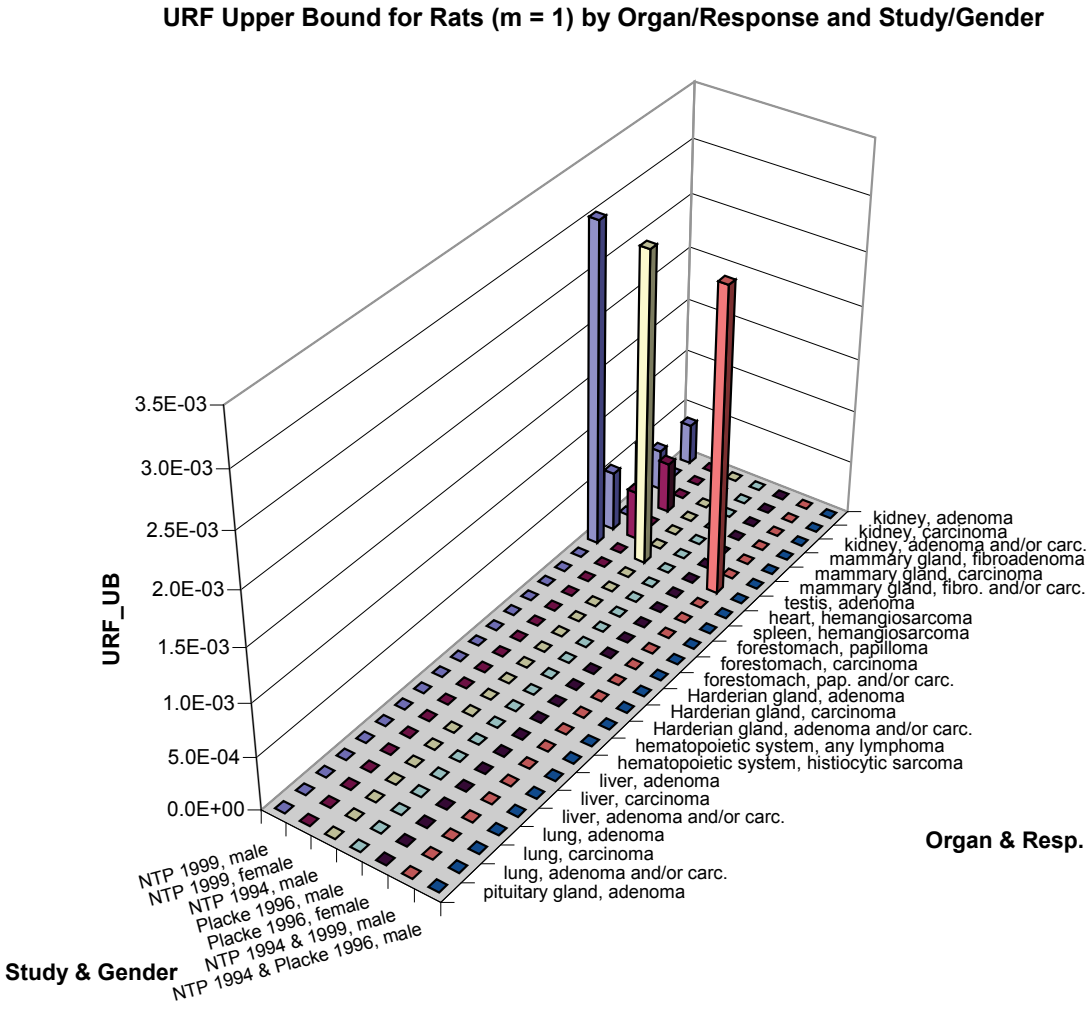


Figure 11. Rat based upper bound URFs (URF_UB=0.10/LEC_{10}) by organ and response (including severity): m=2

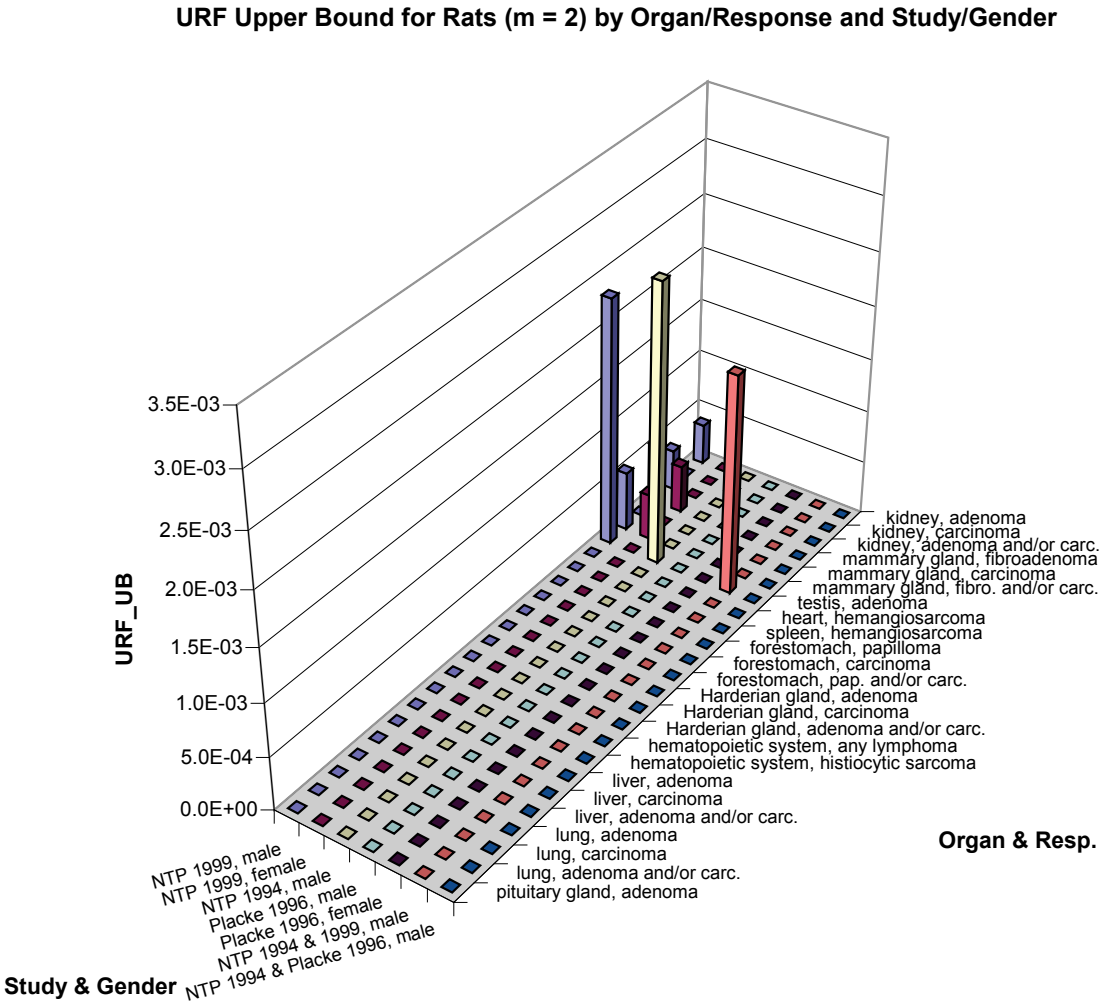


Figure 12. Rat based upper bound URFs (URF_UB=0.10/LEC_{10}) by organ and response (including severity): m=3

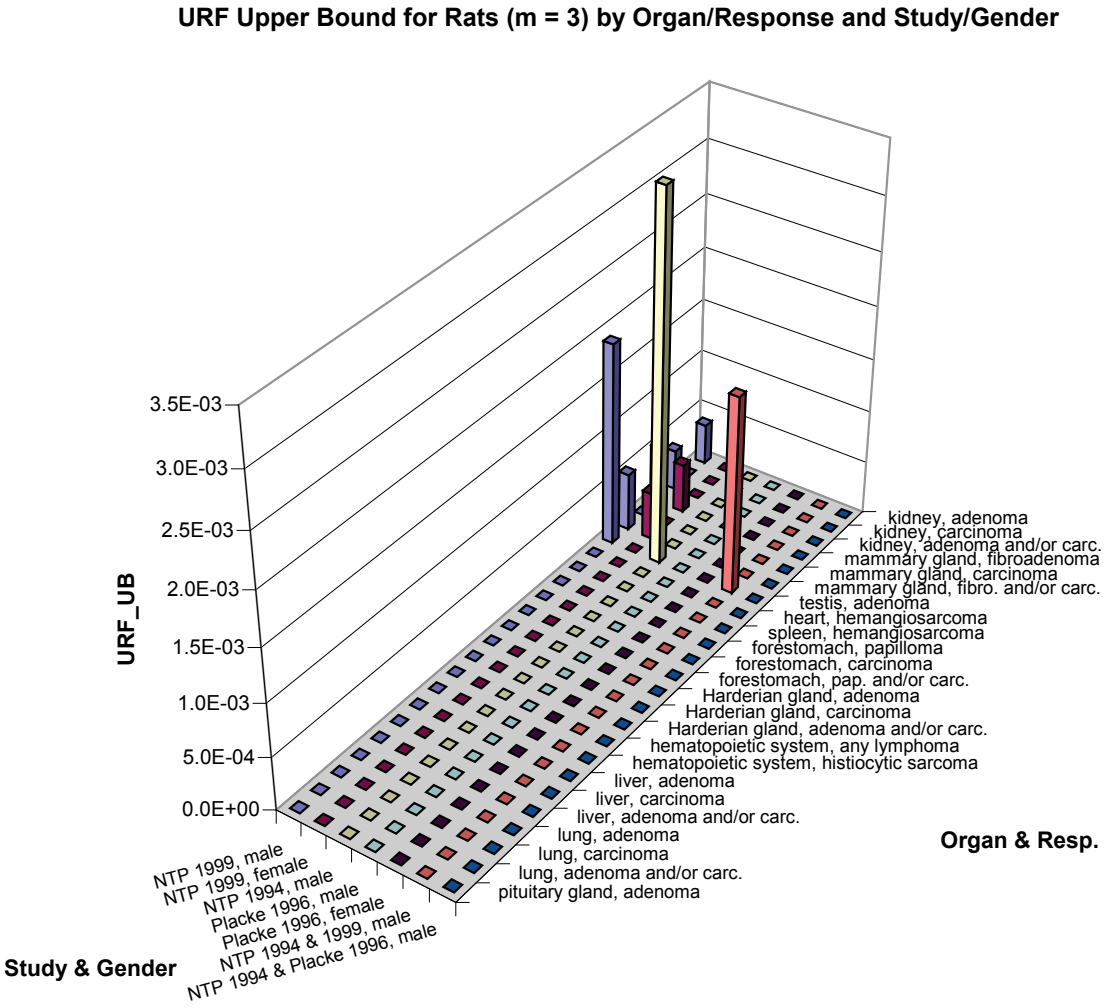


Figure 13. Mouse based URFs (URF=0.10/EC_{10}) by organ and response (including severity): m=1

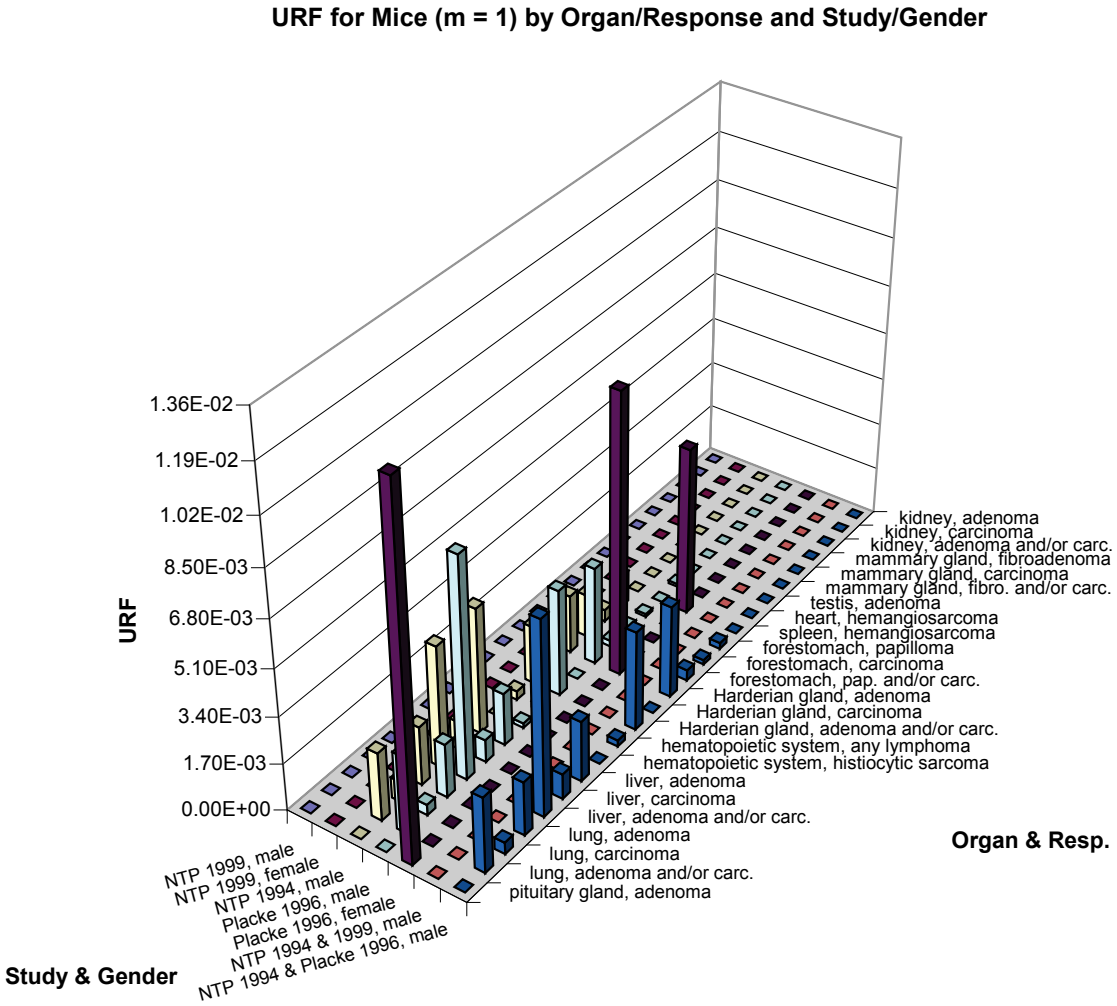


Figure 14. Mouse based URFs (URF=0.10/EC_{10}) by organ and response (including severity): m=2

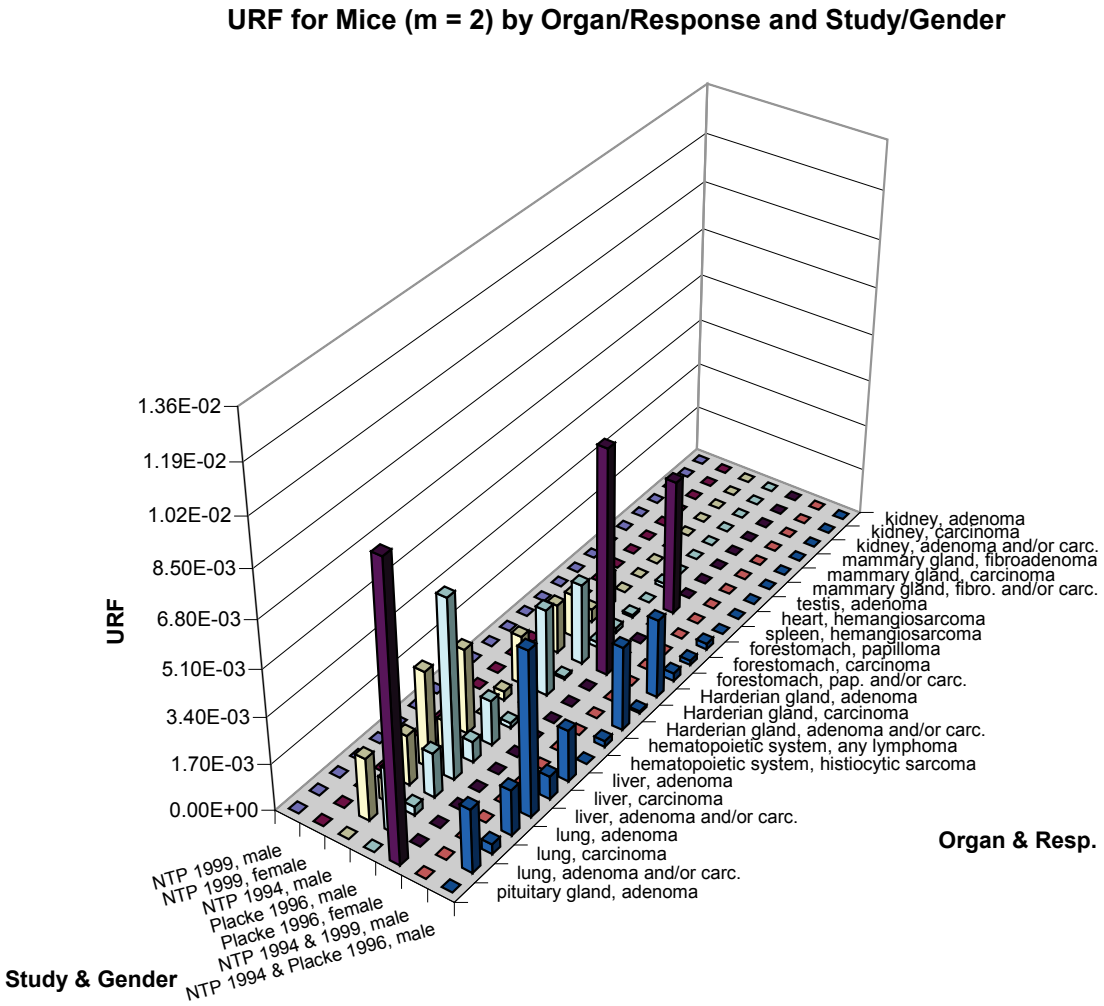


Figure 15. Mouse based URFs (URF=0.10/EC_{10}) by organ and response (including severity: m=3

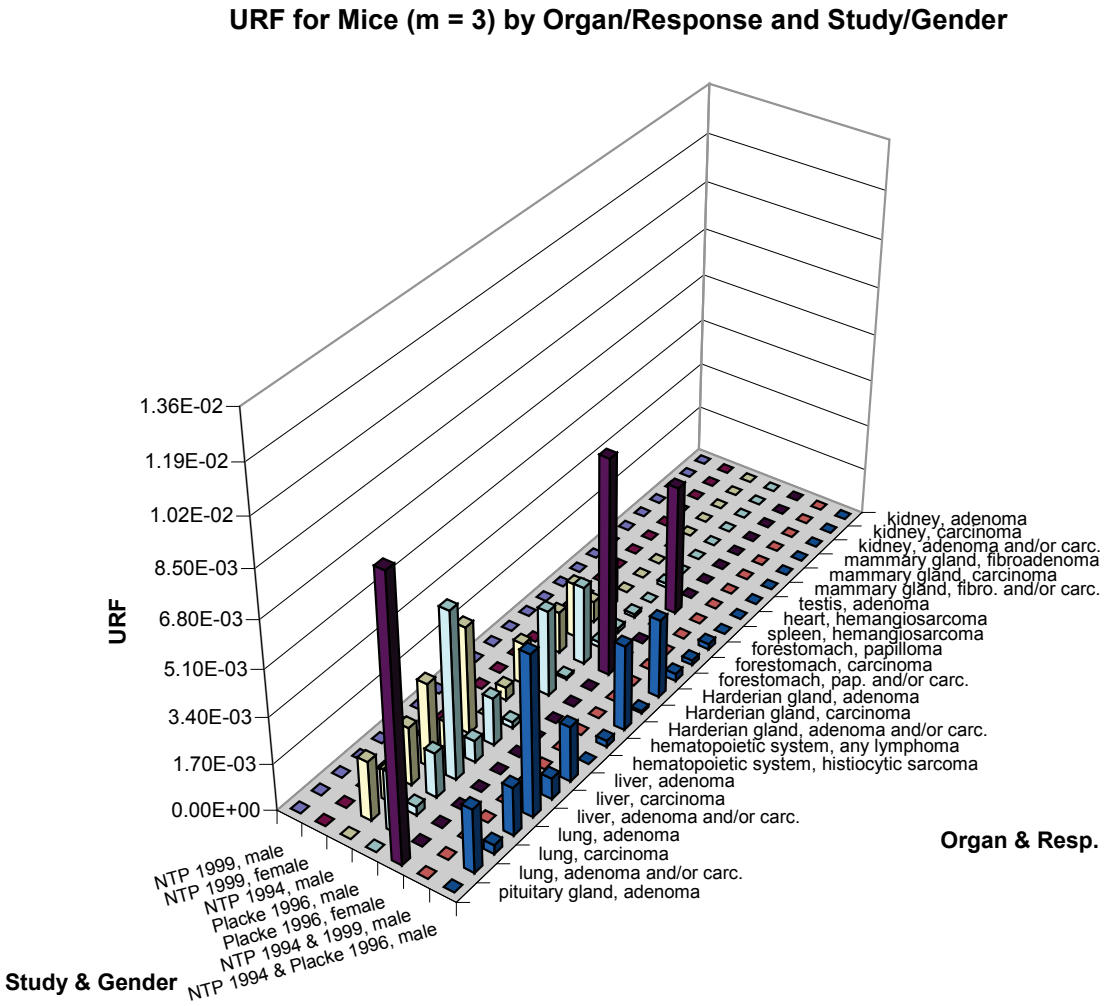


Figure 16. Mouse based upper bound URFs (URF_UB=0.10/LEC_{10}) by organ and response (including severity): m=1

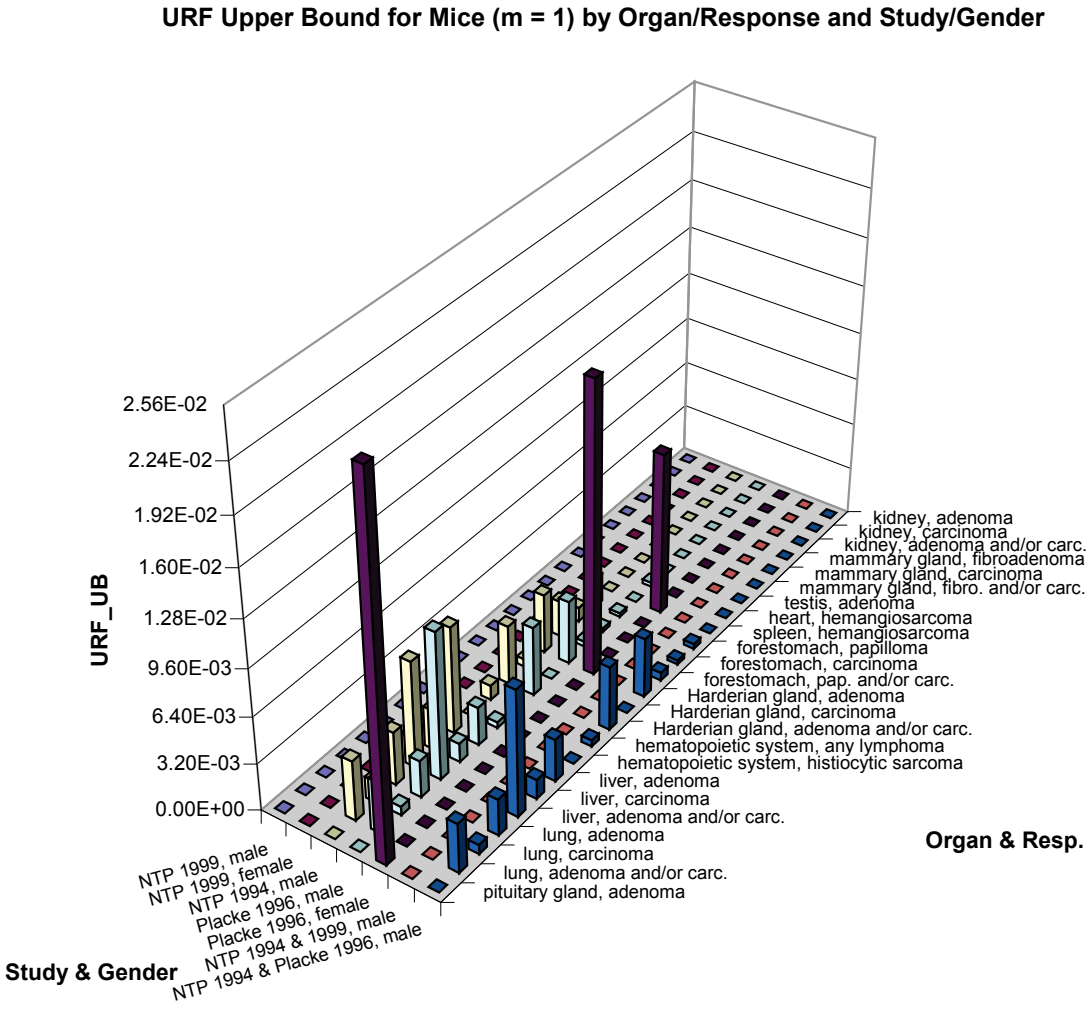


Figure 17. Mouse based upper bound URFs (URF_UB=0.10/LEC_{10}) by organ and response (including severity): m=2

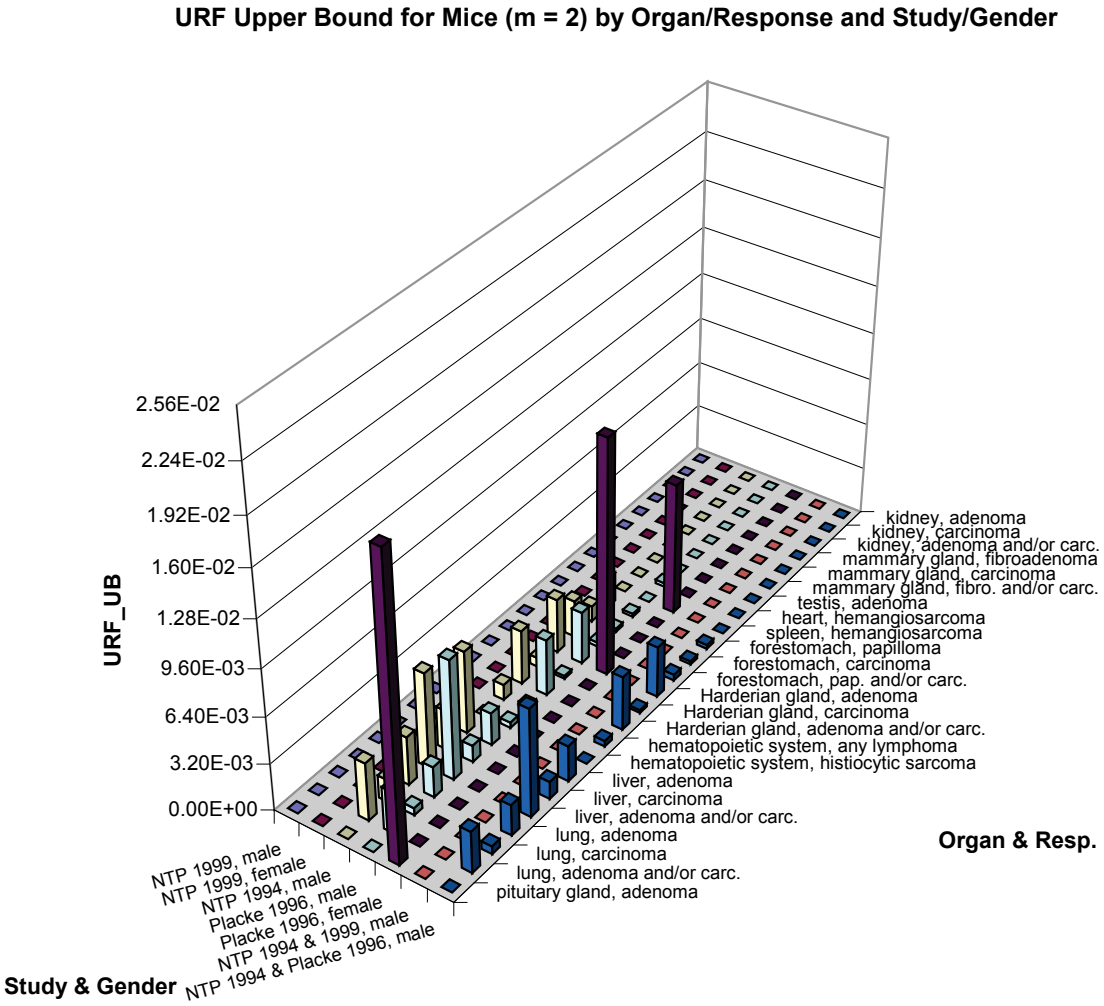
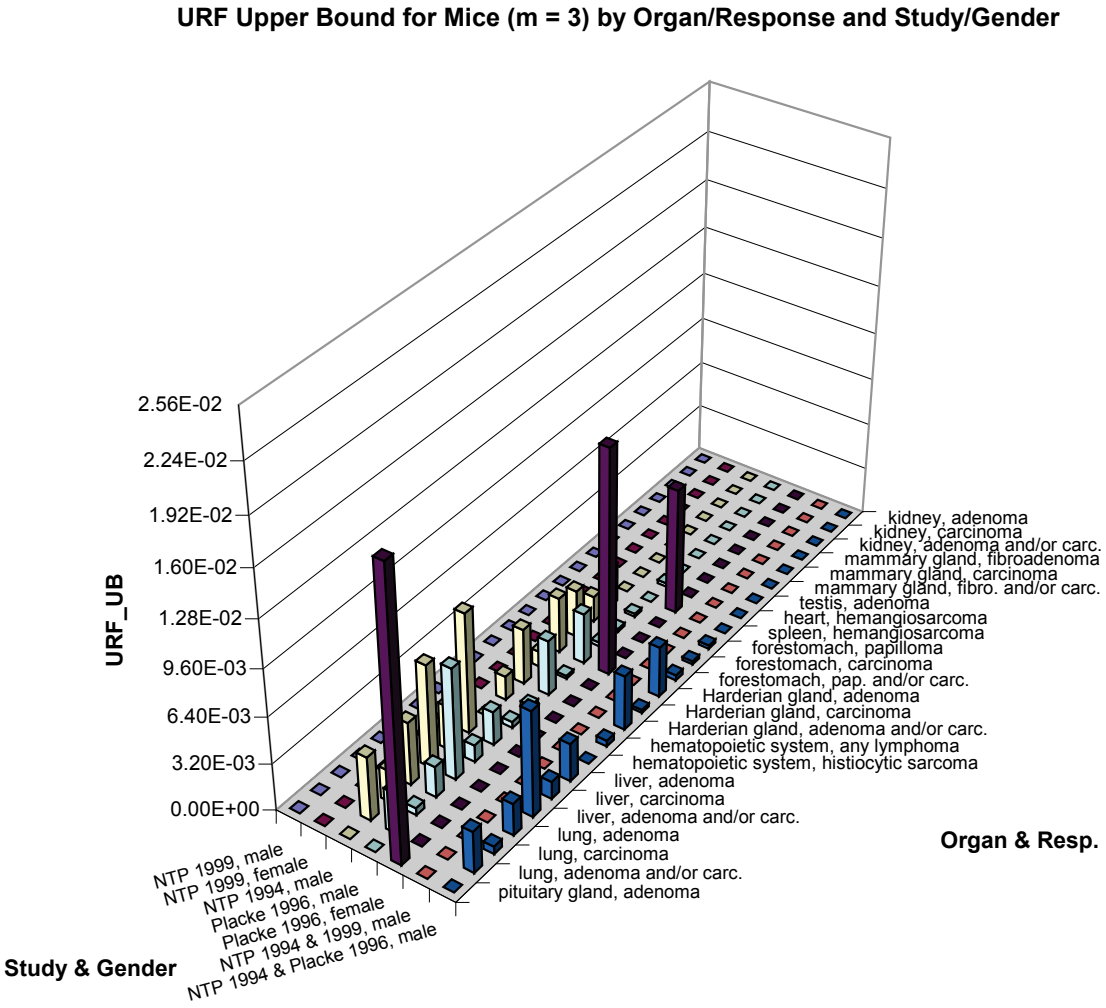


Figure 18. Mouse based upper bound URFs (URF_UB=0.10/LEC_{10}) by organ and response (including severity): m=3



7. Animal-to-Human Extrapolation

The human equivalent concentration (HEC) for inhalation exposures can be calculated by applying a chemical-specific dosimetric adjustment factor (DAF). The purpose of the DAF is to account for any pharmacokinetic differences between experimental species and humans and is applied to the duration-adjusted point of departure (U.S. EPA 1994, 2005).

Although there are no data for isoprene, EPA's risk assessment of chloroprene (U.S. EPA 2010) indicates that "chloroprene is a structural analog of isoprene (2-methyl 1,3-butadiene)." In addition, Himmelstein et al. (2004) points out that "the metabolic and genotoxic profile of chloroprene is consistent with that of the chemical analogs 1,3-butadiene and isoprene." In their toxicological review for chloroprene, EPA derives a DAF of 1.7 for mouse-to-human. Although EPA does not derive a DAF for rat-to-human, from their Table 3-1, the DAF for rat-to-human ranges from 1.6 to 1.8.

Similar to the EPA, in 2012 the Office of Environmental Health Hazard Assessment (OEHHA) reports that the mouse-to-human DAF for 1,3-butadiene is 1.68 based on PBPK modeling.

Because isoprene is structurally similar to chloroprene and butadiene and because the mouse-to-human DAF for chloroprene and butadiene are approximately 1.7, the DAF for isoprene is expected to be approximately 1.7. That is, the ECs, LECs and URFs presented herein are conservative (i.e., health protective) by a factor of approximately 1.7.

8. Sensitivity Analyses

For each of the endpoints analyzed, three forms of the response data were fit by the multistage model. The three forms of the response data correspond to the three forms of the adjusted doses and the three forms of the adjusted numbers of animals at risk. The three forms correspond to (1) m=1 with one transition rate from a normal cell to a first-stage (tumor) cell, (2) m=2 with one transition rate from a normal cell to a first stage cell and a second transition rate from a first-stage cell to a second-stage (tumor) cell, and (3) m=3 with one transition rate from a normal cell to a first stage cell, a second transition rate from a first-stage cell to a second-stage cell, and a third transition rate from a second-stage cell to a third-stage (tumor) cell.

A comparison of the EC_{10}s in Table 19 or Table 20 (or equivalently the URFs in Table 21 and Figures 1 to 3) indicates that the EC_{10}s (or equivalently the URFs) are relatively insensitive to whether m=1, m=2, or m=3. For example, the smallest EC_{10} is 7.68 ppm for m=1 and pituitary gland adenoma in female mice in Placke et al. (1996) compared to 9.46 and 9.87 for m=2 and m=3, respectively. As another example, the smallest EC_{10} for adenoma/carcinoma is 12.53 ppm for m=1 and liver in male mice in Placke et al. (1996) compared to 15.29 and 16.39 for m=2 and m=3, respectively. This insensitivity is also quantified in Table 23 which indicates that the ratios of EC_{10} for m=2 to the EC_{10} for m=1 ranges between 0.7 and 1.8 with an average ratio of 1.14 and, similarly, indicates that the ratios of EC_{10} for m=3 to the EC_{10} for m=1 ranges between 0.5 and 1.7 with an average ratio of 1.10.

Table 23. Estimated EC_{10}s based on the multistage model and three alternative adjustments to the doses and numbers of animals at risk (i.e., m=1, 2, or 3) and the range of these EC_{10}s: ppm is environmental ppm, that is 24 hours per day, 7 days per week

Organ	Severity	EC_{10} (ppm) m=1	Range of EC_{10} for m=1, 2, 3	Ratio: EC_{10}(m=2) to EC_{10}(m=1)	Ratio: EC_{10}(m=3) to EC_{10}(m=1)
NTP 1999: Male F344/N Rats					
Kidney	Adenoma	432.94	432.94 - 432.94	1.0	1.0
	Carcinoma	>1250[1]	>1250		
	Adenoma/Carcinoma	432.94	432.94 - 432.94	1.0	1.0
Mammary Gland	Fibroadenoma	261.68	261.68 - 269.65	1.0	1.0
	Carcinoma	>1250	>1250		
	Fibroadenoma/Carcinoma	265.14	265.14 - 273.63	1.0	1.0
Testis	Adenoma	58.77	58.77 - 89.7	1.3	1.5
NTP 1999: Female F344/N Rats					
Mammary Gland	Fibroadenoma	588.76	588.76 - 653.03	1.1	1.0
	Carcinoma	>1250	>1250		
	Fibroadenoma/Carcinoma	648.87	648.87 - 724.86	1.1	1.0
NTP 1994: Male F344/N Rats					
Testis	Adenoma	69.33	69.33 - 82.82	1.2	1.0
NTP 1994 and NTP 1999 Combined: Male F344/N Rats					
Testis	Adenoma	52.55	52.55 - 95.63	1.8	1.7
NTP 1994: Male B6C3F1 Mice					
Forestomach	Papilloma	86.93	64.02 - 86.93	1.0	0.7
	Carcinoma	234.01	130.03 - 234.01	0.9	0.6
	Papilloma/Carcinoma	64.40	50.29 - 64.4	1.0	0.8
Harderian Gland	Adenoma	47.34	47.34 - 65.94	1.2	1.4
	Carcinoma	713.76	344.34 - 713.76	0.8	0.5
	Adenoma/Carcinoma	47.34	47.34 - 65.94	1.2	1.4
Liver	Adenoma	21.91	21.91 - 32.18	1.5	1.2
	Carcinoma	98.24	95.41 - 99.68	1.0	1.0
	Adenoma/Carcinoma	22.67	22.67 - 32.41	1.3	1.4
Lung	Adenoma	47.07	47.07 - 54.88	1.2	1.0
	Carcinoma	131.92	89.17 - 131.92	0.9	0.7
	Adenoma/Carcinoma	40.45	40.45 - 46.02	1.1	1.1
Hematopoietic System	Any Lymphoma	314.61	211.41 - 314.61	1.0	0.7
Placke et al. 1996: Male B6C3F1 Mice					
Heart	Hemangiosarcoma	976.69	662.78 - 976.69	0.7	0.7
Spleen	Hemangiosarcoma	>403	>403		
Forestomach	Papilloma	1016.32	843 - 1174.77	1.2	0.8
	Carcinoma	476.25	476.25 - 625.44	1.2	1.3

Organ	Severity	EC_{10} (ppm) m=1	Range of EC_{10} for m=1, 2, 3	Ratio: EC_{10}(m=2) to EC_{10}(m=1)	Ratio: EC_{10}(m=3) to EC_{10}(m=1)
	Papilloma/Carcinoma	454.60	454.6 - 535.69	1.2	1.2
Harderian Gland	Adenoma	28.27	28.27 - 34.94	1.2	1.2
	Carcinoma	>403	1342.28-1412.21		
	Adenoma/Carcinoma	25.88	25.88 - 31.82	1.2	1.2
Hematopoietic System	Any Lymphoma	472.10	470.01 - 510.34	1.1	1.0
Hematopoietic System	Histiocytic Sarcoma	600.67	446.69 - 600.67	0.9	0.7
Liver	Adenoma	52.84	52.84 - 60.8	1.2	1.1
	Carcinoma	122.96	122.96 - 131.36	1.1	1.0
	Adenoma/Carcinoma	12.53	12.53 - 16.39	1.2	1.3
Lung	Adenoma	51.75	51.75 - 61.43	1.2	1.2
	Carcinoma	263.11	263.11 - 313.39	1.2	1.2
	Adenoma/Carcinoma	35.70	35.7 - 45.75	1.2	1.3
NTP 1994 and Placke et al. 1996 Combined: Male B6C3F1 Mice					
Forestomach	Papilloma	444.70	444.7 - 485.55	1.0	1.1
	Carcinoma	608.62	585.37 - 608.62	1.0	1.0
	Papilloma/Carcinoma	259.74	259.74 - 379.96	1.3	1.5
Harderian Gland	Adenoma	30.01	30.01 - 34.84	1.2	1.2
	Carcinoma	>403	1185.85-1351.33		
	Adenoma/Carcinoma	27.83	27.83 - 32.68	1.2	1.2
Liver	Adenoma	45.57	45.57 - 53.1	1.2	1.1
	Carcinoma	109.85	109.85 - 129.86	1.1	1.2
	Adenoma/Carcinoma	14.24	14.24 - 17.1	1.2	1.2
Lung	Adenoma	51.22	51.22 - 60.14	1.2	1.1
	Carcinoma	235.00	235 - 299.6	1.2	1.3
	Adenoma/Carcinoma	36.47	36.47 - 43.09	1.2	1.2
Hematopoietic System	Any Lymphoma	440.84	440.84 - 470.84	1.1	1.0
Placke et al. 1996: Female B6C3F1 Mice					
Spleen	Hemangiosarcoma	16.51	16.51 - 21.19	1.2	1.3
Harderian Gland	Adenoma	9.90	9.9 - 12.72	1.2	1.3
Pituitary Gland	Adenoma	7.68	7.68 - 9.87	1.2	1.3
Summary Statistics					
			minimum ratio	0.7	0.5
			maximum ratio	1.8	1.7
			average ratio	1.14	1.10

[1]>#, where # is the highest dose, implies that the EC_{10} or LEC_{10} is at least three times higher than the highest dose

The estimated (fitted) multistage models are used to identify the EC_{10}. The best estimates of these EC_{10}s are shown in Table 19. At the same time that the EC_{10} is calculated, a lower bound (a so-called 95% lower confidence limit) denoted by LEC_{10} on the EC_{10} is calculated. The LEC_{10} is calculated in BMDS using the "standard default" procedure that determines the fit of the multistage model with the largest slope that is not statistically detectable as a bad fit. Then, this largest slope is used to calculate the LEC_{10}. Table 19 includes both the LEC_{10} and the EC_{10} values. Table 24 shows the corresponding best estimate of the URF=$0.10/EC_{10}$ and an upper bound (95% upper confidence limit) or URF_UB =$0.10/LEC_{10}$. Table 24 shows that the URF_UB range between 1.2 and 3.6 times greater than their corresponding URFs. On average, the URF_UBs are approximately 1.8 times greater than their corresponding URFs. The results in Table 24 are almost identical for m=1, m=2, and m=3.

Table 24. Ratio of the 95% upper confidence limit on the URF and the maximum likelihood estimate of the URFs for the endpoints analyzed for three alternative numbers of stages in the tumor-formation process (ppm is environmental ppm, that is 24 hours per day, 7 days per week for a lifetime) arranged by endpoint

Organ	Severity / Study	Ratio: 95% UCL on URF to URF adjusting for 1, 2, or 3 number of stages		
		m=1	m=2	m=3
Male F344/N Rats				
Testis	Adenoma			
	NTP1994	2.0	2.2	2.4
	NTP1999	1.8	1.7	1.7
	NTP1994 & NTP1999	1.5	2.0	1.6
NTP 1999: Male F344/N Rats				
Kidney	Adenoma	1.6	1.6	1.6
	Carcinoma	n/a[1]	n/a	n/a
	Adenoma/Carcinoma	1.6	1.6	1.6
Mammary Gland	Adenoma	1.5	1.5	1.5
	Carcinoma	n/a	n/a	n/a
	Adenoma/Carcinoma	1.5	1.5	1.5
NTP 1999: Female F344/N Rats				
Mammary Gland	Adenoma	2.8	2.9	2.7
	Carcinoma	n/a	n/a	n/a
	Adenoma/Carcinoma	3.0	3.1	2.9
Male $B6C3F_1$ Mice				
Forestomach	Papilloma			
	NTP1994	1.7	1.7	1.7
	Placke1996	2.5	2.5	1.7
	NTP1994 & Placke1996	1.5	1.3	1.2
	Carcinoma			
	NTP1994	2.3	2.3	2.3
	Placke1996	1.3	1.3	1.3
	NTP1994 & Placke1996	1.7	1.4	1.3
	Papilloma/Carcinoma			
	NTP1994	1.6	1.6	1.6
	Placke1996	1.6	1.5	1.5
	NTP1994 & Placke1996	1.4	1.4	1.3
Harderian Gland	Adenoma			
	NTP1994	1.9	2.1	2.5
	Placke1996	1.2	1.2	1.2
	NTP1994 & Placke1996	1.2	1.2	1.2
	Carcinoma			
	NTP1994	3.6	3.6	3.6
	Placke1996	n/a	2.9	2.7
	NTP1994 & Placke1996	n/a	2.6	2.5
	Adenoma/Carcinoma			

Organ	Severity / Study	Ratio: 95% UCL on URF to URF adjusting for 1, 2, or 3 number of stages		
		m=1	m=2	m=3
	NTP1994	1.9	2.1	2.5
	Placke1996	1.2	1.2	1.2
	NTP1994 & Placke1996	1.2	1.2	1.2
Liver	Adenoma			
	NTP1994	1.6	1.8	2.1
	Placke1996	1.4	1.3	1.3
	NTP1994 & Placke1996	1.3	1.3	1.3
	Carcinoma			
	NTP1994	2.7	2.6	3.0
	Placke1996	1.6	1.5	1.5
	NTP1994 & Placke1996	1.5	1.4	1.6
	Adenoma/Carcinoma			
	NTP1994	1.6	1.8	2.3
	Placke1996	1.3	1.3	1.3
	NTP1994 & Placke1996	1.2	1.2	1.2
Lung	Adenoma			
	NTP1994	1.7	1.8	2.0
	Placke1996	1.3	1.3	1.3
	NTP1994 & Placke1996	1.3	1.3	1.3
	Carcinoma			
	NTP1994	1.9	1.9	2.0
	Placke1996	1.6	1.5	1.5
	NTP1994 & Placke1996	1.5	1.5	1.6
	Adenoma/Carcinoma			
	NTP1994	1.7	1.7	2.0
	Placke1996	1.3	1.3	1.3
	NTP1994 & Placke1996	1.2	1.2	1.2
Hematopoietic System	Any Lymphoma			
	NTP1994	3.1	3.3	3.5
	Placke1996	2.1	1.9	1.9
	NTP1994 & Placke1996	1.9	1.8	1.8
Placke et al. 1996: Male B6C3F$_1$ Mice				
Heart	Hemangiosarcoma	2.8	1.7	1.6
Spleen	Hemangiosarcoma	n/a	n/a	n/a
Hematopoietic System	Histiocytic Sarcoma	2.4	2.0	1.8
Placke et al. 1996: Female B6C3F$_1$ Mice				
Spleen	Hemangiosarcoma	1.8	1.8	1.8
Harderian Gland	Adenoma	2.0	2.0	2.0
Pituitary Gland	Adenoma	1.9	1.9	1.9

Organ	Severity / Study	Ratio: 95% UCL on URF to URF adjusting for 1, 2, or 3 number of stages		
		m=1	m=2	m=3
Summary Statistics				
	minimum ratio	1.2	1.2	1.2
	maximum ratio	3.6	3.6	3.6
	average ratio	1.78	1.80	1.81

[1]n/a implies that the URF and 95% UCL on the URF could not be calculated by BMDS and the ratio is undefined

9. Discussion

The highest URFs and URF_UB are for m=1 with one transition rate from a normal cell to a first-stage (tumor) cell. The maximum URFs and maximum URF_UBs for m=2 and m=3 are slightly smaller (Table 21).

In the rats, the organs with candidate responses are the kidney, mammary gland, and testis. In the mice, the organs with candidate responses are forestomach, Harderian gland, hematopoietic system, liver, lung, and pituitary gland. None of the organs with candidate responses in rats have candidate responses in the mice. Also, none of the organs with candidate responses in mice have candidate responses in the rats. That is, none of the organs have candidate responses in both species (Table 21).

The organ and response with the highest URF (0.013 per environmental ppm) and highest URF_UB (0.025 per environmental ppm) is the pituitary gland and adenoma in female mice in Placke et al. (1996). However, this response is not significant in male mice in the same study (Placke et al., 1996) or in the NTP (1994) study. The only study with female mice was Placke et al. (1996) (Table 21). Furthermore, Placke et al. (1996) does not indicate that carcinomas or adenomas/carcinomas were candidate responses – presumably because of the absence of carcinomas (or at least low frequency).

The organ and response with the second highest URF (0.010 per environmental ppm) and second highest URF_UB (0.020 per environmental ppm) is the Harderian gland and adenoma in female mice in Placke et al. (1996) (Table 21).

Among the responses that were not only adenomas, fibroadenomas, or papillomas, the organ and response with the highest URF (0.008 per environmental ppm) and highest URF_UB (0.011 per environmental ppm) is liver and adenoma/carcinoma in male mice in Placke et al. (1996) (Table 21).

Among the responses that were malignant responses (i.e., carcinoma, sarcoma, and lymphoma), the organ and response with the highest URF (0.0061 per environmental ppm) and highest URF_UB (0.0110 per environmental ppm) is spleen and hemangiosarcoma in female mice in Placke et al. (1996) (Table 21).

For rats and male mice (i.e., all animals except the female mice in Placke et al. 1996), the organ and response with the highest URF (0.008 per environmental ppm) and highest URF_UB (0.010 per environmental ppm) is liver and adenoma/carcinoma in male mice in Placke et al. (1996) (Table 21).

For rats and male mice (i.e., all animals except the female mice in Placke et al. 1996), among the responses that were malignant responses (i.e., carcinoma, sarcoma, and lymphoma), the organ and response with the highest URF (0.001 per environmental ppm) and highest URF_UB (0.003 per environmental ppm) is liver and carcinoma in male mice in NTP (1994) (Table 21).

These paragraphs all suggest that a reasonable characterization of the highest URF is approximately 0.010 per environmental ppm based on all endpoints or approximately 0.001 per environmental ppm based on malignant responses (i.e., carcinoma, sarcoma, and lymphoma) in rats and male mice (i.e., all animals except the female mice in Placke et al. 1996).

This discussion has focused on the URFs and URF_UBs calculated herein assuming that rats and mice and humans are equally sensitive when the dose is on the ppm scale. However, OEHHA used a dosimetric adjustment factor (DAF) of 1.68 for butadiene (which is frequently considered to be similar to isoprene). This would mean dividing the URFs and URF_UBs calculated herein by a factor of 1.68. Similarly, the U.S. EPA calculated a DAF for chloroprene (which is also frequently considered to be similar to isoprene) of approximately 1.7, although they used a more conservative DAF=1 in their IRIS document.

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Appendix B: Appendices for the Dose-Response Modeling and Inhalation Toxicity Factors for Isoprene Report

Appendix A. Equivalent Doses

The multistage theory of carcinogenesis assumes that the transformation of a normal cell to a specified neoplastic stage requires the occurrence of m biological events (transitions) and that these events occur in one specific order. Mathematically, if λ_i is the transition rate for a cell from the i-th stage to the i+1-th stage in an m-stage carcinogenic process (i=0, 1, 2, ..., m-1 and i=0 corresponds to the normal or background stage), then **the hazard rate $H(T_e)$** corresponding to a single cell leading to the specified response (tumor) occurring by a specified time T_e under Armitage and Doll (1954) becomes

$$H(T_e) = {}_0\int^{T}{}_e \lambda_{m-1} \times [\, {}_0\int^{t}{}_{m-1} \lambda_{m-2} \times [\, {}_0\int^{t}{}_{m-2} \lambda_{m-3} \times \ldots \times [\, {}_0\int^{t1} \lambda_0 \, dt_0 \,] \, dt_1 \ldots dt_{m-2} \, dt_{m-1} \, dt_m$$

which corresponds to an (m-1)-stage cell having to make the final transition to the m-th stage at some time $t_{(m-1)}$ between time 0 and time T_e, preceded by an (m-2)-th stage cell having to make a transition to the (m-1)-th stage at some time $t_{(m-2)}$ between time 0 and time $t_{(m-1)}$, and so forth back to a normal (0-th stage) stage cell having to make a transition to the 1-th stage at some time t_0 between time 0 and time t_1 (see also Crump and Howe, 1984, Kodell et al., 1987, or Holland and Sielken, 1993). Therefore, if there are N independent normal cell lines, the **probability of developing cancer by age T_e** is the probability of at least one of these cell lines reaching the m-th stage, that is,

$$P(T_e) = 1 - \exp[\, - N \times H(T_e) \,].$$

If λ_i linearly dependent on dose and dose is dependent on time, say d(t), but λ_i is otherwise independent of time, then

$$\lambda_i = \lambda i(t) = \alpha i + \beta_i \times d(t)$$

and

$$H(T_e) = {}_0\int^{T}{}_e \lambda_{m-1} \times {}_0\int^{t}{}_{m-1} \lambda_{m-2} \times {}_0\int^{t}{}_{m-2} \lambda_{m-3} \times \ldots \times {}_0\int^{t1} \lambda_0 \, dt_0 \, dt_1 \ldots dt_{m-2} \, dt_{m-1} \, dt_m$$

depends on which specific λ_i are time dependent and the functional form of d(t). In particular, if only λ_0 is dose dependent and

$$\begin{aligned} d(t) &= 0 \text{ for } t<a \\ &= d \text{ for } a \le t \le b \\ &= 0 \text{ for } t>b \end{aligned}$$

then, as shown in mathematical detail below, the extra risk at time T_e for this situation is equal to the extra risk at time T corresponding to the end of a normal lifetime at a constant dose D from time 0 to time T when

$$D = d \times \{\, [T_e - a]^m - [T_e - b]^m \,\} / T^m.$$

The above formula for D assumes that "d" is for 24 hours per day and 7 days per week. In order to adjust for a d that is n_{hrs} hours per day and n_{days} days per week, the formula for D becomes

$$D = d \times (n_{hrs} / 24) \times (n_{days}/ 7) \times [(T_e - a)^m – (T_e – b)^m] / T^m.$$

In this sense, the constant dose D is equivalent to the time-dependent dose d(t). In the dose-response modeling herein, we transform the intermittent experimental doses d(t) to this equivalent doses D and then do the dose-response modeling and estimate EC_{10}s in units of D (i.e., constant environmental ppm). This same equivalence is alluded to and used in both OEHHA 2004) and OEHHA (2010) and both reference Crouch (1983). Although the basis of this equivalence is only alluded to in these references, it is more clearly stated above and mathematically demonstrated in detail below. Section A.1, Section A.2, and Section A.3 correspond to m=1, 2, and 3, respectively. Section A.4 and Section A.5 contain alternative proofs corresponding to m=2 and 3, respectively. The proofs in Section A.2, and Section A.3 involve a technique involving changing the order of integration. The proofs in Section A.4 and Section A.5 are based on more straight forward integrations but are considerably more tedious. The proofs in Section A.2 and Section A.3 and the proofs in Section A.4 and Section A.5 prove the same results, respectively.

A.1 Proof for m=1

For a constant dose D, the probability of a specified response occurring by time T corresponding to the end of a nominal lifetime is

$$P(T; D) = 1 – \exp[- N \times H(T; \text{dose}=D)]$$

where

$$H(T; \text{dose}=D) = \int_0^T \lambda_0(t_0, D)\, dt_0.$$

When the multistage process consists of two stages (i.e., m=1) and one transition rate, only the transition rate for the first stage (j=1) is dose-dependent, and λ_1 is a constant independent of time and linearly related to D which is also a constant independent of time, say

$$\lambda_0(t_0, D) = \alpha_0 + \beta_0 \times D,$$

it follows from the integral for H(T; D) that

$$H(T; \text{dose}=D) = [\lambda_0(t_0, D) \times t_0 \text{ evaluated at } t_0 \text{ equal to } T] – [\lambda_0(t_0, D) \times t_0 \text{ evaluated at } t_0 \text{ equal to } 0]$$

$$= (\alpha_0 + \beta_0 \times D) \times T - (\alpha_0 + \beta_0 \times D) \times 0 = \alpha_0 \times T + \beta_0 \times D \times T$$

and

$$P(T; dose=D) = 1 - \exp\{ - N \times [\alpha_0 \times T + \beta_0 \times D \times T] \}.$$

Then the extra risk at time T is

$$[P(T; dose=D) - P(T; dose=0)] / [1 - P(T; dose=0)] =$$
$$\{ [1 - \exp(- N \times [\alpha_0 \times T + \beta_0 \times D \times T])] - [1 - \exp(- N \times \alpha_0 \times T)] \} /$$
$$\{ 1 - [1 - \exp(- N \times \alpha_0 \times T)] \}$$

$$= \{ [- \exp(- N \times [\alpha_0 \times T + \beta_0 \times D \times T])] - [- \exp(- N \times \alpha_0 \times T)] \} /$$
$$\{ \exp(- N \times \alpha_0 \times T)] \}$$

$$= \{ \exp(- N \times \alpha_0 \times T)] - \exp(- N \times [\alpha_0 \times T + \beta_0 \times D \times T])] /\{ \exp(- N \times \alpha_0 \times T) \}$$

$= 1 - \exp(- N \times [\beta_0 \times D \times T]).$

Similarly, if the dose is not a constant dose D but rather a time-dependent dose

$d(t) = 0$ for $t<a$
$= d$ for $a \le t \le b$
$= 0$ for $t>b$

and

$$\lambda_0[t_0, d(t)] = \alpha_1 + \beta_1 \times d(t),$$

then the probability of a specified response occurring by time T_e corresponding to the observation time (necropsy time) in the experiment is

$$P[T_e; d(t)] = 1 - \exp\{ - N \times H[T_e; dose=d(t)] \}$$

where

$$H[T_e; dose=d(t)] = {}_0\int^{T}_{e} \lambda_0[t_0, d(t_0)] \, dt_0.$$

It follows from the integral for $H[T_e; dose=d(t)]$ that with both a and b $\le T_e$

$$H[T_e; dose=d(t)] = {}_0\int^{T} [\alpha_0 + \beta_0 \times d(t_0)] \, dt_0$$

$$= {}_0\int^{a} [\alpha_0 + \beta_0 \times 0] \, dt_0 + {}_a\int^{b} [\alpha_0 + \beta_0 \times d] \, dt_0 + + {}_b\int^{T}_{e} [\alpha_0 + \beta_0 \times 0] \, dt_0$$

$$= {}_0\int^{T}_{e} [\alpha_0] \, dt_0 + {}_a\int^{b} [\beta_0 \times d] \, dt_0$$

$$= \alpha_0 \times T_e + [\beta_0 \times d] \times (b-a).$$

Therefore,

$$P[T_e; d(t)] = 1 - \exp\{ - N \times [\alpha_0 \times T_e + (\beta_0 \times d)] \times (b-a)] \},$$

and the extra risk at time T_e is

$$\{ P[T_e; \text{dose}=d(t)] - P(T_e; \text{dose}=0) \} / [1 - P(T_e; \text{dose}=0)]$$

$$= \{ [1 - \exp(- N \times [\alpha_0 \times T_e + (\beta_0 \times d)] \times (b-a)])] - [1 - \exp(- N \times \alpha_0 \times T_e)] \} / \{ 1 - [1 - \exp(- N \times \alpha_0 \times T_e)] \}$$

$$= \{ - \exp(- N \times [\alpha_0 \times T_e + (\beta_0 \times d)] \times (b-a)]) + \exp(- N \times \alpha_0 \times T_e) \} / \exp(- N \times \alpha_0 \times T_e)$$

$$= 1 - \exp[- N \times (\beta_0 \times d) \times (b-a)].$$

Now in order for the extra risk at the end T of a nominal lifetime at a constant dose D, namely

$$1 - \exp(- N \times \beta_0 \times D \times T),$$

to be equal to the extra risk at time T_e corresponding to the observation time (necropsy time) in the experiment with a time-dependent dose d(t), namely

$$1 - \exp[- N \times \beta_0 \times d \times (b-a)] ,$$

it must be true that

$$[D \times T] = [d \times (b-a)]$$

or, equivalently,

$$D = d \times (b-a) / T.$$

For m=1, this is equivalent to

$$D = d \times \{ (T_e-a)^1 - (T_e-b)^1 \} / T^1 = d \times \{ (T_e-a)^m - (T_e-b)^m \} / T^m.$$

A.2 Proof for m=2

If the multistage process consists of three stages (i.e., m=2) and two transition rates, then, for a constant dose D, the probability of a specified response occurring by time T corresponding to the end of a nominal lifetime is

$$P(T; D) = 1 - \exp[- N \times H(T; dose=D)]$$

where

$$H(T; dose=D) = {}_0\int^T \lambda_1(t_0, D) \times [{}_0\int^{t}_1 \lambda_0(t_0, D)\, dt_0]\, dt_1$$

When the transition rate λ_1 for the transition from the first stage to the second stage is independent of both time and dose, and the transition rate λ_0 for the transition from the normal stage (0-th stage) to the first stage is a constant independent of time and linearly related to D which is also a constant independent of time, say

$$\lambda_0(t_0, D) = \alpha_0 + \beta_0 \times D,$$

it follows from the integral for H(T; D) that

$$H(T; dose=D) = {}_0\int^T \lambda_1 \times [{}_0\int^{t}_1 (\alpha_0 + \beta_0 \times D)\, dt_0]\, dt_1$$

$$= \lambda_1 \times {}_0\int^T [{}_0\int^{t}_1 (\alpha_0 + \beta_0 \times D)\, dt_0]\, dt_1$$
$$= \lambda_1 \times {}_0\int^T (\alpha_0 + \beta_0 \times D) \times t_1\, dt_1$$
$$= \lambda_1 \times (\alpha_0 + \beta_0 \times D) \times {}_0\int^T t_1\, dt_1$$
$$= \lambda_1 \times (\alpha_0 + \beta_0 \times D) \times T^2/2$$

and

$$P(T; dose=D) = 1 - \exp[- N \times \lambda_1 \times (\alpha_0 + \beta_0 \times D) \times T^2/2] \}.$$

Then the extra risk at time T is

$$[P(T; dose=D) - P(T; dose=0)] / [1 - P(T; dose=0)] =$$
$$\{ [1-\exp(-N \times \lambda_1 \times (\alpha_0 + \beta_0 \times D) \times T^2/2)] - [1-\exp(-N \times \lambda_1 \times \alpha_0 \times T^2/2)] \} /$$
$$\{ 1 - [1 - \exp(- N \times \lambda_1 \times \alpha_1 \times T^2/2)] \}$$

$$= \{ [- \exp(- N \times \lambda_1 \times \{\alpha_0 + \beta_0 \times D) \times T^2/2 \})] - [- \exp(- N \times \lambda_1 \times \alpha_0 \times T^2/2)] \} /$$
$$\{ \exp(- N \times \lambda_1 \times \alpha_0 \times T^2/2)] \}$$

$$= \{ \exp(- N \times \lambda_1 \times \alpha_0 \times T^2/2)] - \exp(- N \times [\lambda_1 \times \alpha_0 \times T^2/2 + \beta_0 \times D \times T^2/2])]$$
$$/\{ \exp(- N \times \lambda_1 \times \alpha_0 \times T^2/2) \}$$

$$= 1 - \exp(- N \times \lambda_1 \times \beta_0 \times D \times T^2/2).$$

Similarly, if the dose is not a constant dose D but rather a time-dependent dose

$d(t) = 0$ for $t<a$
$= d$ for $a \le t \le b$
$= 0$ for $t>b$

and

$$\lambda_0[t_0, d(t_0)] = \alpha_0 + \beta_0 \times d(t_0),$$

then the probability of a specified response occurring by time T_e corresponding to the observation time (necropsy time) in the experiment is

$$P[T_e; d(t)] = 1 - \exp\{ - N \times H[T_e; \text{dose}=d(t)] \}$$

where

$$H[T_e; \text{dose}=d(t)] = {}_0\int^{T_e} \lambda_1 \times [{}_0\int^{t_1} \lambda_0[t_0, d(t_0)] dt_0 \, dt_1$$

It follows from the integral for $H[T_e; \text{dose}=d(t)]$ that with both $a \le T_e$ and $b \le T_e$

$$H[T_e; \text{dose}=d(t)] = \lambda_1 \times {}_0\int^{T_e} [{}_0\int^{t_1} [\alpha_0 + \beta_0 \times d(t_0)] dt_0] dt_1$$

$$= \lambda_1 \times {}_0\int^{T_e} [{}_0\int^{t_1} [\alpha_0] dt_0] dt_1 + \lambda_1 \times {}_0\int^{T_e} [{}_0\int^{t_1} [\beta_0 \times d(t_0)] dt_0] dt_1$$

$$= \lambda_1 \times \alpha_0 \times {}_0\int^{T_e} t_1 \, dt_1 + \lambda_1 \times \beta_0 \times {}_0\int^{T_e} [{}_0\int^{t_1} [d(t_0)] dt_0] dt_1$$

$$= \lambda_1 \times \alpha_0 \times T_e^2/2 + \lambda_1 \times \beta_0 \times {}_0\int^{T_e} [{}_0\int^{t_1} [d(t_0)] dt_0] dt_1$$

$$= \lambda_1 \times \alpha_0 \times T_e^2/2 + \lambda_1 \times \beta_0 \times d \times \{ (T_e - a)^2 - (T_e - b)^2 \}/2$$

since

$${}_0\int^{T_e} [{}_0\int^{t_1} [d(t_0)] dt_0] dt_1 = {}_0\int^{T_e} [{}_{t0}\int^{T_e} [d(t_0)] dt_1] dt_0$$

$$= {}_0\int^{T_e} [d(t_0)] [{}_{t0}\int^{T_e} dt_1] dt_0$$

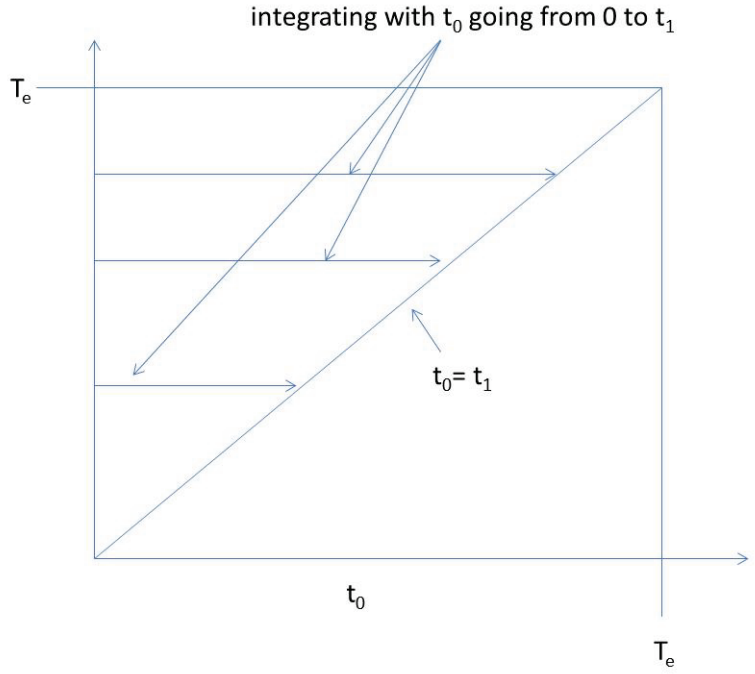
$$= {}_0\int^{T_e} [d(t_0)] [T_e - t_0] dt_0$$

$$= d \times {}_a\int^{b} [T_e - t_0] dt_0$$

$$= d \times \{ (T_e - a)^2 - (T_e - b)^2 \}/2$$

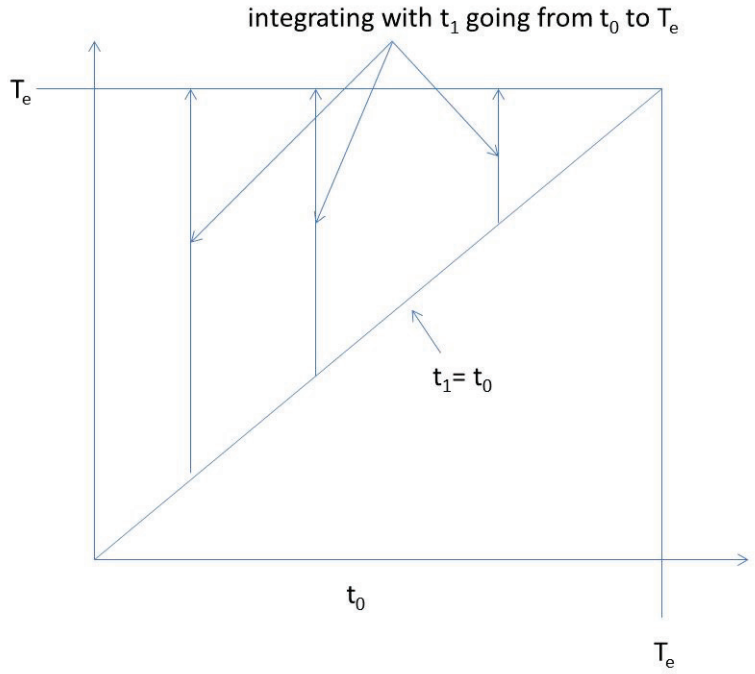
after the order of integration has been changed. Pictorially, changing the order of integration goes from

Integrating over t_0 first and then integrating over t_1 second, implies



to

Integrating over t_1 first and then integrating over t_0 second, implies



Therefore,

$$H[T_e; dose=d(t)] = \lambda_1 \times \alpha_0 \times T_e^2/2 + \lambda_1 \times \beta_0 \times d \times \{ (T_e - a)^2 - (T_e - b)^2 \}/2$$

and

$$P[T_e; d(t)] = 1 - \exp\{- N \times [\lambda_1 \times \alpha_0 \times T_e^2/2 + \lambda_1 \times \beta_0 \times d \times \{ (T_e - a)^2 - (T_e - b)^2 \}/2\}]$$

and the extra risk at time T_e is

$$\{ P[T_e; dose=d(t)] - P(T_e; dose=0) \} / [1 - P(T_e; dose=0)]$$

$$= \{ [1-\exp(-N \times [\lambda_1 \times \alpha_0 \times T_e^2/2 + \lambda_1 \times \beta_0 \times d \times (b-a) \times \{(T_e-a)^2 - (T_e-b)^2 \}/2\}]) - [1 - \exp(- N \times \lambda_1 \times \alpha_0 \times T_e^2/2)] \} / \{ 1 - [1 - \exp(- N \times \lambda_1 \times \alpha_0 \times T_e^2/2)] \}$$

$$= \{ - \exp(- N \times [\lambda_1 \times \alpha_0 \times T_e^2/2 + \lambda_1 \times \beta_0 \times d \times \{(T_e-a)^2 - (T_e-b)^2 \}/2\}]) + \exp(- N \times \lambda_1 \times \alpha_0 \times T_e^2/2) \} / \exp(- N \times \lambda_1 \times \alpha_0 \times T_e^2/2)$$

$$= 1 - \exp\{ - N \times \lambda_1 \times \beta_0 \times d \times [(T_e-a)^2 - (T_e-b)^2]/2] \}$$

Now in order for the extra risk at the end T of a nominal lifetime at a constant dose D, namely

$$1 - \exp(- N \times \lambda_1 \times \beta_0 \times D \times T^2/2)$$

to be equal to the extra risk at time T_e corresponding to the observation time (necropsy time) in the experiment with a time-dependent dose d(t), namely

$$1 - \exp\{ - N \times \lambda_1 \times \beta_0 \times d \times [(T_e-a)^2 - (T_e-b)^2]/2] \},$$

it must be true that

$$[D \times T^2/2] = [d \times [(T_e-a)^2 - (T_e-b)^2 \}/2]$$

or, equivalently,

$$D = d \times [(T_e-a)^2 - (T_e-b)^2] / T^2$$

and , for m=2, this is equivalent to

$$D = d \times [(T_e-a)^m - (T_e-b)^m] / T^m.$$

A.3 Proof for m=3

If the multistage process consists of four stages (i.e., m=3) and three transition states, then, for a constant dose D, the probability of a specified response occurring by time T corresponding to the end of a nominal lifetime is

$$P(T; D) = 1 - \exp[- N \times H(T; dose=D)]$$

where

$$H(T; dose=D) = {}_0\int^T \lambda_2(t_2, D) \times [{}_0\int^{t_2} \lambda_1(t_1, D) \times [{}_0\int^{t_1} \lambda_0(t_0, D)\, dt_0]\, dt_1]\, dt_2$$

When the transition rates λ_2 and λ_1 for the transitions from the second stage to the third stage and from the first stage to the second stage, respectively, are independent of both time and dose, and the transition rate λ_0 for the transition from the normal stage (0-th stage) to the first stage is a constant independent of time and linearly related to D which is also a constant independent of time, say

$$\lambda_0(t_0, D) = \alpha_0 + \beta_0 \times D,$$

it follows from the integral for H(T; D) that

$$H(T; dose=D) = {}_0\int^T \lambda_2 \times [{}_0\int^{t_2} \lambda_1 \times [{}_0\int^{t_1} (\alpha_0 + \beta_0 \times D)\, dt_0]\, dt_1]\, dt_2$$

$$= \lambda_2 \times \lambda_1 \times {}_0\int^T {}_0\int^{t_2} [{}_0\int^{t_1} (\alpha_0 + \beta_0 \times D)\, dt_0\, dt_1\, dt_2$$
$$= \lambda_2 \times \lambda_1 \times {}_0\int^T {}_0\int^{t_2} (\alpha_0 + \beta_0 \times D) \times t_1\, dt_1\, dt_2$$
$$= \lambda_2 \times \lambda_1 \times (\alpha_0 + \beta_0 \times D) \times {}_0\int^T {}_0\int^{t_2} t_1\, dt_1\, dt_2$$
$$= \lambda_2 \times \lambda_1 \times (\alpha_0 + \beta_0 \times D) \times {}_0\int^T t_2^2/2$$

$$= \lambda_2 \times \lambda_1 \times (\alpha_0 + \beta_0 \times D) \times T^3/6$$

and

$$P(T; dose=D) = 1 - \exp[- N \times \lambda_2 \times \lambda_1 \times (\alpha_0 + \beta_0 \times D) \times T^3/6] \}.$$

Then the extra risk at time T is

$$[P(T; dose=D) - P(T; dose=0)] / [1 - P(T; dose=0)] =$$
$$\{ [1-\exp(-N \times \lambda_2 \times \lambda_1 \times (\alpha_0 + \beta_0 \times D) \times T^3/6)]$$
$$- [1-\exp(-N \times \lambda_2 \times \lambda_1 \times \alpha_0 \times T^3/6)] \} /$$
$$\{ 1 - [1 - \exp(- N \times \lambda_2 \times \lambda_1 \times \alpha_0 \times T^3/6)] \}$$

$$= \{ [- \exp(- N \times \lambda_2 \times \lambda_1 \times (\alpha_0 + \beta_0 \times D) \times T^3/6\})]$$
$$- [- \exp(- N \times \lambda_2 \times \lambda_1 \times \alpha_0 \times T^3/6)] \} /$$
$$\{ \exp(- N \times \lambda_2 \times \lambda_1 \times \alpha_0 \times T^3/6)] \}$$

$$= \{ \exp(- N \times \lambda_2 \times \lambda_1 \times \alpha_0 \times T^3/6)]$$
$$- \exp(- N \times \lambda_2 \times \lambda_1 \times [\alpha_0 \times T^3/6 + \beta_0 \times D \times T^3/6])]$$
$$/\{ \exp(- N \times \lambda_2 \times \lambda_1 \times \alpha_0 \times T^3/6) \}$$

$$\mathbf{= 1 - \exp(- N \times \lambda_2 \times \lambda_1 \times \beta_0 \times D \times T^3/6).}$$

Similarly, if the dose is not a constant dose D but rather a time-dependent dose

$d(t)$ = 0 for $t<a$
= d for $a \le t \le b$
= 0 for $t>b$

and

$$\lambda_0[t_0, d(t_0)] = \alpha_0 + \beta_0 \times d(t_0),$$

then the probability of a specified response occurring by time T_e corresponding to the observation time (necropsy time) in the experiment is

$$P[T_e; d(t)] = 1 - \exp\{ - N \times H[T_e; \text{dose}=d(t)] \}$$

where

$$H[T_e; \text{dose}=d(t)] = {}_0\int^{T_e} \lambda_2 \times {}_0\int^{t_2} \lambda_1 \times \{ {}_0\int^{t_1} \lambda_0[t_0, d(t_0)]\, dt_0 \}\, dt_1\, dt_2$$

It follows from the integral for $H[T_e; \text{dose}=d(t)]$ that with both $a \le T_e$ and $b \le T_e$

$$H[T_e; \text{dose}=d(t)] = \lambda_2 \times \lambda_1 \times {}_0\int^{T_e} {}_0\int^{t_2} {}_0\int^{t_1} [\alpha_0 + \beta_0 \times d(t_0)]\, dt_0\, dt_1\, dt_2$$

$$= \lambda_2 \times \lambda_1 \times {}_0\int^{T_e} {}_0\int^{t_2} {}_0\int^{t_1} [\alpha_0]\, dt_0\, dt_1\, dt_2 + \lambda_1 \times {}_0\int^{T_e} {}_0\int^{t_1} [\beta_0 \times d(t_0)]\, dt_0\, dt_1\, dt_2$$

$$= \lambda_2 \times \lambda_1 \times \alpha_0 \times {}_0\int^{T_e} {}_0\int^{t_2} {}_0\int^{t_1} dt_0\, dt_1\, dt_2 + \lambda_2 \times \lambda_1 \times \beta_0 \times {}_0\int^{T_e} {}_0\int^{t_2} {}_0\int^{t_1} [d(t_0)]\, dt_0\, dt_1\, dt_2$$

$$= \lambda_2 \times \lambda_1 \times \alpha_0 \times T_e^3/6 + \lambda_2 \times \lambda_1 \times \beta_0 \times {}_0\int^{T_e} {}_0\int^{t_2} {}_0\int^{t_1} [d(t_0)]\, dt_0\, dt_1\, dt_2.$$

As in the proof for m=2, changing the order of integration implies

$$H[T_e; \text{dose}=d(t)]$$

$$= \lambda_2 \times \lambda_1 \times \alpha_0 \times T_e^3/6 + \lambda_2 \times \lambda_1 \times \beta_0 \times {}_0\int^{T_e} {}_0\int^{t_2} {}_{t0}\int^{t_2} [d(t_0)]\, dt_1\, dt_0\, dt_2$$

$$= \lambda_2 \times \lambda_1 \times \alpha_0 \times T_e^3/6 + \lambda_2 \times \lambda_1 \times \beta_0 \times {}_0\int^{T_e} {}_0\int^{t_2} [d(t_0)]\, {}_{t0}\int^{t_2} dt_1\, dt_0\, dt_2$$

$$= \lambda_2 \times \lambda_1 \times \alpha_0 \times T_e^3/6 + \lambda_2 \times \lambda_1 \times \beta_0 \times {}_0\int^{T_e} {}_0\int^{t_2} [d(t_0)] \times [t_2 - t_0]\, dt_0\, dt_2$$

$$= \lambda_2 \times \lambda_1 \times \alpha_0 \times T_e^3/6 + \lambda_2 \times \lambda_1 \times \beta_0 \times \int_0^{T_e} [d(t_0)] \int_{t0}^{T_e} [t_2 - t_0]\, dt_2\, dt_0$$

$$= \lambda_2 \times \lambda_1 \times \alpha_0 \times T_e^3/6 + \lambda_2 \times \lambda_1 \times \beta_0 \times \int_0^{T_e} [d(t_0)] \times (T_e - t_0)^2/2\, dt_0$$

$$= \lambda_2 \times \lambda_1 \times \alpha_0 \times T_e^3/6 + \lambda_2 \times \lambda_1 \times \beta_0 \times \int_a^b d \times (T_e - t_0)^2/2\, dt_0$$

$$= \lambda_2 \times \lambda_1 \times \alpha_0 \times T_e^3/6 + \lambda_2 \times \lambda_1 \times \beta_0 \times d \times [\,(T_e - a)^3 - (T_e - b)^3\,]/6.$$

Therefore,

$$H[T_e;\ dose=d(t)] = \lambda_2 \times \lambda_1 \times \alpha_0 \times T_e^3/6 + \lambda_2 \times \lambda_1 \times \beta_0 \times d \times [\,(T_e - a)^3 - (T_e - b)^3\,]/6$$

and

$$P[T_e;\ d(t)]$$

$$= 1 - \exp(-N \times \{\lambda_2 \times \lambda_1 \times \alpha_0 \times T_e^3/6 + \lambda_2 \times \lambda_1 \times \beta_0 \times d \times [(T_e - a)^3 - (T_e - b)^3\,]/6] \}\,)$$

and the extra risk at time T_e is

$$\{ P[T_e;\ dose=d(t)] - P(T_e;\ dose=0) \} / [1 - P(T_e;\ dose=0)]$$

$$= \{ [1 - \exp(-N \times \{\lambda_2 \times \lambda_1 \times \alpha_0 \times T_e^3/6 + \lambda_2 \times \lambda_1 \times \beta_0 \times d \times [(T_e - a)^3 - (T_e - b)^3\,]/6] \}\,) - [1 - \exp(-N \times \lambda_2 \times \lambda_1 \times \alpha_0 \times T_e^3/6\,)\} / \{ 1 - [1 - \exp(-N \times \lambda_2 \times \lambda_1 \times \alpha_0 \times T_e^3/6)\,] \}$$

$$= \{ -\exp(-N \times \{\lambda_2 \times \lambda_1 \times \alpha_0 \times T_e^3/6 + \lambda_2 \times \lambda_1 \times \beta_0 \times d \times [(T_e - a)^3 - (T_e - b)^3\,]/6] \}\,) + \exp(-N \times \lambda_2 \times \lambda_1 \times \alpha_0 \times T_e^3/6)\} / \exp(-N \times \lambda_2 \times \lambda_1 \times \alpha_0 \times T_e^3/6)$$

$$\mathbf{= 1 - \exp\{ - N \times \lambda_2 \times \lambda_1 \times \beta_0 \times d \times [(T_e - a)^3 - (T_e - b)^3\,]/6] \}}$$

Now in order for the extra risk at the end T of a nominal lifetime at a constant dose D, namely

$$\mathbf{1 - \exp(-N \times \lambda_2 \times \lambda_1 \times \beta_0 \times D \times T^3/6)}$$

to be equal to the extra risk at time T_e corresponding to the observation time (necropsy time) in the experiment with a time-dependent dose d(t), namely

$$\mathbf{1 - \exp\{ - N \times \lambda_2 \times \lambda_1 \times \beta_0 \times d \times [(T_e - a)^3 - (T_e - b)^3\,]/6] \}}$$

it must be true that

$$[D \times T^3/6] = [\, d \times [(T_e - a)^3 - (T_e - b)^3\,\}/6\,]$$

or, equivalently,

$$D = d \times [(T_e - a)^3 - (T_e - b)^3] / T^3$$

and , for m=3, this is equivalent to

$$D = d \times [(T_e - a)^m - (T_e - b)^m] / T^m.$$

A.4 Alternative Proof for m=2

If the multistage process consists of three stages (i.e., m=2) and two transition rates, then, for a constant dose D, the probability of a specified response occurring by time T corresponding to the end of a nominal lifetime is

$$P(T; D) = 1 - \exp[- N \times H(T; dose=D)]$$

where

$$H(T; dose=D) = {}_0\int^T \lambda_1(t_0, D) \times [{}_0\int^{t}_1 \lambda_0(t_0, D)\, dt_0]\, dt_1$$

When the transition rate λ_1 for the transition from the first stage to the second stage is independent of both time and dose, and the transition rate λ_0 for the transition from the normal stage (0-th stage) to the first stage is a constant independent of time and linearly related to D which is also a constant independent of time, say

$$\lambda_0(t_0, D) = \alpha_0 + \beta_0 \times D,$$

it follows from the integral for H(T; D) that

$$H(T; dose=D) = {}_0\int^T \lambda_1 \times [{}_0\int^{t}_1 (\alpha_0 + \beta_0 \times D)\, dt_0]\, dt_1$$

$$= \lambda_1 \times {}_0\int^T [{}_0\int^{t}_1 (\alpha_0 + \beta_0 \times D)\, dt_0]\, dt_1$$
$$= \lambda_1 \times {}_0\int^T (\alpha_0 + \beta_0 \times D) \times t_1\, dt_1$$
$$= \lambda_1 \times (\alpha_0 + \beta_0 \times D) \times {}_0\int^T t_1\, dt_1$$
$$= \lambda_1 \times (\alpha_0 + \beta_0 \times D) \times T^2/2$$

and

$$P(T; dose=D) = 1 - \exp[- N \times \lambda_1 \times (\alpha_0 + \beta_0 \times D) \times T^2/2] \}.$$

Then the extra risk at time T is

$$[P(T; dose=D) - P(T; dose=0)] / [1 - P(T; dose=0)] =$$
$$\{ [1-\exp(-N \times \lambda_1 \times (\alpha_0 + \beta_0 \times D) \times T^2/2)] - [1-\exp(-N \times \lambda_1 \times \alpha_0 \times T^2/2)] \} /$$
$$\{ 1 - [1 - \exp(- N \times \lambda_1 \times \alpha_1 \times T^2/2)] \}$$

$$= \{ [- \exp(- N \times \lambda_1 \times \{\alpha_0 + \beta_0 \times D) \times T^2/2 \})] - [- \exp(- N \times \lambda_1 \times \alpha_0 \times T^2/2)] \} /$$
$$\{ \exp(- N \times \lambda_1 \times \alpha_0 \times T^2/2)] \}$$

$$= \{ \exp(- N \times \lambda_1 \times \alpha_0 \times T^2/2)] - \exp(- N \times [\lambda_1 \times \alpha_0 \times T^2/2 + \beta_0 \times D \times T^2/2])]$$
$$/\{ \exp(- N \times \lambda_1 \times \alpha_0 \times T^2/2) \}$$

$$= 1 - \exp(- N \times \lambda_1 \times \beta_0 \times D \times T^2/2).$$

Similarly, if the dose is not a constant dose D but rather a time-dependent dose

$d(t) = 0$ for $t<a$
$= d$ for $a \le t \le b$
$= 0$ for $t>b$

and

$$\lambda_0[t_0, d(t_0)] = \alpha_0 + \beta_0 \times d(t_0),$$

then the probability of a specified response occurring by time T_e corresponding to the observation time (necropsy time) in the experiment is

$$P[T_e; d(t)] = 1 - \exp\{ - N \times H[T_e; \text{dose}=d(t)] \}$$

where

$$H[T_e; \text{dose}=d(t)] = {}_0\int^{T}{}_e \lambda_1 \times [{}_0\int^{t}{}_1 \lambda_0[t_0, d(t_0)] dt_0 \, dt_1$$

It follows from the integral for $H[T_e; \text{dose}=d(t)]$ that with both $a \le T_e$ and $b \le T_e$

$$H[T_e; \text{dose}=d(t)] = \lambda_1 \times {}_0\int^{T}{}_e [{}_0\int^{t}{}_1 [\alpha_0 + \beta_0 \times d(t_0)] dt_0] dt_1$$

$$= \lambda_1 \times {}_0\int^{T}{}_e [{}_0\int^{t}{}_1 [\alpha_0] dt_0] dt_1 + \lambda_1 \times {}_0\int^{T}{}_e [{}_0\int^{t}{}_1 [\beta_0 \times d(t_0)] dt_0] dt_1$$

$$= \lambda_1 \times \alpha_0 \times {}_0\int^{T}{}_e [{}_0\int^{t}{}_1 dt_0] dt_1 + \lambda_1 \times \beta_0 \times {}_0\int^{T}{}_e [{}_0\int^{t}{}_1 [d(t_0)] dt_0] dt_1$$

$$= \lambda_1 \times \alpha_0 \times T_e^2/2 + \lambda_1 \times \beta_0 \times {}_0\int^{T}{}_e [{}_0\int^{t}{}_1 [d(t_0)] dt_0] dt_1$$

$$= \lambda_1 \times \alpha_0 \times T_e^2/2 + \lambda_1 \times \beta_0 \times$$

$$\{ {}_0\int^{a} [{}_0\int^{t}{}_1 [0] dt_0] dt_1 + {}_a\int^{b} [{}_a\int^{t}{}_1 [d] dt_0] dt_1 + {}_b\int^{T}{}_e [{}_a\int^{b} [d] dt_0] dt_1 \}$$

because

if $0 \le t_1 \le a$ and $t_0 \le t_1$, then $t_0 \le a$ and $d(t_0) = 0$,
if $a \le t_1 \le b$ and $t_0 \le t_1$ and $t_0 \le a$, then $d(t_0) = 0$,
if $a \le t_1 \le b$ and $t_0 \le t_1$ and $a \le t_0 \le t_1$, then $d(t_0) = d$,
if $t_1 > b$ and $t_0 \le t_1$ and $t_0 \le a$, then $d(t_0) = 0$,
if $t_1 > b$ and $t_0 \le t_1$ and $a \le t_0 \le b$, then $d(t_0) = d$,
if $t_1 > b$ and $t_0 \le t_1$ and $t_0 > b$, then $d(t_0) = 0$.

Therefore,

$$H[T_e; \text{dose}=d(t)] = \lambda_1 \times \alpha_0 \times T_e^2/2 + \lambda_1 \times \beta_0 \times \{ {}_a\int^{b} {}_a\int^{t}{}_1 [d] dt_0 \, dt_1 + {}_b\int^{T}{}_e {}_a\int^{b} [d] dt_0 \, dt_1 \}$$

$$= \lambda_1 \times \alpha_0 \times T_e^2/2 + \lambda_1 \times \beta_0 \times [\{ \int_a^b [d] \times (t_1\text{-}a)\, dt_1 \} + \int_b^{T_e} [d] \times (b\text{-}a)\, dt_1]$$

$$= \lambda_1 \times \alpha_0 \times T_e^2/2 + \lambda_1 \times \beta_0 \times [[d] \times (t_1\text{-}a)^2/2 \text{ evaluated at } t_1 = b - [d] \times (t_1\text{-}a)^2/2 \text{ evaluated at } t_1 = a] + [d] \times (b\text{-}a) \times (T_e - b) \}$$

$$= \lambda_1 \times \alpha_0 \times T_e^2/2 + \lambda_1 \times \beta_0 \times [[d] \times (b\text{-}a)^2/2 - 0] + [d] \times (b\text{-}a) \times (T_e - b) \}$$

$$= \lambda_1 \times \alpha_0 \times T_e^2/2 + \lambda_1 \times \beta_0 \times d \times (b\text{-}a) \times \{ (b\text{-}a)/2 + (T_e - b) \}$$

Therefore,

$$P[T_e; d(t)]$$

$$= 1 - \exp\{- N \times [\lambda_1 \times \alpha_0 \times T_e^2/2 + \lambda_1 \times \beta_0 \times d \times (b\text{-}a) \times \{ (b\text{-}a)/2 + (T_e - b) \}] \},$$

and the extra risk at time T_e is

$$\{ P[T_e; \text{dose}=d(t)] - P(T_e; \text{dose}=0) \} / [1 - P(T_e; \text{dose}=0)]$$

$$= \{ [1 - \exp(-N \times [\lambda_1 \times \alpha_0 \times T_e^2/2 + \lambda_1 \times \beta_0 \times d \times (b\text{-}a) \times \{ (b\text{-}a)/2 + (T_e - b) \}]) - [1 - \exp(- N \times \lambda_1 \times \alpha_0 \times T_e^2/2)] \} / \{ 1 - [1 - \exp(- N \times \lambda_1 \times \alpha_0 \times T_e^2/2)] \}$$

$$= \{ - \exp(- N \times [\lambda_1 \times \alpha_0 \times T_e^2/2 + \lambda_1 \times \beta_0 \times d \times (b\text{-}a) \times \{ (b\text{-}a)/2 + (T_e - b) \}]) + \exp(- N \times \lambda_1 \times \alpha_0 \times T_e^2/2) \} / \exp(- N \times \lambda_1 \times \alpha_0 \times T_e^2/2)$$

$$\mathbf{= 1 - \exp\{ - N \times \lambda_1 \times \beta_0 \times d \times (b\text{-}a) \times [(b\text{-}a)/2 + (T_e - b)] \}}$$

Now in order for the extra risk at the end T of a nominal lifetime at a constant dose D, namely

$$\mathbf{1 - \exp(- N \times \lambda_1 \times \beta_0 \times D \times T^2/2)}$$

to be equal to the extra risk at time T_e corresponding to the observation time (necropsy time) in the experiment with a time-dependent dose d(t), namely

$$\mathbf{1 - \exp\{ - N \times \lambda_1 \times \beta_0 \times d \times (b\text{-}a) \times [(b\text{-}a)/2 + (T_e - b)] \}},$$

it must be true that

$$[D \times T^2/2] = [d \times (b\text{-}a) \times \{ (b\text{-}a)/2 + (T_e - b) \}]$$

or, equivalently,

$$D = d \times [\, 2 \times (b\text{-}a) \times \{\, (b\text{-}a)/2 + (T_e - b) \,\} \,] / T^2$$

$$= d \times [\, (b\text{-}a) \times \{\, (b\text{-}a) + 2 \times (T_e - b) \,\} \,] / T^2$$

$$= d \times [\, (b\text{-}a) \times \{\, (b\text{-}a) + 2 \times T_e - 2 \times b) \,\} \,] / T^2$$

$$= d \times [\, (b\text{-}a) \times \{\, (2 \times T_e - a - b) \,\} \,] / T^2$$

For m=2, this is equivalent to

$$D = d \times \{\, (T_e\text{-}a)^m - (T_e\text{-}b)^m \,\} / T^m = d \times [\, (T_e\text{-}a)^2 - (T_e\text{-}b)^2 \,] / T^2.$$

because

$$(T_e\text{-}a)^2 - (T_e\text{-}b)^2 = (T_e^2 - 2 \times T_e \times a + a^2) - (T_e^2 - 2 \times T_e \times b + b^2)$$

$$= -2 \times T_e \times a + a^2 + 2 \times T_e \times b - b^2$$

$$= (a^2 - b^2) + 2 \times T_e \times (b\text{-}a)$$

$$= (a - b) \times (a + b) + 2 \times T_e \times (b\text{-}a)$$

$$= (b\text{-}a) \times \{\, - (a + b) + 2 \times T_e \,\}$$

$$= (b\text{-}a) \times \{\, 2 \times T_e - a - b \,\}.$$

A.5 Alternative Proof for m=3

If the multistage process consists of four stages (i.e., m=3) and three transition states, then, for a constant dose D, the probability of a specified response occurring by time T corresponding to the end of a nominal lifetime is

$$P(T; D) = 1 - \exp[- N \times H(T; dose=D)]$$

where

$$H(T; dose=D) = {}_0\int^T \lambda_2(t_2, D) \times [{}_0\int^{t_2} \lambda_1(t_1, D) \times [{}_0\int^{t_1} \lambda_0(t_0, D)\, dt_0]\, dt_1]\, dt_2$$

When the transition rates λ_2 and λ_1 for the transitions from the second stage to the third stage and from the first stage to the second stage, respectively, are independent of both time and dose, and the transition rate λ_0 for the transition from the normal stage (0-th stage) to the first stage is a constant independent of time and linearly related to D which is also a constant independent of time, say

$$\lambda_0(t_0, D) = \alpha_0 + \beta_0 \times D,$$

it follows from the integral for H(T; D) that

$$H(T; dose=D) = {}_0\int^T \lambda_2 \times [{}_0\int^{t_2} \lambda_1 \times [{}_0\int^{t_1} (\alpha_0 + \beta_0 \times D)\, dt_0]\, dt_1]\, dt_2$$

$$= \lambda_2 \times \lambda_1 \times {}_0\int^T {}_0\int^{t_2} [{}_0\int^{t_1} (\alpha_0 + \beta_0 \times D)\, dt_0\, dt_1\, dt_2$$
$$= \lambda_2 \times \lambda_1 \times {}_0\int^T {}_0\int^{t_2} (\alpha_0 + \beta_0 \times D) \times t_1\, dt_1\, dt_2$$
$$= \lambda_2 \times \lambda_1 \times (\alpha_0 + \beta_0 \times D) \times {}_0\int^T {}_0\int^{t_2} t_1\, dt_1\, dt_2$$
$$= \lambda_2 \times \lambda_1 \times (\alpha_0 + \beta_0 \times D) \times {}_0\int^T t_2^2/2$$
$$= \lambda_2 \times \lambda_1 \times (\alpha_0 + \beta_0 \times D) \times T^3/6$$

and

$$P(T; dose=D) = 1 - \exp[- N \times \lambda_2 \times \lambda_1 \times (\alpha_0 + \beta_0 \times D) \times T^3/6] \}.$$

Then the extra risk at time T for a constant dose D is

$$[P(T; dose=D) - P(T; dose=0)] / [1 - P(T; dose=0)] =$$
$$\{ [1-\exp(-N \times \lambda_2 \times \lambda_1 \times (\alpha_0 + \beta_0 \times D) \times T^3/6)]$$
$$- [1-\exp(-N \times \lambda_2 \times \lambda_1 \times \alpha_0 \times T^3/6)] \} /$$
$$\{ 1 - [1 - \exp(- N \times \lambda_2 \times \lambda_1 \times \alpha_0 \times T^3/6)] \}$$

$$= \{ [- \exp(- N \times \lambda_2 \times \lambda_1 \times (\alpha_0 + \beta_0 \times D) \times T^3/6\})]$$
$$- [- \exp(- N \times \lambda_2 \times \lambda_1 \times \alpha_0 \times T^3/6)] \} /$$
$$\{ \exp(- N \times \lambda_2 \times \lambda_1 \times \alpha_0 \times T^3/6)] \}$$

$$= \{ \exp(- N \times \lambda_2 \times \lambda_1 \times \alpha_0 \times T^3/6)]$$

$$– \exp(- N \times \lambda_2 \times \lambda_1 \times [\alpha_0 \times T^3/6 + \beta_0 \times D \times T^3/6])]$$
$$/\{ \exp(- N \times \lambda_2 \times \lambda_1 \times \alpha_0 \times T^3/6) \}$$

$$= 1 - \exp(- N \times \lambda_2 \times \lambda_1 \times \beta_0 \times D \times T^3/6). \quad \textbf{(Equality 1)}$$

Similarly, if the dose is not a constant dose D but rather a time-dependent dose

$d(t)$ = 0 for t<a
= d for a≤t≤ b
= 0 for t>b

and

$$\lambda_0[t_0, d(t_0)] = \alpha_0 + \beta_0 \times d(t_0),$$

then the probability of a specified response occurring by time T_e corresponding to the observation time (necropsy time) in the experiment is

$$P[T_e; d(t)] = 1 – \exp\{ - N \times H[T_e; \text{dose}=d(t)] \}$$

where

$$H[T_e; \text{dose}=d(t)] = {}_0\int^{T}_{e}\ \lambda_2 \times {}_0\int^{t}_{2}\ \lambda_1 \times \{ {}_0\int^{t}_{1}\ \lambda_0[t_0, d(t_0)]\ dt_0 \}\ dt_1\ dt_2$$

It follows from the integral for $H[T_e; \text{dose}=d(t)]$ that with both $a \le T_e$ and $b \le T_e$

$$H[T_e; \text{dose}=d(t)] = \lambda_2 \times \lambda_1 \times {}_0\int^{T}_{e}\ {}_0\int^{t}_{2}\ {}_0\int^{t}_{1}\ [\alpha_0 + \beta_0 \times d(t_0)]\ dt_0\ dt_1\ dt_2$$

$$= \lambda_2 \times \lambda_1 \times {}_0\int^{T}_{e}\ {}_0\int^{t}_{2}\ {}_0\int^{t}_{1}\ [\alpha_0]\ dt_0\ dt_1\ dt_2 + \lambda_1 \times {}_0\int^{T}_{e}\ {}_0\int^{t}_{1}\ [\beta_0 \times d(t_0)]\ dt_0\ dt_1\ dt_2$$

$$= \lambda_2 \times \lambda_1 \times \alpha_0 \times {}_0\int^{T}_{e}\ {}_0\int^{t}_{2}\ {}_0\int^{t}_{1}\ dt_0\ dt_1\ dt_2 + \lambda_2 \times \lambda_1 \times \beta_0 \times {}_0\int^{T}_{e}\ {}_0\int^{t}_{2}\ {}_0\int^{t}_{1}\ [d(t_0)]\ dt_0\ dt_1\ dt_2$$

$$= \lambda_2 \times \lambda_1 \times \alpha_0 \times T_e^3/6 + \lambda_2 \times \lambda_1 \times \beta_0 \times {}_0\int^{T}_{e}\ {}_0\int^{t}_{2}\ {}_0\int^{t}_{1}\ [d(t_0)]\ dt_0\ dt_1\ dt_2. \quad \textbf{(Equality 2)}$$

The last integral is broken up into three parts as follows:

$${}_0\int^{T}_{e}\ {}_0\int^{t}_{2}\ {}_0\int^{t}_{1}\ [d(t_0)]\ dt_0\ dt_1\ dt_2 = \text{Part 1} + \text{Part 2} + \text{Part 3}$$

where

$$\text{Part 1} = {}_0\int^{a}\ {}_0\int^{t}_{2}\ {}_0\int^{t}_{1}\ [d(t_0)]\ dt_0\ dt_1\ dt_2,$$

$$\text{Part 2} = {}_a\int^{b}\ {}_0\int^{t}_{2}\ {}_0\int^{t}_{1}\ [d(t_0)]\ dt_0\ dt_1\ dt_2, \text{ and}$$

$$\text{Part 3} = {}_b\int^{T}_{e}\ {}_0\int^{t}_{2}\ {}_0\int^{t}_{1}\ [d(t_0)]\ dt_0\ dt_1\ dt_2.$$

Then

Part 1 = 0

because $t_0 \le t_1 \le t_2 \le a$ implies $t_0 \le a$ and $d(t_0)$ equals 0.

Next, Part 2 is broken up into three parts as follows:

Part 2 = $\int_a^b \int_0^{t_2} \int_0^{t_1} [d(t_0)]\, dt_0\, dt_1\, dt_2$

= $\int_a^b \int_0^{a} \int_0^{t_1} [d(t_0)]\, dt_0\, dt_1\, dt_2 + \int_a^b \int_a^{t_2} \int_0^{t_1} [d(t_0)]\, dt_0\, dt_1\, dt_2$

= $\int_a^b \int_0^{a} \int_0^{t_1} [0]\, dt_0\, dt_1\, dt_2 + \int_a^b \int_a^{t_2} \int_0^{t_1} [d(t_0)]\, dt_0\, dt_1\, dt_2$

= $\int_a^b \int_a^{t_2} \int_0^{t_1} [d(t_0)]\, dt_0\, dt_1\, dt_2$

= $\int_a^b \int_a^{t_2} \int_0^{a} [d(t_0)]\, dt_0\, dt_1\, dt_2 + \int_a^b \int_a^{t_2} \int_a^{t_1} [d(t_0)]\, dt_0\, dt_1\, dt_2$

= $\int_a^b \int_a^{t_2} \int_0^{a} [0]\, dt_0\, dt_1\, dt_2 + \int_a^b \int_a^{t_2} \int_a^{t_1} [d]\, dt_0\, dt_1\, dt_2$

= $d \times \int_a^b \int_a^{t_2} \int_a^{t_1} 1\, dt_0\, dt_1\, dt_2$

= $d \times \int_a^b \int_a^{t_2} (t_1-a)\, dt_1\, dt_2$

= $d \times \int_a^b (t_2-a)^2/2\, dt_2$

and

Part 2 = $d \times (b-a)^3/6$.

Finally, Part 3 is broken up into three parts as follows:

Part 3 = = $\int_b^T \int_0^{t_2} \int_0^{t_1} [d(t_0)]\, dt_0\, dt_1\, dt_2$ = Part 3.1 + Part 3.2 + Part 3.3

where

Part 3.1 = $\int_b^T \int_0^{a} \int_0^{t_1} [d(t_0)]\, dt_0\, dt_1\, dt_2$,

Part 3.2 = $\int_b^T \int_a^{b} \int_0^{t_1} [d(t_0)]\, dt_0\, dt_1\, dt_2$, and

Part 3.3 = $\int_b^T \int_b^{t_2} \int_0^{t_1} [d(t_0)]\, dt_0\, dt_1\, dt_2$.

Then

Part 3.1 = 0

because $t_0 \le t_1 \le a$ implies $t_0 \le a$ and $d(t_0)$ equals 0.

Also

$$\text{Part 3.2} = \int_b^{T_e}\int_a^b\int_0^{t_1} [d(t_0)]\, dt_0\, dt_1\, dt_2$$

$$= \int_b^{T_e}\int_a^b\int_0^{a} [d(t_0)]\, dt_0\, dt_1\, dt_2 + \int_b^{T_e}\int_a^b\int_a^{t_1} [d(t_0)]\, dt_0\, dt_1\, dt_2$$

$$= \int_b^{T_e}\int_a^b\int_0^{a} [0]\, dt_0\, dt_1\, dt_2 + \int_b^{T_e}\int_a^b\int_a^{t_1} [d]\, dt_0\, dt_1\, dt_2$$

$$= d \times \int_b^{T_e}\int_a^b\int_a^{t_1} 1\, dt_0\, dt_1\, dt_2$$

$$= d \times \int_b^{T_e}\int_a^b (t_1 - a)\, dt_1\, dt_2$$

$$= d \times \int_b^{T_e} (b - a)^2/2\, dt_1\, dt_2$$

$$= d \times (b - a)^2/2 \times \int_b^{T_e} 1\, dt_2$$

and

$\text{Part 3.2} = d \times (b - a)^2/2 \times (T_e - b).$

Also

$$\text{Part 3.3} = \int_b^{T_e}\int_b^{t_2}\int_0^{t_1} [d(t_0)]\, dt_0\, dt_1\, dt_2$$

$$= \int_b^{T_e}\int_b^{t_2}\int_0^{a} [d(t_0)]\, dt_0\, dt_1\, dt_2 + \int_b^{T_e}\int_b^{t_2}\int_a^{b} [d(t_0)]\, dt_0\, dt_1\, dt_2 + \int_b^{T_e}\int_b^{t_2}\int_b^{t_1} [d(t_0)]\, dt_0\, dt_1\, dt_2$$

$$= \int_b^{T_e}\int_b^{t_2}\int_0^{a} [0]\, dt_0\, dt_1\, dt_2 + \int_b^{T_e}\int_b^{t_2}\int_a^{b} [d]\, dt_0\, dt_1\, dt_2 + \int_b^{T_e}\int_b^{t_2}\int_b^{t_1} [0]\, dt_0\, dt_1\, dt_2$$

$$= \int_b^{T_e}\int_b^{t_2}\int_a^{b} [d]\, dt_0\, dt_1\, dt_2$$

$$= d \times \int_b^{T_e}\int_b^{t_2}\int_a^{b} 1\, dt_0\, dt_1\, dt_2$$

$$= d \times \int_b^{T_e}\int_b^{t_2} (b\text{-}a)\, dt_1\, dt_2$$

$$= d \times (b\text{-}a) \times \int_b^{T_e}\int_b^{t_2} 1\, dt_1\, dt_2$$

$$= d \times (b\text{-}a) \times \int_b^{T_e} (t_2\text{-}b)\, dt_2$$

and

$\text{Part 3.3} = d \times (b\text{-}a) \times (T_e - b)^2/2.$

Combining Part 1, Part 2, and Parts 3.1, 3.2 and 3.3 yields

$$\int_0^{T_e}\int_0^{t_2}\int_0^{t_1} [d(t_0)]\, dt_0\, dt_1\, dt_2$$

$$= \mathbf{0 + d \times (b\text{-}a)^3/6 + 0 + d \times (b - a)^2/2 \times (T_e - b) + d \times (b\text{-}a) \times (T_e - b)^2/2}$$

$$= d \times \{ (b\text{-}a)^3/6 + (b - a)^2/2 \times (T_e - b) + (b\text{-}a) \times (T_e - b)^2/2 \}$$

$$= d \times \{ (b\text{-}a)^3/6 + (b - a)^2/2 \times (T_e - b) + (b\text{-}a) \times (T_e - b)^2/2 \}$$

$$= d \times (b\text{-}a) \times \{ (b\text{-}a)^2/6 + (b - a)/2 \times (T_e - b) + (T_e - b)^2/2 \}$$

$$= d \times (b\text{-}a) \times \{ (b\text{-}a)^2/6 + (b - a)/2 \times T_e - b \times (b - a)/2 + (T_e^2 - 2 \times b \times T_e + b^2)/2 \}$$

$$= d \times (b\text{-}a) \times \{ T_e^2 / 2 - T_e \times (a+b)/2 + (b\text{-}a)^2/6 - b \times (b - a)/2 + b^2/2 \}$$

$$= d \times (b\text{-}a) \times \{ T_e^2/2 - T_e \times (a+b)/2 + b^2/6 - 2 \times a \times b / 6 + a^2/6 - b^2/2 + a \times b/2 + b^2/2 \}$$

$$= d \times (b\text{-}a) \times \{ T_e^2 / 2 - T_e \times (a+b)/2 + b^2/6 + a \times b / 6 + a^2/6\} \qquad \textbf{(Equality 3)}$$

Now, from Equality 1, the extra risk for a constant dose D for a nominal lifetime is

$$\mathbf{1 - \exp(- N \times \lambda_2 \times \lambda_1 \times \beta_0 \times D \times T^3/6).}$$

With

$$P[T_e; d(t)] = 1 - \exp\{ - N \times H[T_e; \text{dose}=d(t)] \}$$

and (using Equality 2)

$$H[T_e; \text{dose}=d(t)] =$$

$$= \lambda_2 \times \lambda_1 \times \alpha_0 \times T_e^3/6 + \lambda_2 \times \lambda_1 \times \beta_0 \times \int_0^{T_e}\int_0^{t_2}\int_0^{t_1} [d(t_0)]\, dt_0\, dt_1\, dt_2,$$

then the extra risk at time T_e for an intermittent dose d(t) is

$$\{ P[T_e; d(t)] - P[T_e; 0] \} / \{ 1 - P[T_e; 0] \}$$

$$= \{ [1 - \exp\{ -N \times (\lambda_2 \times \lambda_1 \times \alpha_0 \times T_e^3/6 + \lambda_2 \times \lambda_1 \times \beta_0 \times \int_0^{T_e}\int_0^{t_2}\int_0^{t_1} [d(t_0)]\, dt_0\, dt_1\, dt_2]) - [1 - \exp\{ - N \times \lambda_2 \times \lambda_1 \times \alpha_0 \times T_e^3/6 \}] \} / \{ 1 - [1 - \exp\{ - N \times \lambda_2 \times \lambda_1 \times \alpha_0 \times T_e^3/6 \}] \}$$

$$= \{ - \exp\{ - N \times (\lambda_2 \times \lambda_1 \times \alpha_0 \times T_e^3/6 + \lambda_2 \times \lambda_1 \times \beta_0 \times \int_0^{T_e}\int_0^{t_2}\int_0^{t_1} [d(t_0)]\, dt_0\, dt_1\, dt_2]) + \exp\{ - N \times \lambda_2 \times \lambda_1 \times \alpha_0 \times T_e^3/6 \}] \}$$

$$/ \{ \exp\{ - N \times \lambda_2 \times \lambda_1 \times \alpha_0 \times T_e^3/6 \}] \}$$

$$= \{ \exp\{ - N \times \lambda_2 \times \lambda_1 \times \alpha_0 \times T_e^3/6 \}] \}$$
$$- \exp\{-N \times (\lambda_2 \times \lambda_1 \times \alpha_0 \times T_e^3/6 + \lambda_2 \times \lambda_1 \times \beta_0 \times {}_0\int^{T_e}\, {}_0\int^{t_2}\, {}_0\int^{t_1}\, [d(t_0)]\, dt_0\, dt_1\, dt_2])$$
$$/ \{ \exp\{ - N \times \lambda_2 \times \lambda_1 \times \alpha_0 \times T_e^3/6 \}] \}$$

$$= \{ 1 - \exp\{ - N \times \lambda_2 \times \lambda_1 \times \beta_0 \times {}_0\int^{T_e}\, {}_0\int^{t_2}\, {}_0\int^{t_1}\, [d(t_0)]\, dt_0\, dt_1\, dt_2 \}.$$

Using Equality 3 and the equation immediately above, the extra risk at time T_e for an intermittent dose d(t) is

$$\{ 1 - \exp\{ - N \times \lambda_2 \times \lambda_1 \times \beta_0 \times ({}_0\int^{T_e}\, {}_0\int^{t_2}\, {}_0\int^{t_1}\, [d(t_0)]\, dt_0\, dt_1\, dt_2) \}$$

$$= 1 - \exp\{ - N \times \lambda_2 \times \lambda_1 \times \beta_0 \times (d \times (b-a) \times \{T_e^2/2 - T_e \times (a+b)/2 + b^2/6 + a \times b/6 + a^2/6\}) \}$$

(Equality 4)

Therefore, using Equality 1 and Equality 4, in order for the extra risk at time T for a constant dose D, namely,

$$\mathbf{1 - \exp(- N \times \lambda_2 \times \lambda_1 \times \beta_0 \times D \times T^3/6),}$$

to be equal to the extra risk at time T_e for an intermittent dose d(t), namely

$$1 - \exp\{ - N \times \lambda_2 \times \lambda_1 \times \beta_0 \times (d \times (b-a) \times \{T_e^2/2 - T_e \times (a+b)/2 + b^2/6 + a \times b/6 + a^2/6\}) \}$$

it must be true that

$$\mathbf{D \times T^3/6 =} \; d \times (b-a) \times \{T_e^2/2 - T_e \times (a+b)/2 + b^2/6 + a \times b/6 + a^2/6\}. \qquad \text{(Equality 5)}$$

In order for Equality 5 to be true, it must be true that

$$D = d \times \{ 6 \times (b-a) \times [T_e^2/2 - T_e \times (a+b)/2 + b^2/6 + a \times b/6 + a^2/6] \} / T^3 .$$

$$= d \times \{ 6 \times (b-a) \times [T_e^2/2 - T_e \times (a+b)/2 + b^2/6 + a \times b/6 + a^2/6] \} / T^3$$

$$= d \times \{ (b-a) \times [3 \times T_e^2 - 3 \times T_e \times (a+b) + b^2 + a \times b + a^2] \} / T^3$$

$$= d \times \{ T_e^2 \times [3 \times (b-a)] - T_e \times 3 \times (a+b) \times (b-a) + (b-a) \times (b^2 + a \times b + a^2) \} / T^3$$

$$= d \times \{ T_e^2 \times [-3 \times a + 3 \times b] + T_e \times [-3 \times (a+b) \times (b-a)] + [b^3 - a^3] \} / T^3$$

$$= d \times \{ T_e^2 \times [-3 \times a + 3 \times b] + T_e \times [-3 \times (b^2 - a^2)] + [b^3 - a^3] \} / T^3$$

$$= d \times \{ T_e^2 \times [-3 \times a + 3 \times b] + T_e \times [3 \times a^2 - 3 \times b^2)] + [b^3 - a^3] \} / T^3$$

$$= d \times \{ (T_e - a)^3 - (T_e - b)^3 \} / T^3$$

since

$$(T_e - a)^3 - (T_e - b)^3$$

$$= (T_e^3 - 3\times a\times T_e^2 + 3\times a^2\times T_e - a^3) - (T_e^3 - 3\times b\times T_e^2 + 3\times b^2\times T_e - b^3)$$

$$= T_e^2\times[\ -3\times a + 3\times b\ ] + T_e\times[\ 3\times a^2 - 3\times b^2\ ] + b^3 - a^3$$

Appendix B

Comparison of the Weibull and Multistage Models Fit to the NTP 1999 Two-Years Study on Male and Female F344/N Rats

Executive Summary

NTP(1999) presents the findings of a two-year experiment on rats exposed to isoprene. The NTP fitted the dose-response data after adjusting the number of animals at risk for the early mortality of non-responding rats. The NTP fit the quantal Weibull model to the experimental data with a shape parameter that was restricted to values between zero and 10. Shape parameter values less than one result in supralinear dose-response relationships while shape parameter values greater than one result in sublinear dose-response relationships. Although NTP claimed that they tested whether the estimation of the shape parameter made a statistical significant difference in the fit of the model to the data, they did not present or discuss those results. The NTP presented only the results of the quantal Weibull model and the estimates of the shape parameter. (They did not present results for the quantal multistage model.)

The hypothesis suggested by the NTP (that the shape parameter in the Weibull model made a statistically significant difference in the fit compared to a one-stage multistage model) is evaluated herein and the results of such hypothesis tests are presented. The results of the one-stage multistage model (i.e., NTP Weibull model with the shape parameter fixed at one) are also presented here.

We find that, in general, estimating the shape parameter of the Weibull model does not result in a statistically significant better fit of the model to the data. This result is true for adjusted and unadjusted number of animals at risk and the three endpoints in male rats. Although for female rats the shape parameter seems to be relevant, the data and the model fit seem biologically implausible. Furthermore, the risk measures calculated without the shape parameter are less variable and result in more stable characterization of risks.

Introduction

The NTP 1999 report presents the findings of a 2-year study on male and female rats exposed to isoprene. The NTP fitted a dose-response model to the “neoplasms showing chemical-related effects.” The NTP adjusted the number of animals at risk at each dose group and each endpoint using the Poly-3 adjustment proposed by Portier et al. (1986). The Poly-3 adjustment considers that the number of animals at risk is equal to the number of animals responding plus the number of animals that died multiplied by the cubic power of the ratio of the time of death and the time of the end of the study. That is,

$$n_{adjusted} = Responses + \sum_{i=1}^{NonResponses} \left(\frac{t_{death}(i)}{t}\right)^3$$

where *Responses* is the number of animals with the response by the end of the experiment, *NonResponses* is the number of animals without the response by the end of the experiment, $t_{death(i)}$ is the time of death of the i-th animal that did not have the response, and t is the duration of the experiment.

The NTP 1999 study exposed 50 male and 50 female rats to four concentration levels of isoprene. The NTP study also measured the isoprene and isoprene monoepoxide levels in the blood as alternative dose metrics. Table B.1 lists the dose groups used in the NTP 1999 study.

After adjusting for the number of animals at risk using the *Poly-3* adjustment, NTP focused their analyses on mammary gland neoplasms in male and female rats, and renal tubule adenomas and testicular adenomas in male rats. Table B.2 shows the number of responses and number of animals at risk adjusted using the *Poly-3* adjustment for each of the dose groups and each response analyzed by the NTP 1999. Figure B.1, Figure B.2, and Figure B.3 show the incidence, adjusted for early deaths using the *Poly-3* adjustment, of the four responses and using the three dose scales, respectively.

The NTP fit the following Weibull model to the dose-response data

$$P(dose) = 1 - e^{-(intercept + scale \times dose^{shape})}$$

where the parameters *intercept*, *scale*, and *shape* are estimated from the data using maximum likelihood. The NTP indicates that "A likelihood ratio test is used to test the hypothesis that the shape parameter equals 1. The test statistic is given as -2 times the differences in the log likelihoods. A one-sided test was used so that the critical values are 2.706 for P=0.05 and 5.410 for P=0.01 (these are the squares of the critical regions from standard normal distribution)."

Testing the Hypothesis that the Shape Parameter is Equal to One

The NTP does not show and does not discuss the results of the statistical hypothesis that the *shape* parameter of the Weibull model is equal to 1. Herein, the *Shape* parameter in the Weibull model was tested, as it should have been done by the NTP, by fitting the following one-stage multistage model (which is the same as a Weibull model with shape parameter equal to 1)

$$P(dose) = 1 - e^{-(intercept + slope \times dose)}$$

where the parameters are as before (*slope* is similar to *scale* with the *shape* fixed to 1) and the *shape* parameters is fixed to 1. The *intercept* and *slope* of the multistage model

were estimated using maximum likelihood. Because the multistage model is nested within the Weibull model (i.e., the multistage model is a special case of the Weibull model with the *shape* parameter fixed to 1), the maximum likelihood of the multistage model is less than or equal to the maximum likelihood of the Weibull model. The test statistic given by -2 times the difference given by logarithm of the maximum likelihood for the multistage model minus the logarithm of the maximum likelihood for the Weibull model is approximately distributed as a Chi-square distribution with one degree of freedom. That is,

$$-2 \times (\log Likelihood\ Multistage\ Model - \log Likelihood\ Weibull\ Model) \sim \chi_1^2$$

where χ_1^2 is the Chi-square distribution with one degree of freedom. If the probability of values greater than the test statistic (p-value) is greater than 0.05, then there is not enough statistical evidence that the log-likelihood of the Weibull model is greater than the log-likelihood of the multistage model (i.e., there is no statistical evidence that estimating the *shape* parameter in the Weibull model significantly improves the fit of the model to the data). In other words, the statistic tests the hypothesis that the *shape* parameter in the Weibull model is equal to 1.

Table B.3 shows for each of the four endpoints analyzed, each of the dose-metrics used, and each of the two models fitted (Weibull and multistage), the EC_{10} (the dose corresponding to an extra risk of 10%), the LEC_{10} (the 95% lower confidence limit on the EC_{10}), the log-likelihood, and the p-value for the lack of fit for the Weibull and multistage models. Table B.3 also shows the estimate of *shape* parameter for the Weibull model and the p-value of the statistical test for the hypothesis that the *shape* parameter is equal to 1. The p-value for the lack of fit statistic indicates how well the model fits the observed data. A small p-value indicates that the difference in the log-likelihoods of fitting the data with a model that goes through the observed frequencies and the model fit to the data is statistical significant. On the other hand, a large p-value indicates that difference in the log-likelihoods of fitting the data with a model that goes through the observed frequencies and the model fit to the data are not statistically significantly different.

The p-values for the *shape* parameter of the Weibull model are greater than 0.05 for all three endpoints in male rats and all three dose metrics. That is, the *shape* parameter of the Weibull model does not improve significantly the fit of the model to the observed data.

The p-values for the *shape* parameter of the Weibull model are less than 0.05 for two of the three dose metrics in the one female rat endpoint analyzed. The dose-response data for mammary gland neoplasms in female rats is such that the frequency of tumors decreases with dose in the three exposed groups and there is a 60% increase in the frequency of response between the control group and the first dose group. In addition, the estimates of the *shape* parameter and EC_{10} values are unrealistic and biologically implausible.

Figure B.4, Figure B.5, and Figure B.6 show the incidence of the four responses using the three dose scales, respectively. The incidences in Figure B.4, Figure B.5, and Figure B.6 are not adjusted for early deaths. Table B.4 shows the results when the numbers of animals at risk are not adjusted for early mortality. The results in Table B.4 (with unadjusted numbers of animals at risk) are very similar to the results in Table B.3 (with adjusted numbers of animals at risk).

Figure B.7 and Figure B.8 show the relationship between the experimental air concentrations of isoprene and the internal dose metrics used in the NTP 1999 study. The relationships shown in Figure B.7 and Figure B.8 can be used to convert the EC_{10} and LEC_{10} values from the internal doses to the equivalent isoprene air concentrations of the experimental animals.

Conclusion

In general, the inclusion of the *shape* parameter in the Weibull model does not result in a statistically significant improvement in the fit of the model to the observed data compared to a one-stage model (or, equivalently the Weibull model with shape parameter fixed equal to 1). This is true whether or not the numbers of animals at risk are adjusted for early mortality of non-responding animals.

References

National Toxicology Program (NTP) (1999). Toxicology and Carcinogenesis Studies of Isoprene (Cas No. 78-79-5) in F344/N Rats (Inhalation Studies). Technical Report No. 486. NIH Publication No. 99-3976. U.S. Department Of Health And Human Services. Public Health Service, National Institutes of Health, Research Triangle Park, NC.

Portier, C.J., Hedges, J.C., and Hoel, D.G. (1986). Age-specific models of mortality and tumor onset for historical control animals in the National Toxicology Program's carcinogenicity experiments. Cancer Res. 46, 4372-4378.

Table B.1. NTP 1999 experimental design, number of animals per group and dose metrics

Dose Group	Isoprene Exposure (ppm)	Blood Isoprene (μmol/L·7 days)	Blood Isoprene Monoepoxide (μmol/L·7 days)	Number of Animals at Risk
1	0	38.7	426	50
2	220	584	4,920	50
3	700	2,160	9,620	50
4	7,000	26,200	17,400	50

Table B.2. Number of responses and adjusted number of animals at risk using the *Poly-3* adjustment to account for non-responding animals that died before the end of the experiment for the endpoints analyzed in the NTP 1999 report

Dose Group	Male F344/N Rats						Female F344/N Rats	
	Mammary Gland Neoplasms		Renal Tubule Adenoma		Testicular Adenoma		Mammary Gland Neoplasms	
	Adjusted Number of Animals at Risk	Number of Animals with the Response	Adjusted Number of Animals at Risk	Number of Animals with the Response	Adjusted Number of Animals at Risk	Number of Animals with the Response	Adjusted Number of Animals at Risk	Number of Animals with the Response
1	37.04	2	37.04	2	42.09	33	44.25	20
2	38.17	5	38.10	4	43.38	37	47.11	35
3	38.04	7	38.10	8	46.61	44	43.42	32
4	38.75	21	38.96	15	48.05	48	43.72	32

Table B.3. Dose response modeling of the Melnick et al. 1999 data adjusted for early deaths using BMDS and comparing the Multistage Weibull model and the Multistage model

Endpoint	Parameter	Dose Scale					
		Isoprene Exposure (ppm)		Blood Isoprene (µmol/L·7 days)		Blood Isoprene Monoepoxide (µmol/L·7 days)	
		MSW[1]	MS[2]	MSW	MS	MSW	MS
Male F344/N Rats							
Mammary Gland Neoplasms	EC_{10}	378.33	988.62	838.50	3751.28	7858.06	3297.52
	LEC_{10}	63.48	668.42	78.17	2522.65	3725.99	2400.95
	LogL	-67.512	-68.063	-67.541	-68.235	-67.849	-69.581
	Shape (S.E.)	0.658 (0.219)	n/a	0.565 (0.240)	n/a	2.365 (n/a)[3]	n/a
	Lack of Fit[4]	0.8231	0.5621	0.7424	0.4733	0.3948	0.1232
	p-value[5]	0.2938		0.2387		0.0627	
Renal Tubule Adenoma	EC_{10}	349.49	1694.31	313.30	6506.24	6911.67	4521.70
	LEC_{10}	16.89	1032.05	31.60	3937.37	1435.83	3120.24
	LogL	-66.332	-67.458	-66.258	-67.615	-66.134	-66.472
	Shape (S.E.)	0.480 (0.199)	n/a	0.348 (0.242)	n/a	1.540 (0.739)	n/a
	Lack of Fit	0.5220	0.2642	0.6087	0.2258	0.9058	0.7082
	p-value	0.1334		0.0995		0.4110	
Testicular Adenoma	EC_{10}	24.04	47.04	31.62	139.69	2886.41	564.14
	LEC_{10}	0.08	25.57	(<0.01)[6]	74.44	(21.00)[6]	358.18
	LogL	-50.638	-51.048	-50.576	-51.248	-50.527	-51.394
	Shape (S.E.)	0.724 (0.376)	n/a	0.607 (n/a)[6]	n/a	2.145 (n/a)[6]	n/a
	Lack of Fit	0.6390	0.5945	0.7567	0.4868	1.0000	0.4206
	p-value	0.3652		0.2463		0.1879	
Female F344/N Rats							
Mammary Gland Neoplasms	EC_{10}	700000.0	1814.57	<0.1	7170.56	0.111	2081.37
	LEC_{10}	Infinite	?[7]	<0.01	2949.26	<0.01	1245.21
	LogL	-107.759	-112.790	-110.042	-112.858	-108.554	-110.467
	Shape (S.E.)	0 (n/a)[8]	n/a	0.108 (0.041)	n/a	0.222 (0.073)	n/a
	Lack of Fit	0.9058	0.0065**	0.0323*	0.0061**	0.2053	0.0662
	p-value	0.0015*		0.0176*		0.0505	

[1]Multistage Weibull model $p(d) = 1 - \exp\{-(\text{intercept} + \text{scale}\times d^{\text{shape}})\}$

[2]Multistage linear model $p(d) = 1 - \exp\{-(\text{intercept} + \text{slope}\times d)\}$

[3]BMDS could not fit the model and the standard error of the shape was not calculated.

[4]The p-value for the lack of fit is the probability that twice the difference between the logarithm of the likelihood of the full model and the logarithm of the likelihood of the fitted model is too large relative to a Chi distribution.

[5]The p-value compares the fit of the MSW model versus the MS model.

[6]BMDS could not fit the model and the standard error of the shape was not calculated and the LCL on the BMD reported here is the 95% LCL reported by Melnick et al. 1999.
[7]BMDS could not fit the data so that a BMDL could not be calculated.
[8]BMDS could not calculate the standard error of the shape parameter for the MSW model
* statistically significant at the 5% significance level
** statistically significant at the 1% significance level

Table B.4. Dose response modeling of the Melnick et al. 1999 data unadjusted for early deaths using BMDS and comparing the Multistage Weibull model and the Multistage model

Endpoint	Parameter	Isoprene Exposure (ppm)		Blood Isoprene (µmol/L·7 days)		Blood Isoprene Monoepoxide (µmol/L·7 days)	
		MSW[1]	MS[2]	MSW	MS	MSW	MS
Male F344/N Rats							
Mammary Gland Neoplasms	EC_{10}	589.54	1484.81	1338.21	5643.09	8756.42	4661.79
	LEC_{10}	123.21	1002.15	181.5	3790.36	4534.91	3389.95
	LogL	-78.937	-79.555	-78.966	-79.728	-79.2614	-80.691
	Shape (S.E.)	0.630 (0.214)	n/a	0.533 (0.238)	n/a	2.208 (???)[3]	n/a
	Lack of Fit[4]	0.8302	0.5268	0.7471	0.4431	0.4045	0.1691
	p-value[5]	0.2662		0.2170		0.0909	
Renal Tubule Adenoma	EC_{10}	642.87	2424.48	704.16	9313.62	8348.29	6267.46
	LEC_{10}	61.66	1470.81	126.02	5614.24	2415.86	4317.11
	LogL	-75.055	-76.204	-74.983	-76.360	-74.869	-75.170
	Shape (S.E.)	0.472 (0.198)	n/a	0.342 (0.094)	n/a	1.512 (0.734)	n/a
	Lack of Fit	0.5344	0.2613	0.6228	0.2236	0.9058	0.7349
	p-value	0.1295		0.0970		0.4378	
Testicular Adenoma	EC_{10}	9.29	329.10	1.23	1274.93	2117.02	908.27
	LEC_{10}	0.02	184.68	??	710.70	??	624.38
	LogL	-87.996	-89.701	-87.840	-89.946	-87.542	-87.854
	Shape (S.E.)	0.465 (0.175)	n/a	0.322 (???)[3]	n/a	1.455 (???)[3]	n/a
	Lack of Fit	0.2951	0.1051	0.3759	0.0822	0.6646	0.6663
	p-value	0.0648		0.0401*		0.4296	
Female F344/N Rats							
Mammary Gland Neoplasms	EC_{10}	700000	3633.67	<0.01	14481.20	0.06	3511.32
	LEC_{10}	Infinite	???[6]	<0.01	4694.87	<0.01	1888.67
	LogL	-129.806	-134.564	-132.453	-135.00	-131.03	-132.97
	Shape (S.E.)	0 (???)[7]	n/a	0.089 (0.040)	n/a	0.193 (0.071)	n/a
	Lack of Fit	0.4624	0.0066**	0.0157*	0.0042**	0.0839	0.0323*
	p-value	0.0020**		0.0240*		0.0489*	

[1]Multistage Weibull model $p(d) = 1 - \exp\{-(\text{intercept} + \text{scale}\times d^{\text{shape}})\}$

[2]Multistage linear model $p(d) = 1 - \exp\{-(\text{intercept} + \text{slope}\times d)\}$

[3]BMDS could not fit the model and the standard error of the shape was not calculated

[4]The p-value for the lack of fit is the probability that twice the difference between the logarithm of the likelihood of the full model and the logarithm of the likelihood of the fitted model is too large relative to a Chi distribution.

[5]The p-value compares the fit of the MSW model versus the MS model

[6]BMDS could not fit the data so than a BMDL could not be calculated

[7]BMDS could not calculate the standard error of the shape parameter for the MSW model

* statistically significant at the 5% significance level
** statistically significant at the 1% significance level

Figure B.1. Tumor incidence adjusted for early deaths for isoprene exposure (ppm) – Melnick et al. 1999

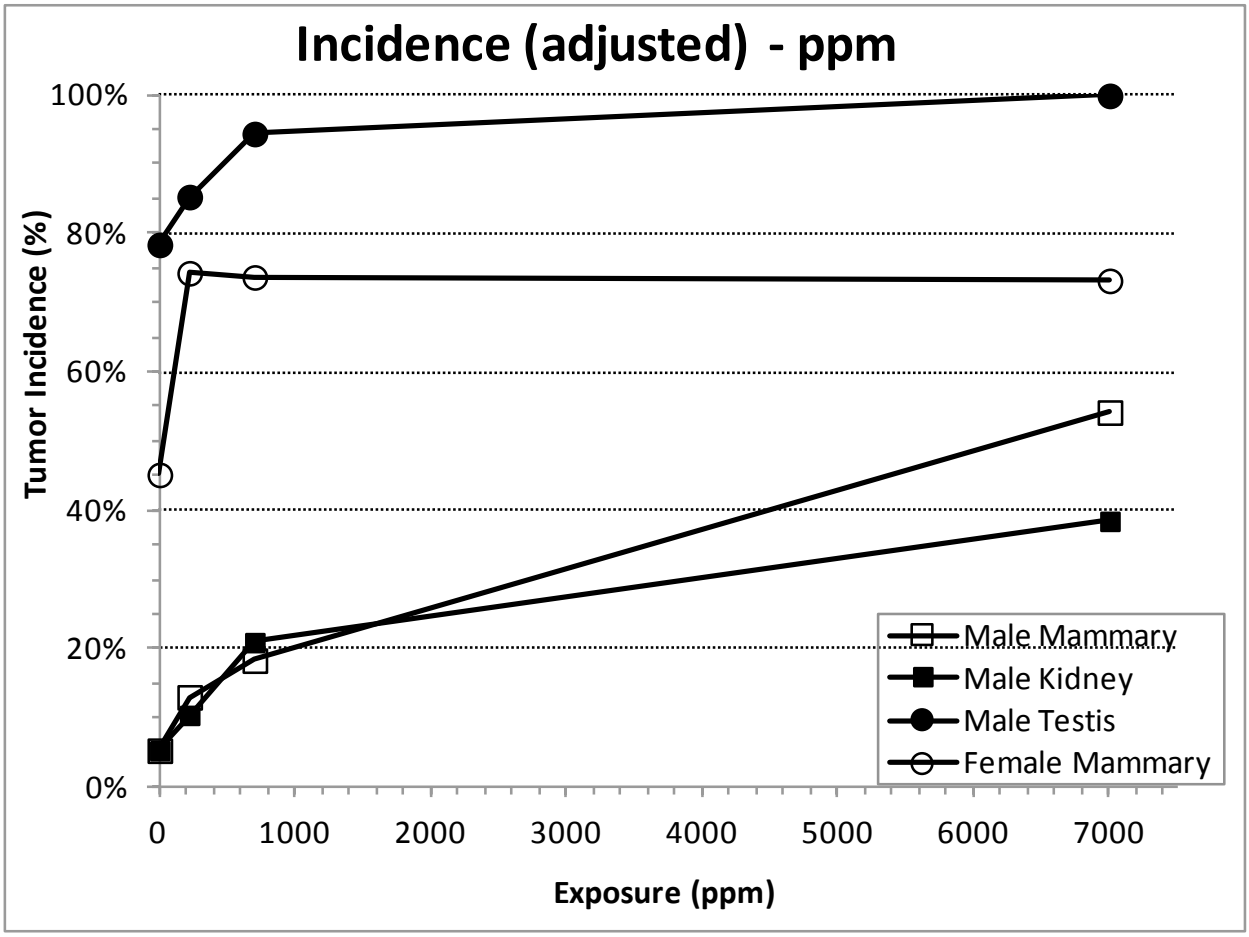


Figure B.2. Tumor incidence adjusted for early deaths for blood isoprene (µmol/L·7 days) – Melnick et al. 1999

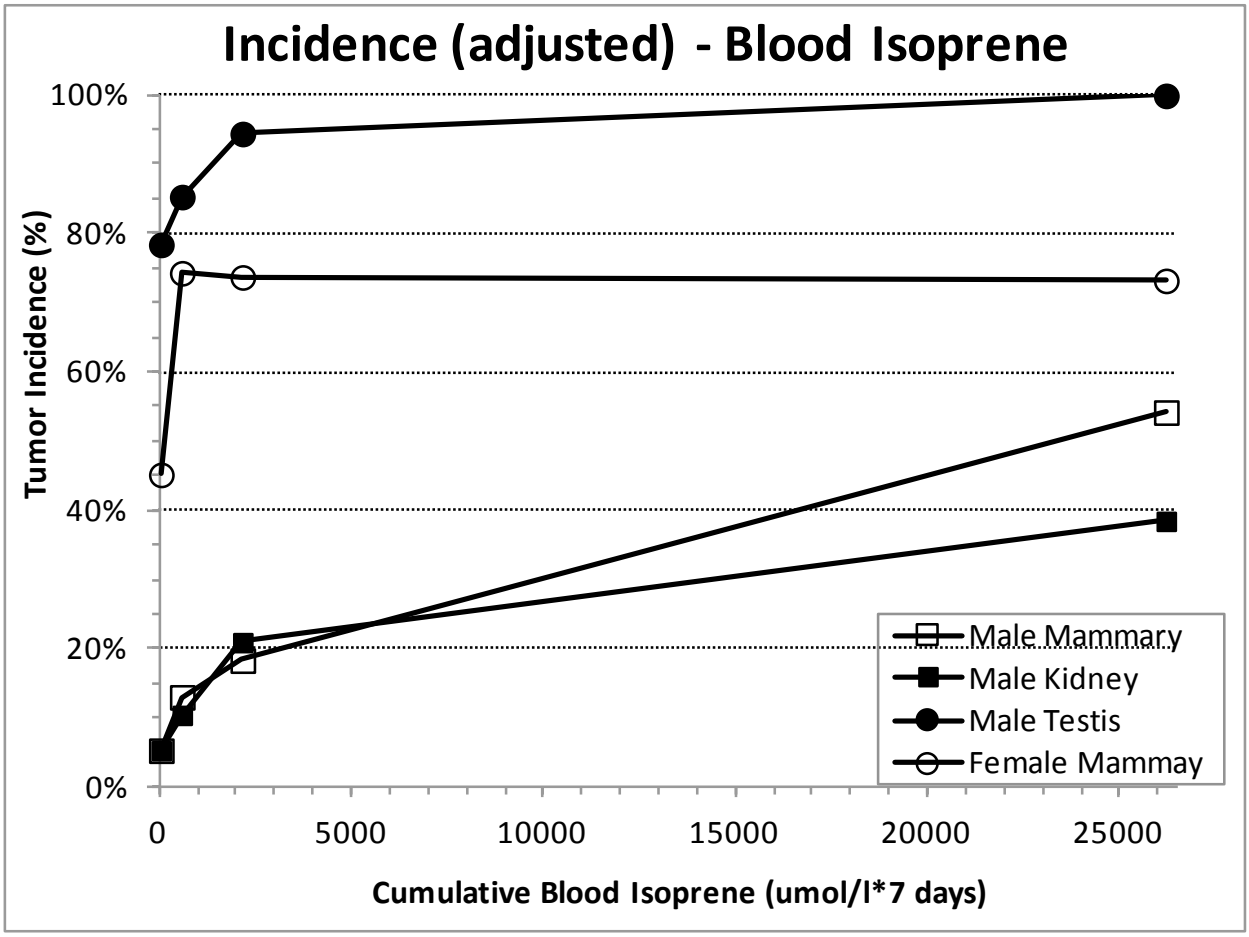


Figure B.3. Tumor incidence adjusted for early deaths for blood isoprene monoepoxide (μmol/L·7 days) – Melnick et al. 1999

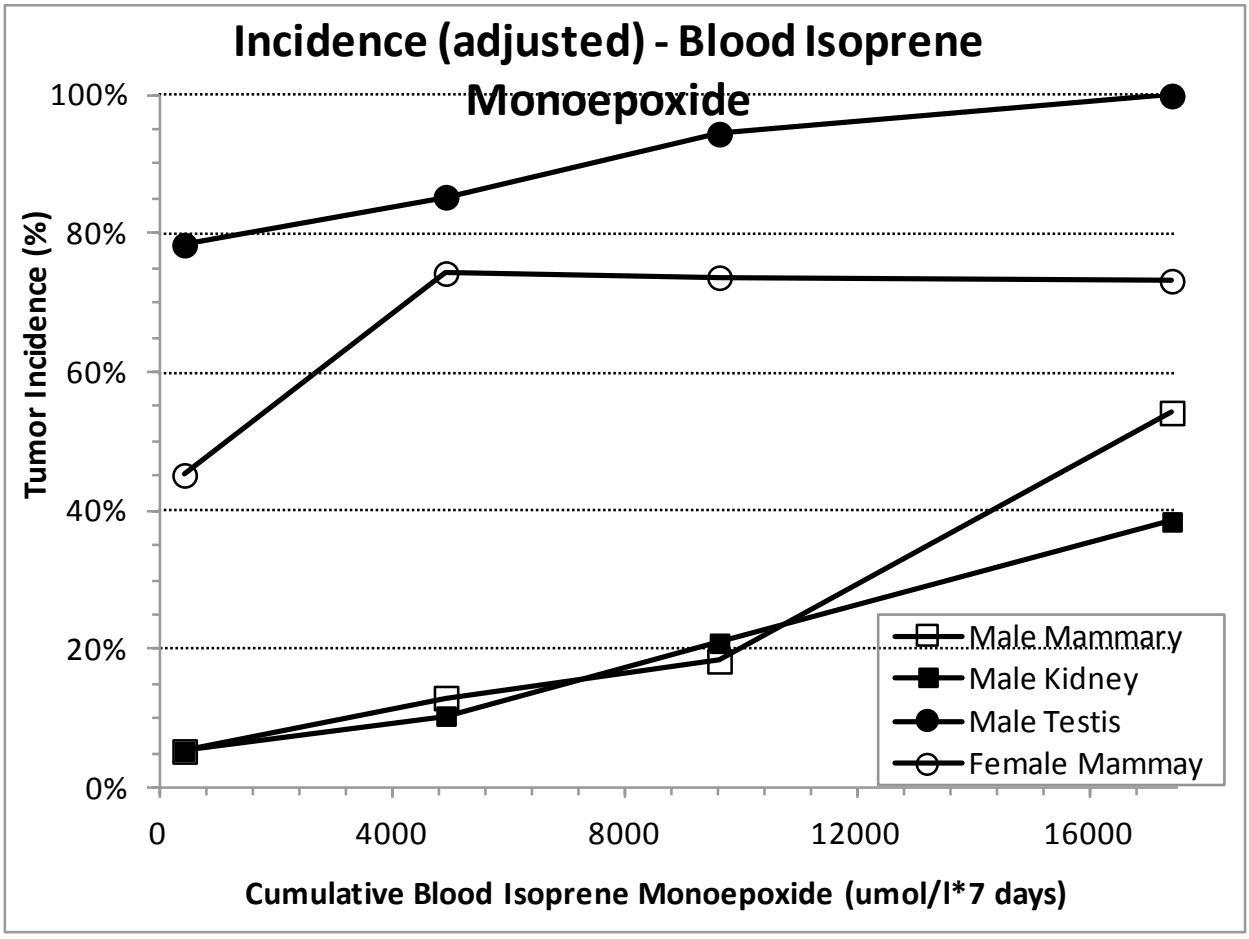


Figure B.4. Tumor incidence unadjusted for early deaths for isoprene exposure (ppm) – Melnick et al. 1999

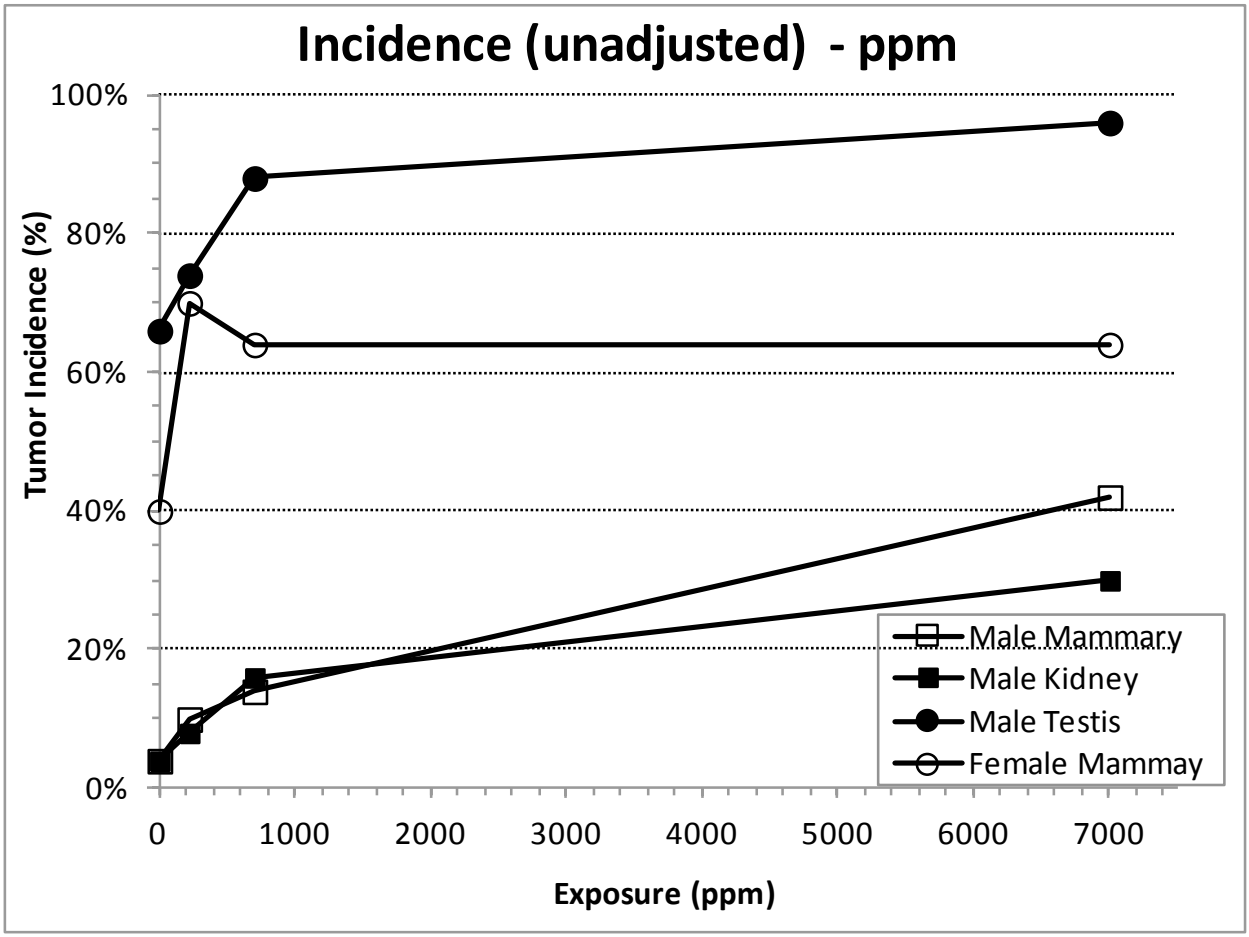


Figure B.5. Tumor incidence unadjusted for early deaths for blood isoprene (µmol/L·7 days) – Melnick et al. 1999

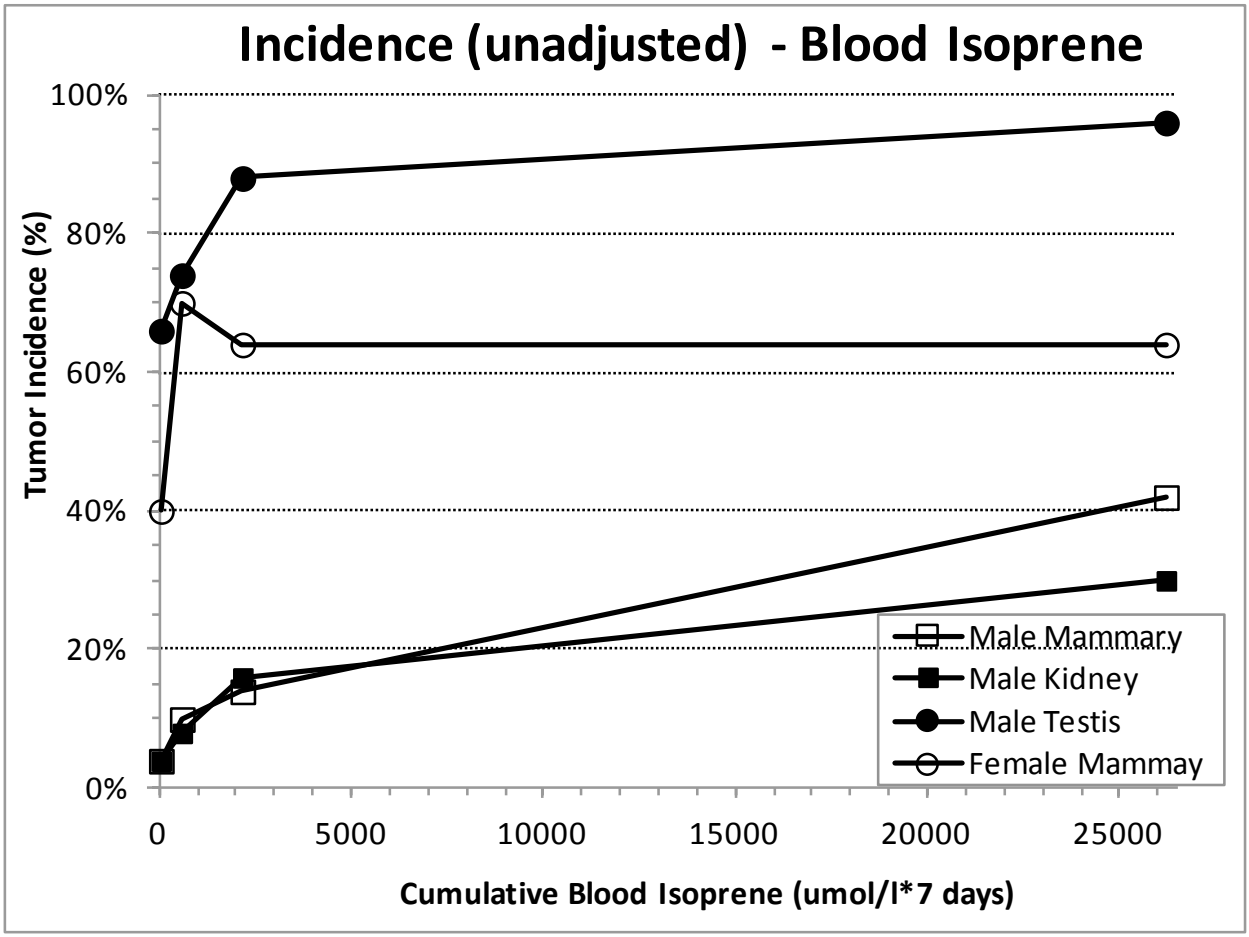


Figure B.6. Tumor incidence unadjusted for early deaths for blood isoprene monoepoxide (µmol/L·7 days) – Melnick et al. 1999

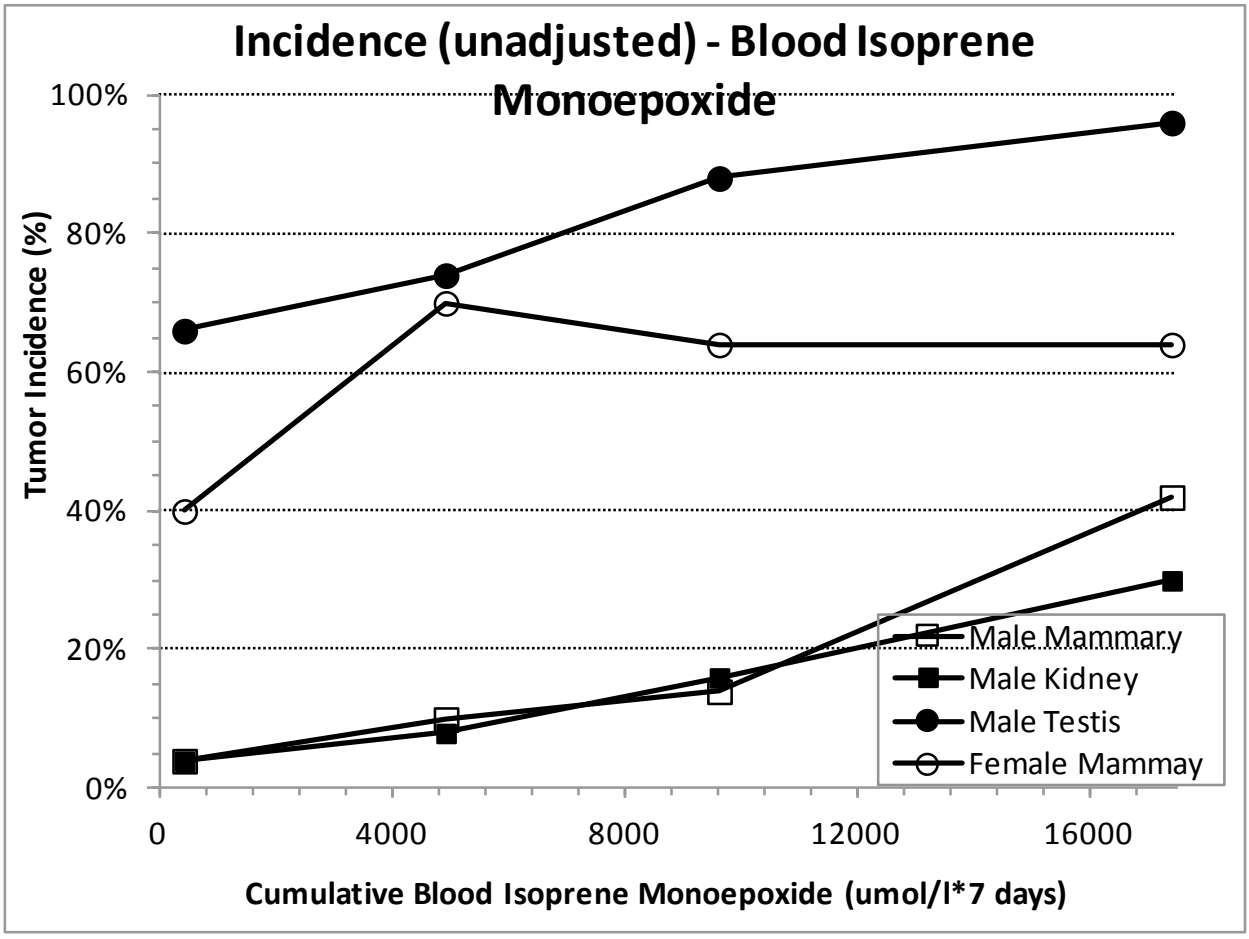


Figure B.7. Relationship between blood isoprene (µmol/L·7 days) and isoprene exposure (ppm)

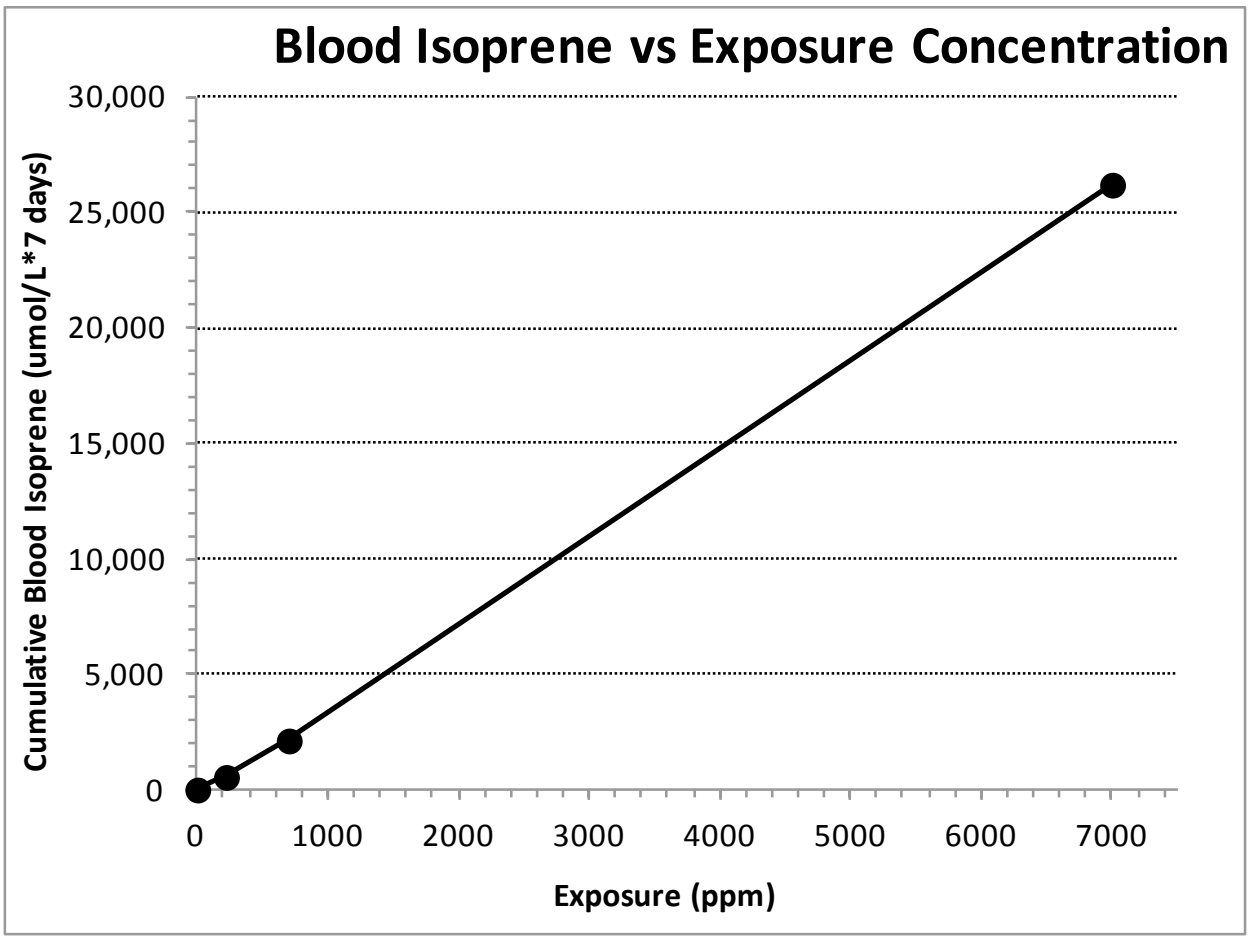
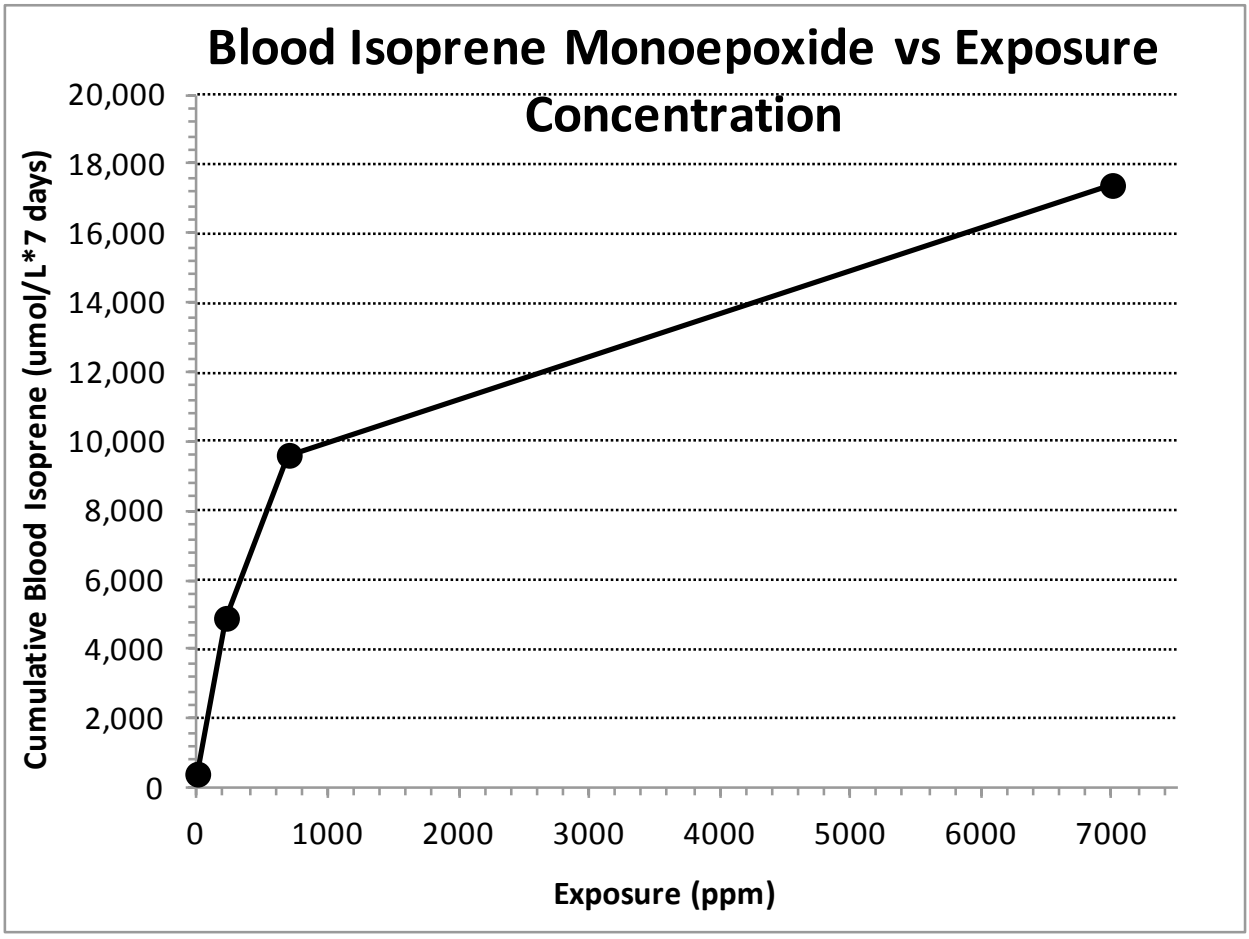


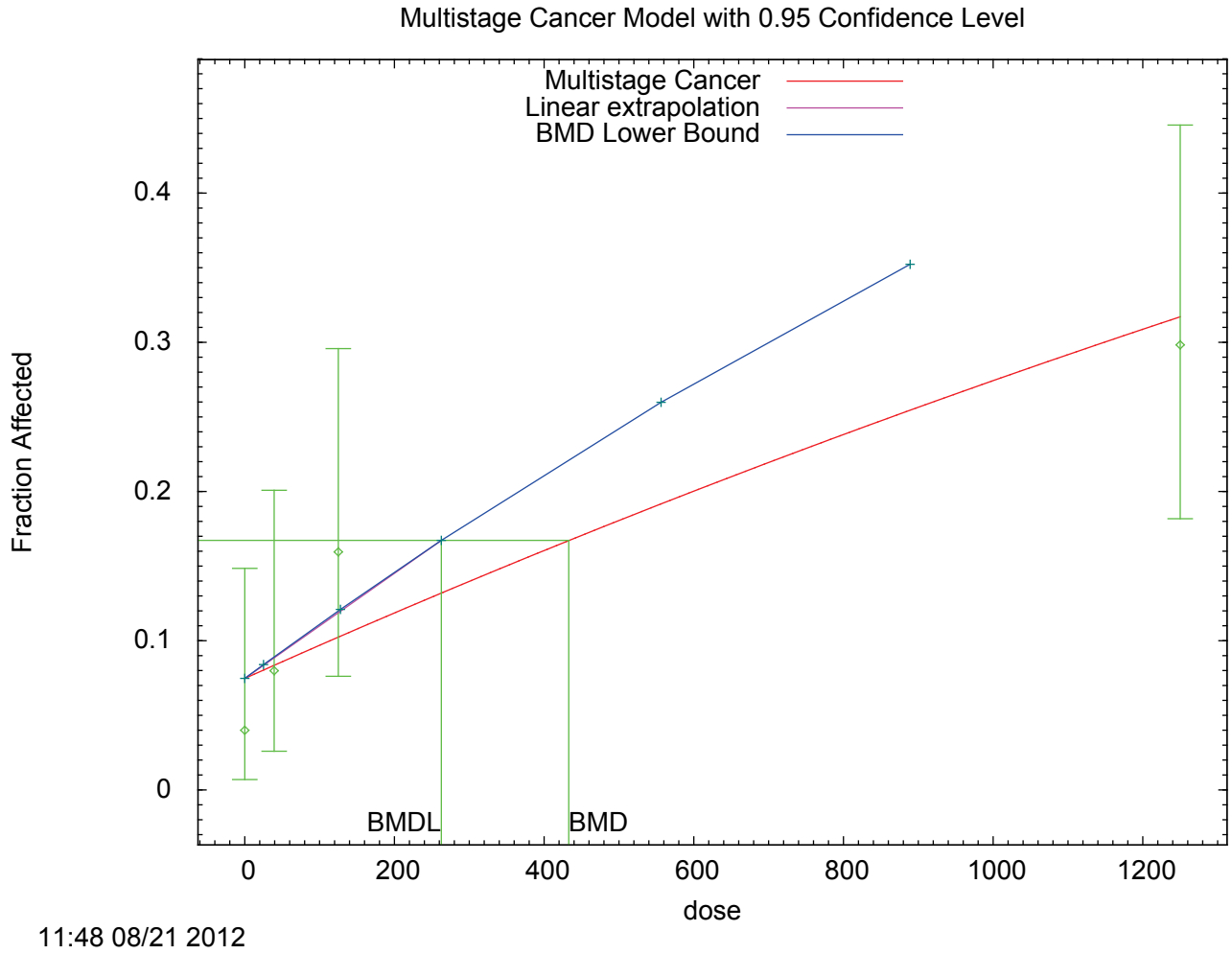
Figure B.8. Relationship between blood isoprene monoepoxide (µmol/L·7 days) and isoprene exposure (ppm)



Appendix C

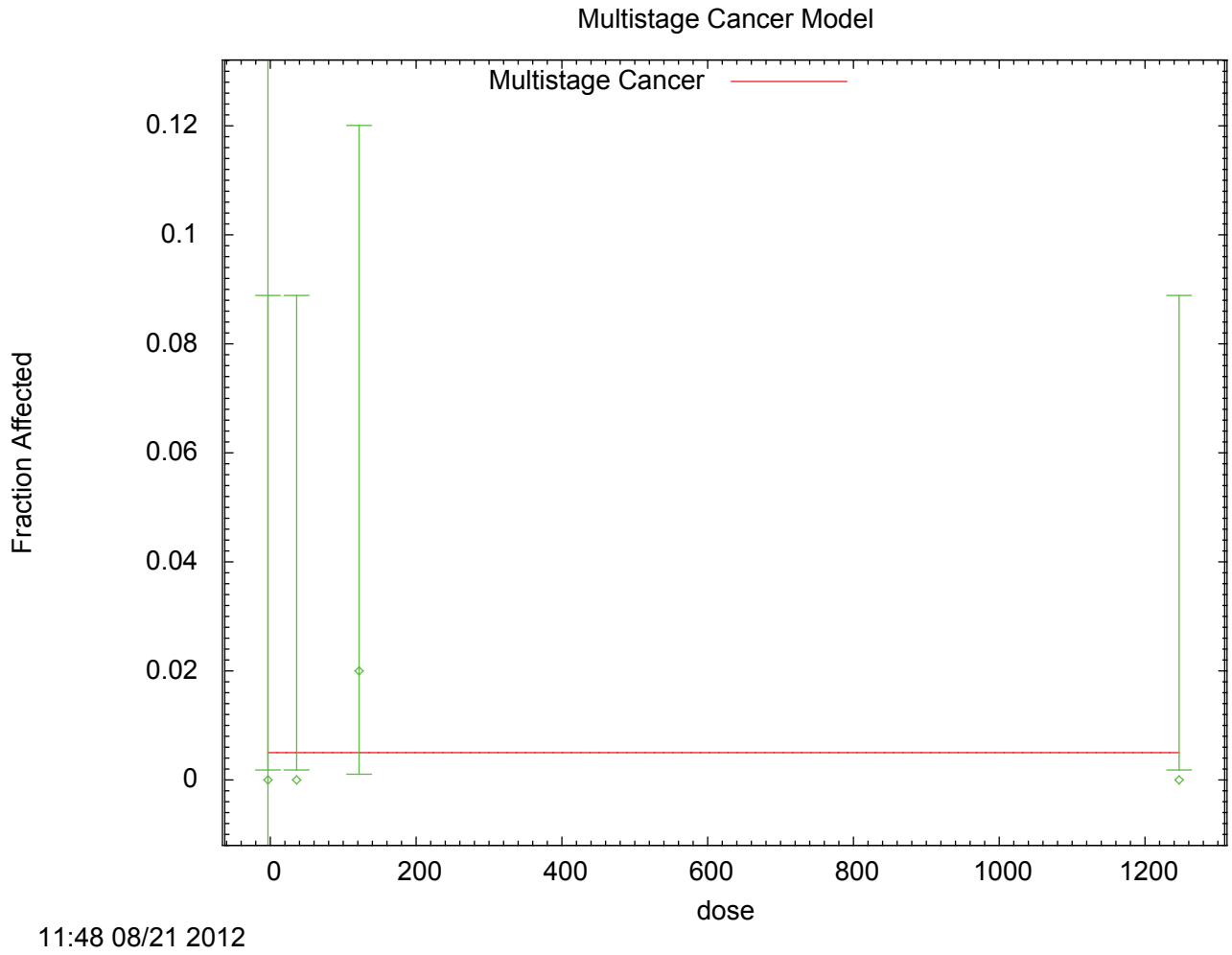
Figures from BMDS Showing the Fits of the Multistage Models

Run 1
Species: Rat
Gender: Male
Organ: Kidney
Response: Adenoma
Study: NTP 1999 m = 1

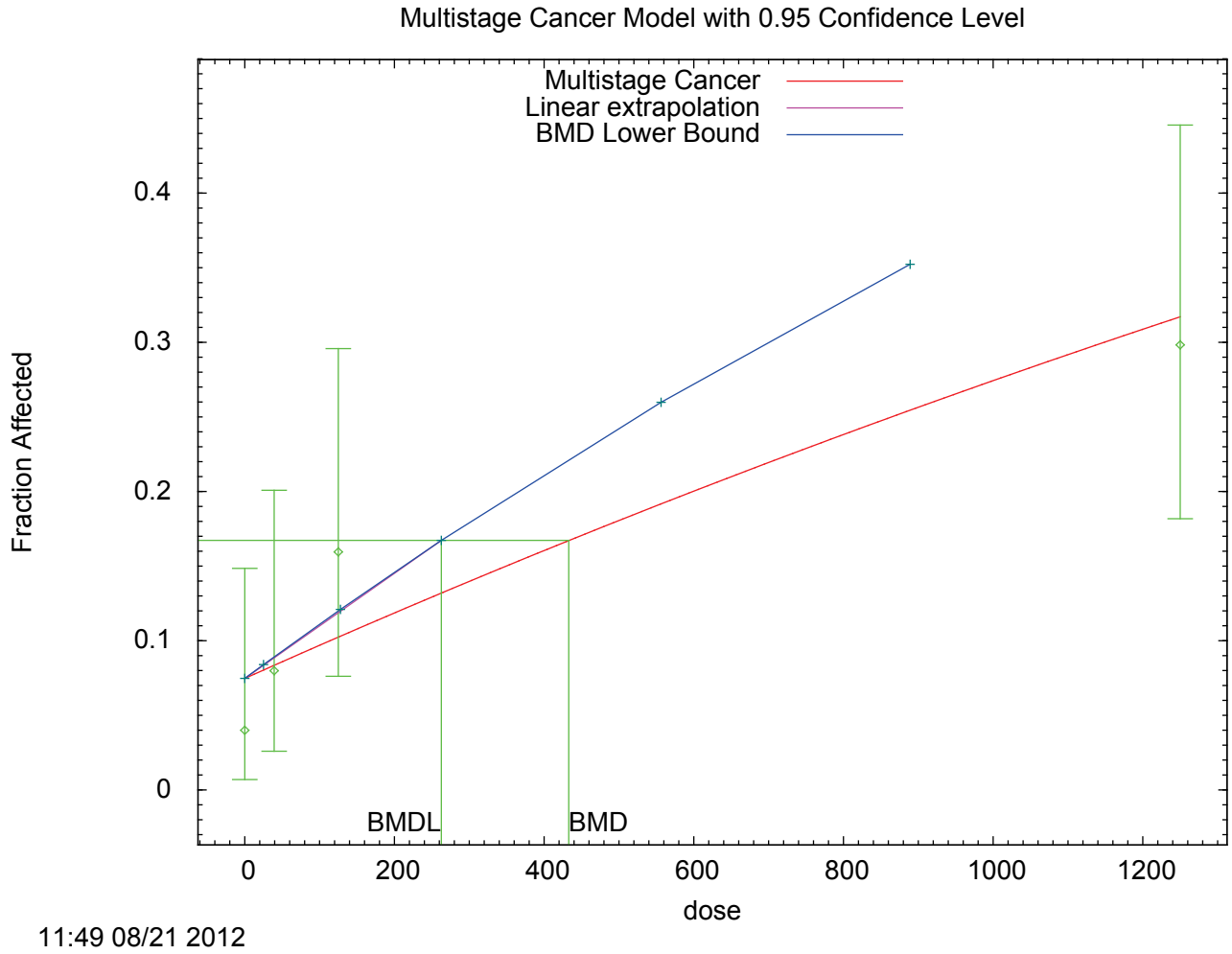


Run 2
Species: Rat
Gender: Male
Organ: Kidney
Response: Carcinoma
Study: NTP 1999 m = 1

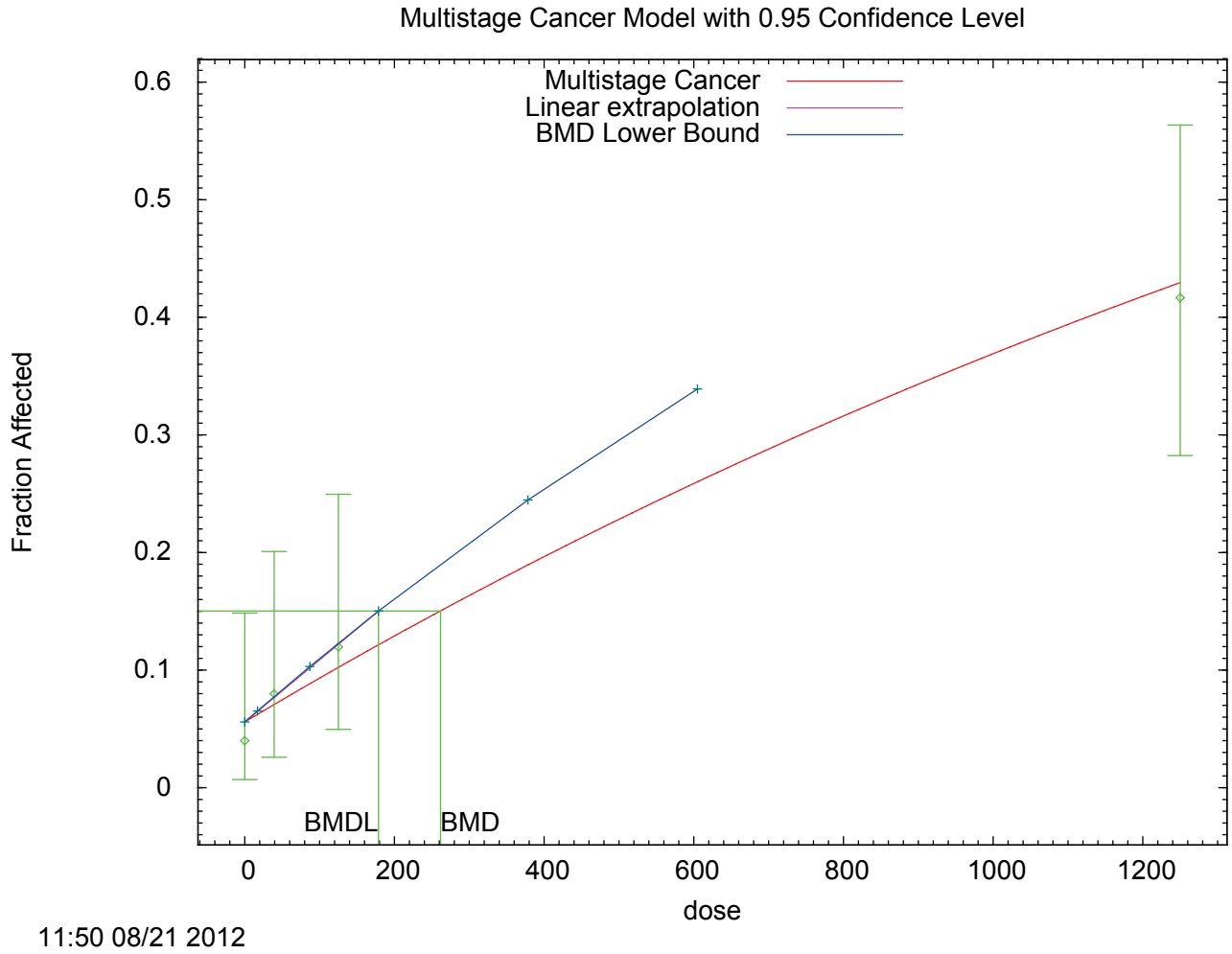
```
BMD computation failed. BMD is larger than three times maximum input doses.
```



Run 3
Species: Rat
Gender: Male
Organ: Kidney
Response: Adenoma/Carcinoma
Study: NTP 1999 m = 1

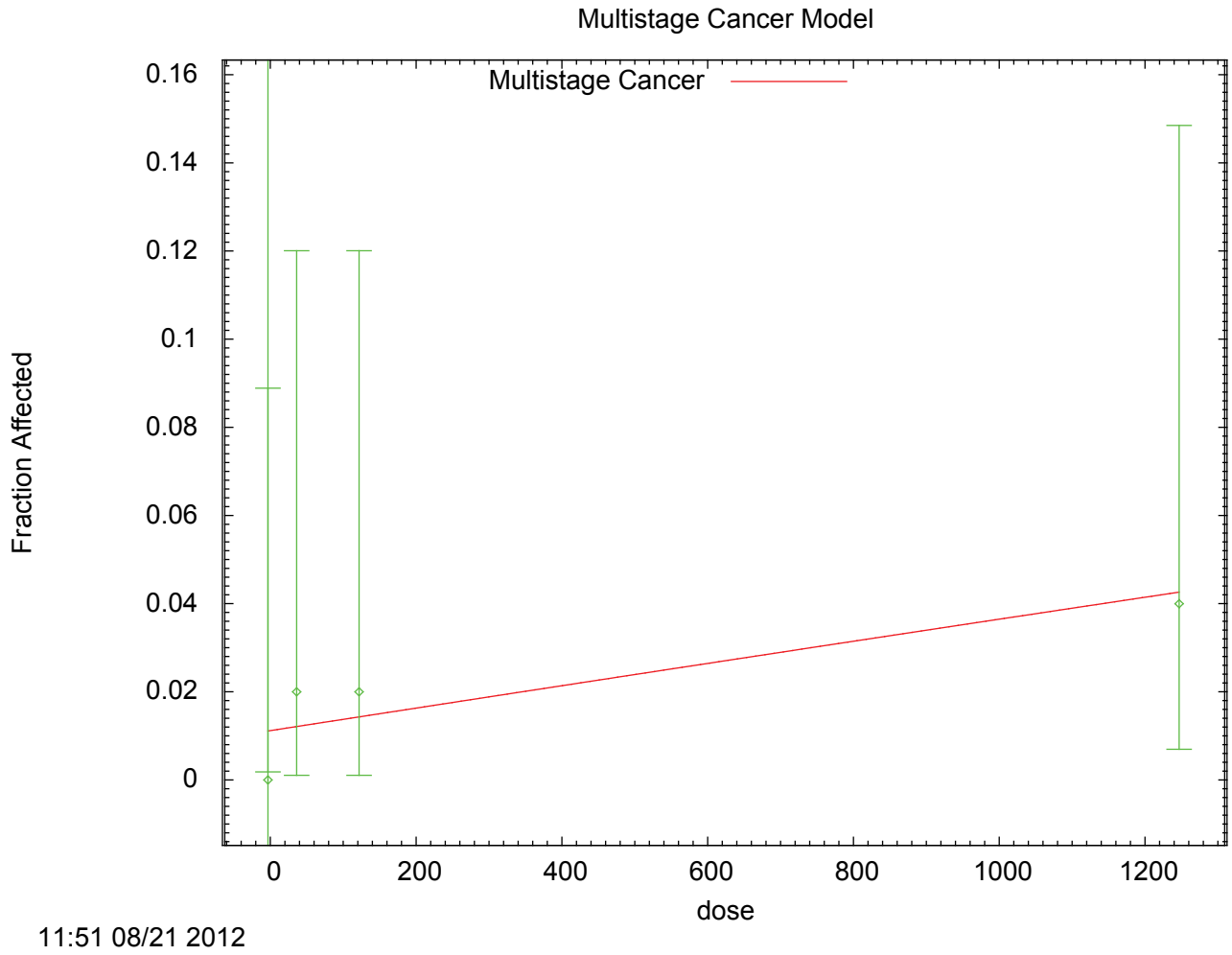


Run 4
Species: Rat
Gender: Male
Organ: Mammary gland
Response: Fibroadenoma
Study: NTP 1999 m = 1

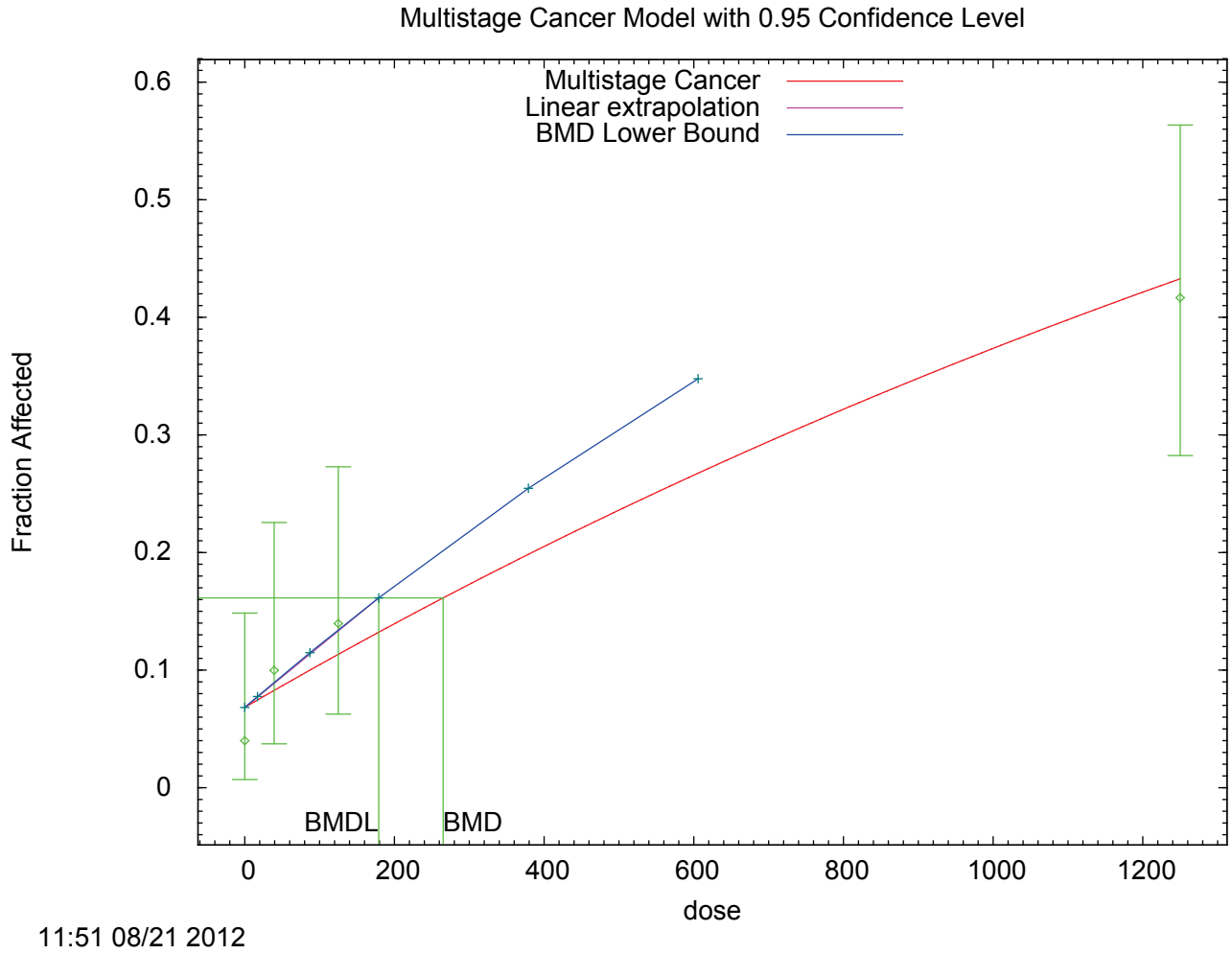


Run 5
Species: Rat
Gender: Male
Organ: Mammary gland
Response: Carcinoma
Study: NTP 1999 m = 1

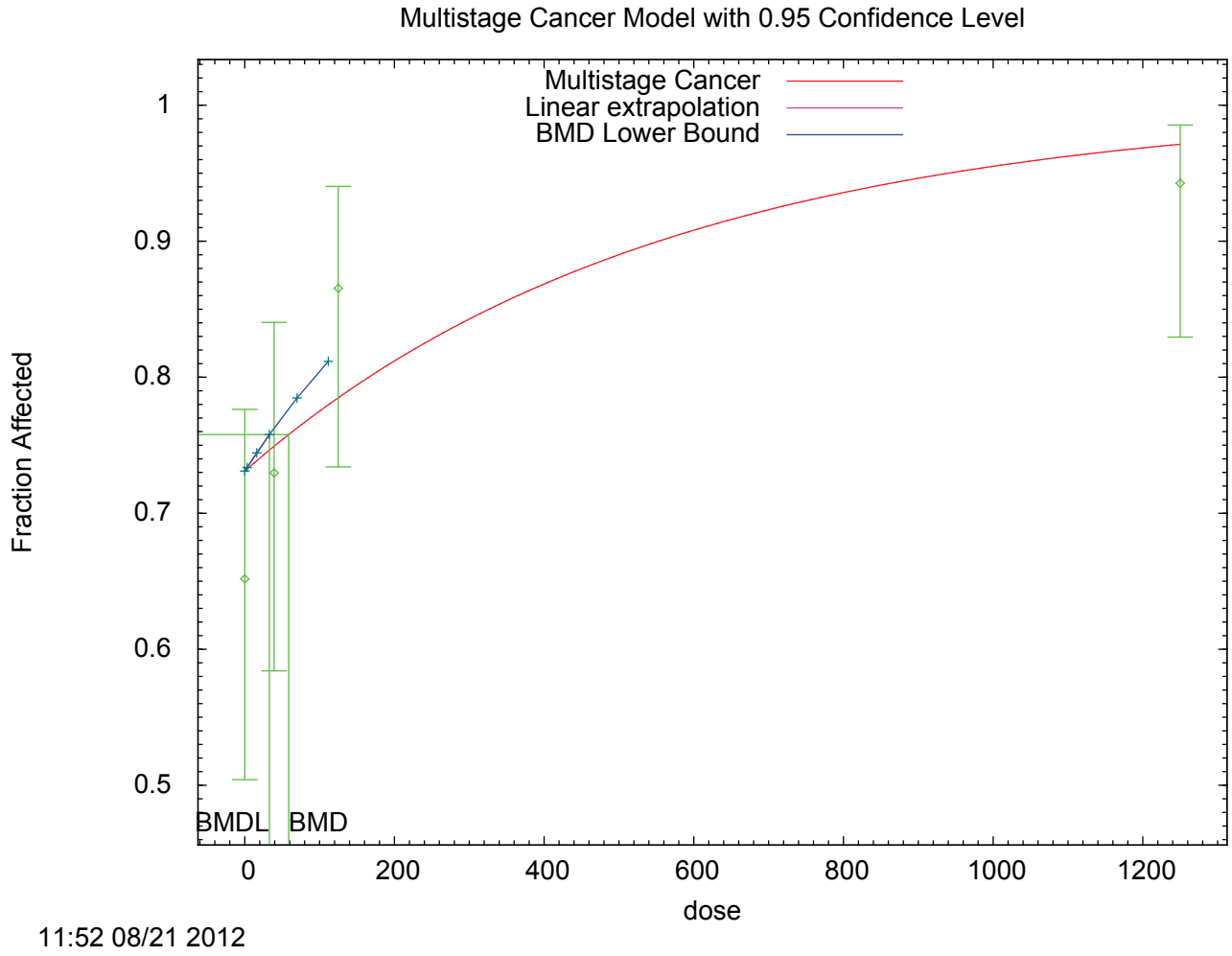
```
BMD computation failed. BMD is larger than three times maximum input doses.
```



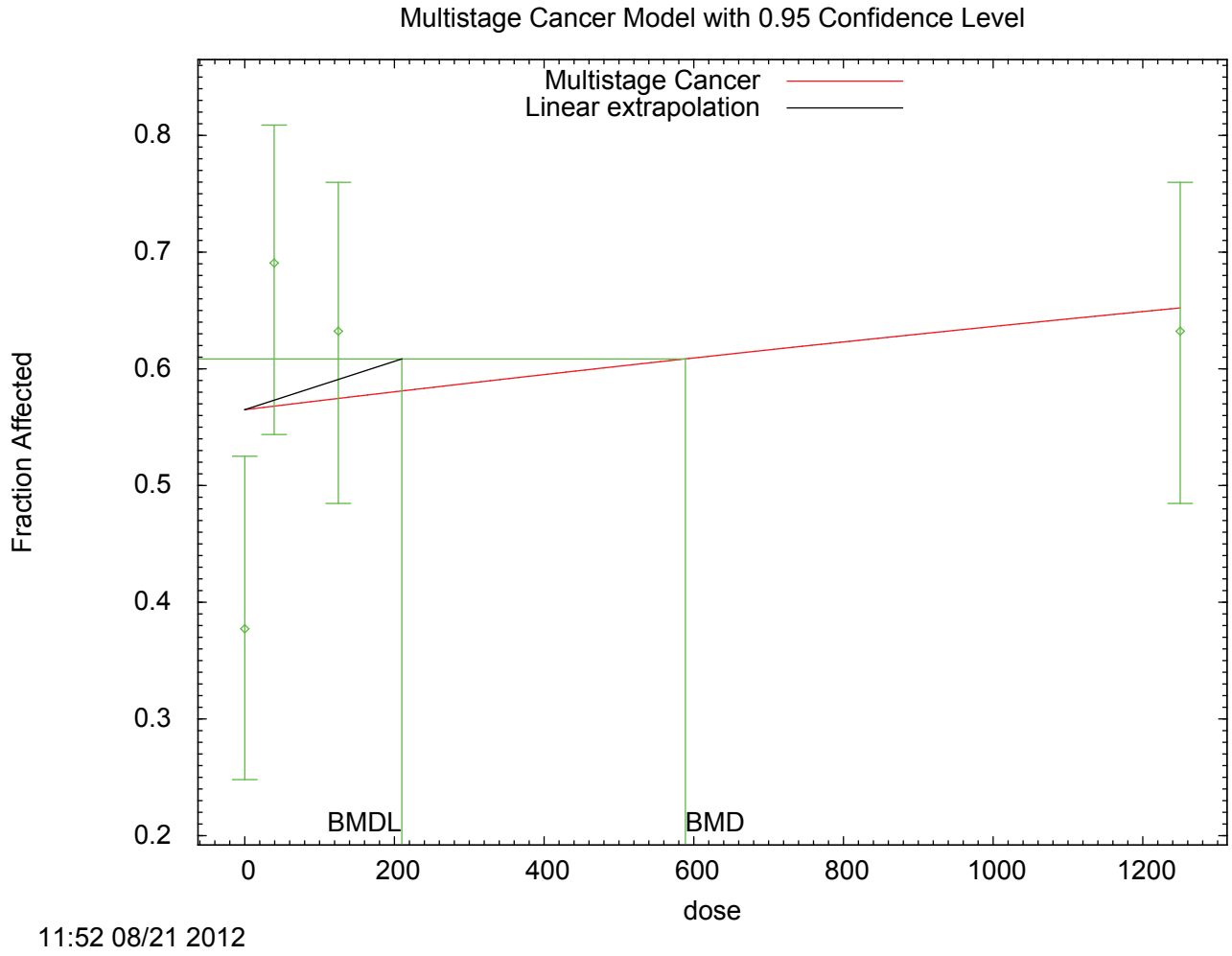
Run 6
Species: Rat
Gender: Male
Organ: Mammary gland
Response: Fibroadenoma/Carcinoma
Study: NTP 1999 m = 1



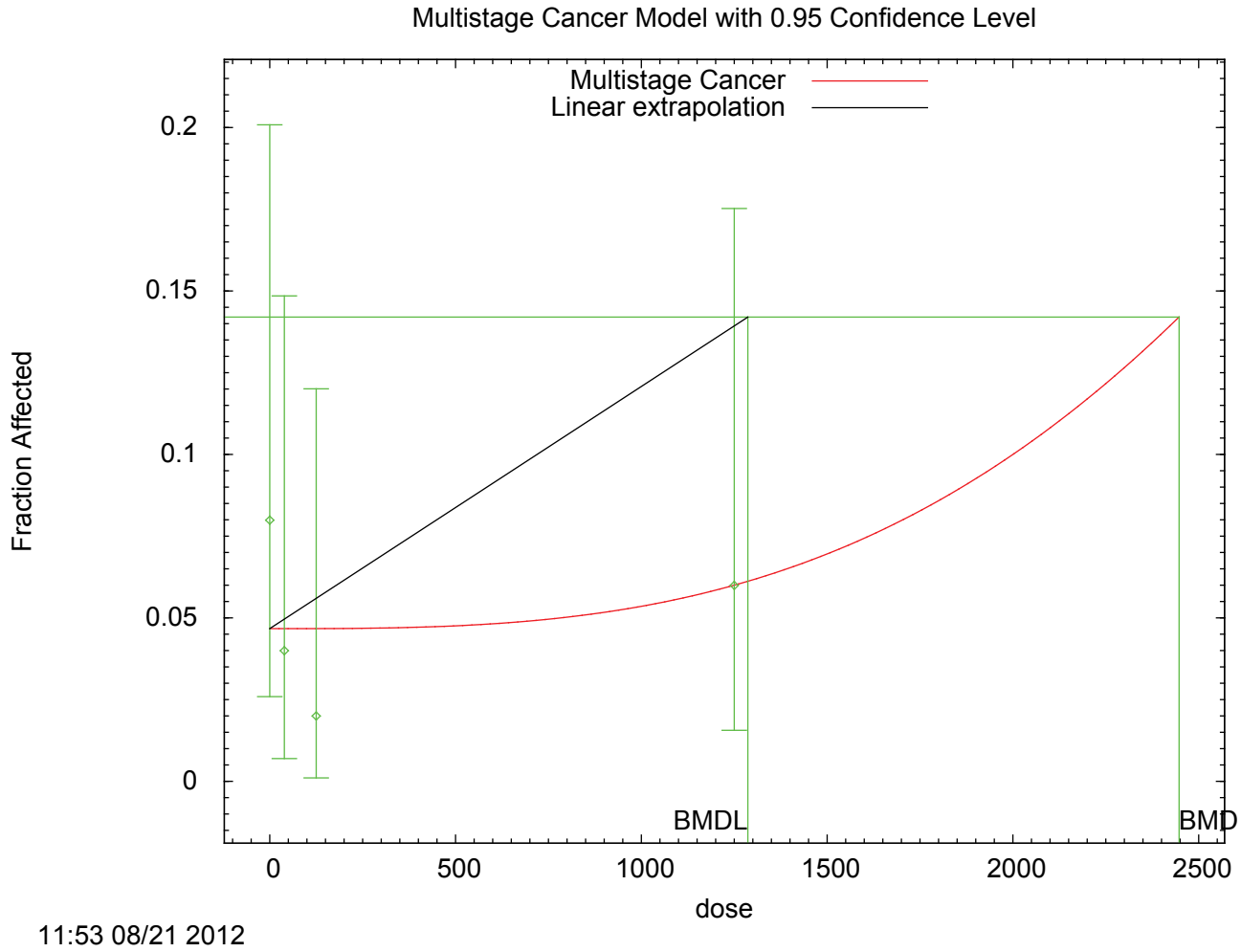
Run 7
Species: Rat
Gender: Male
Organ: Testis
Response: Adenoma
Study: NTP 1999 m = 1



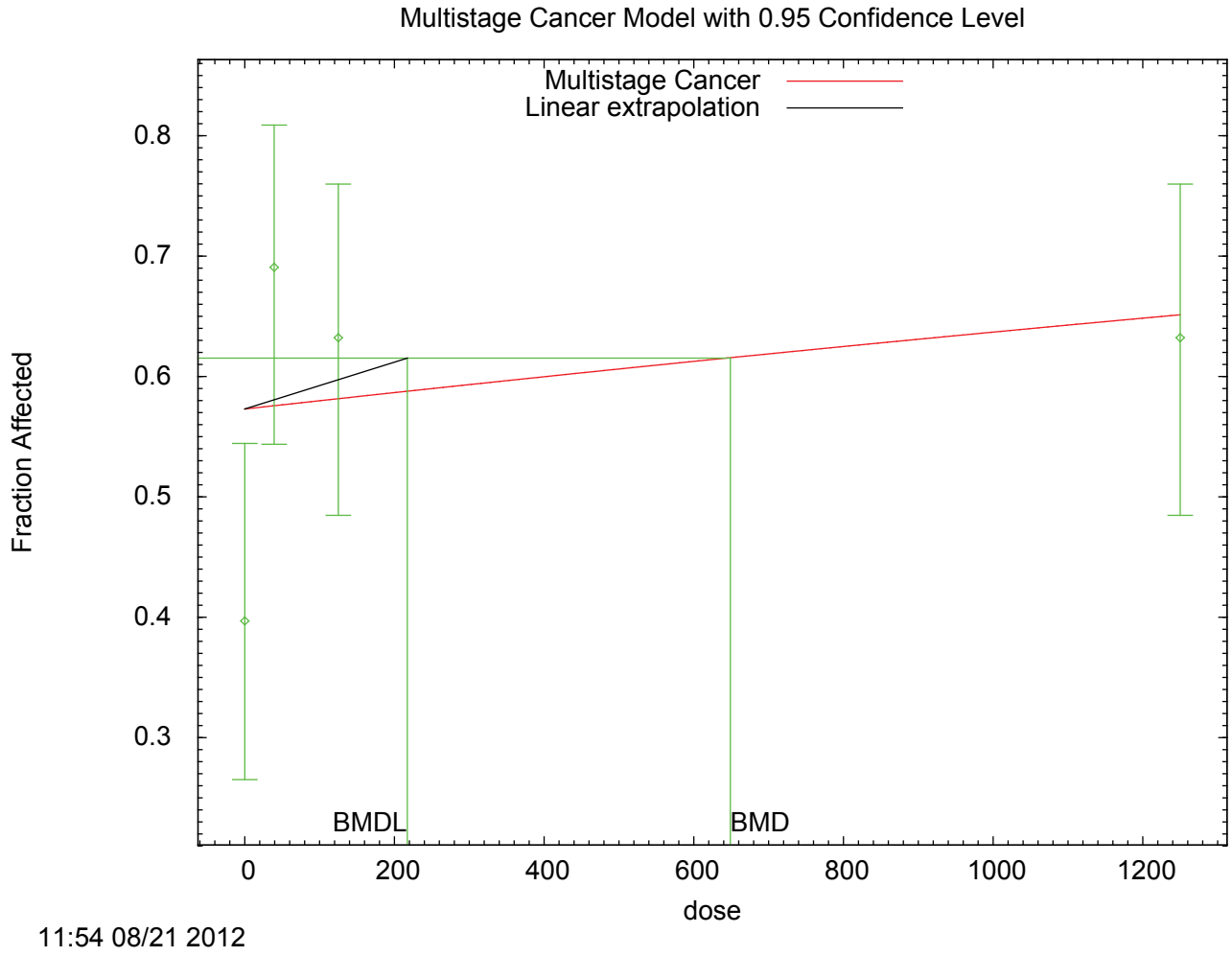
Run 8
Species: Rat
Gender: Female
Organ: Mammary gland
Response: Fibroadenoma
Study: NTP 1999 m = 1



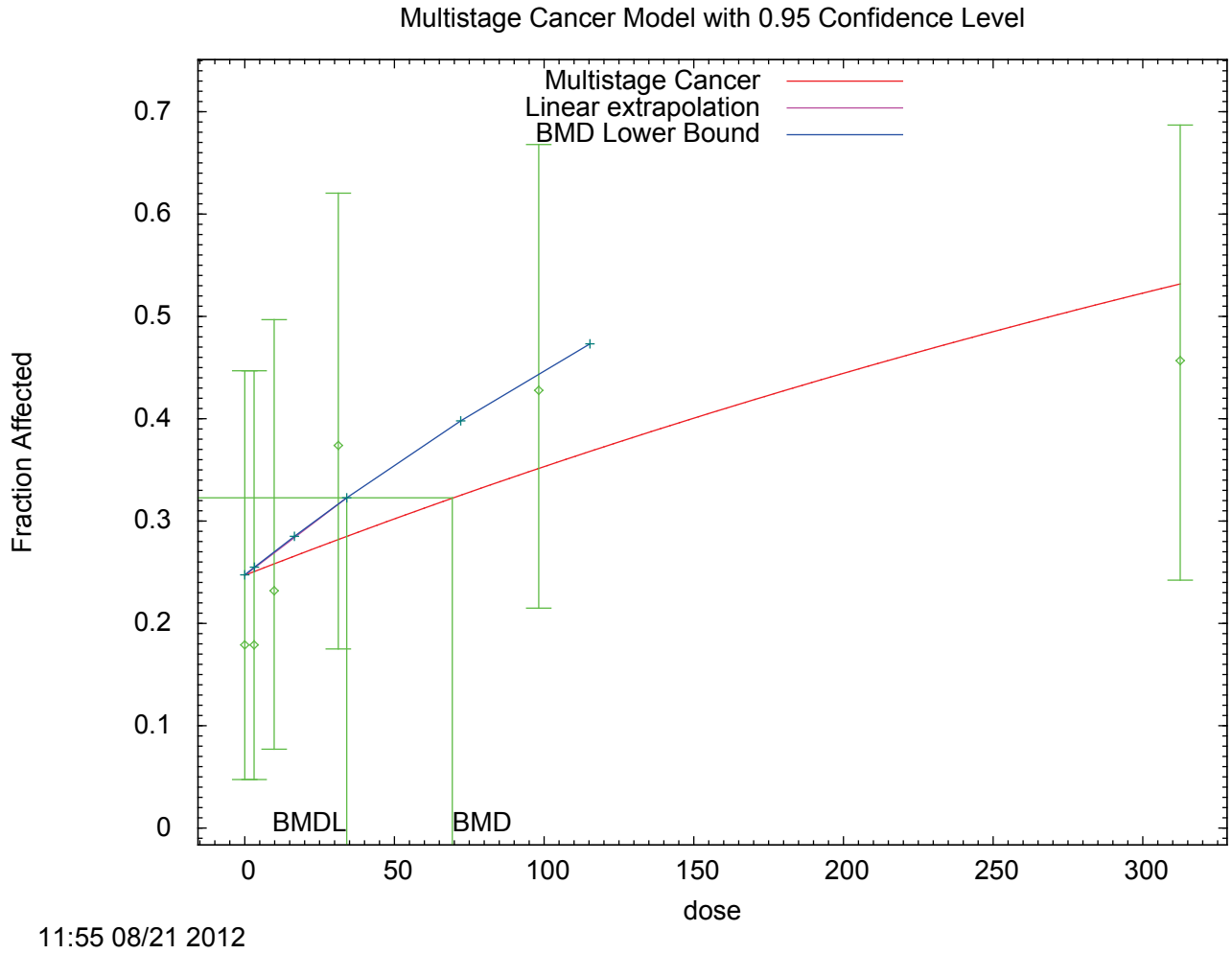
Run 9
Species: Rat
Gender: Female
Organ: Mammary gland
Response: Carcinoma
Study: NTP 1999 m = 1



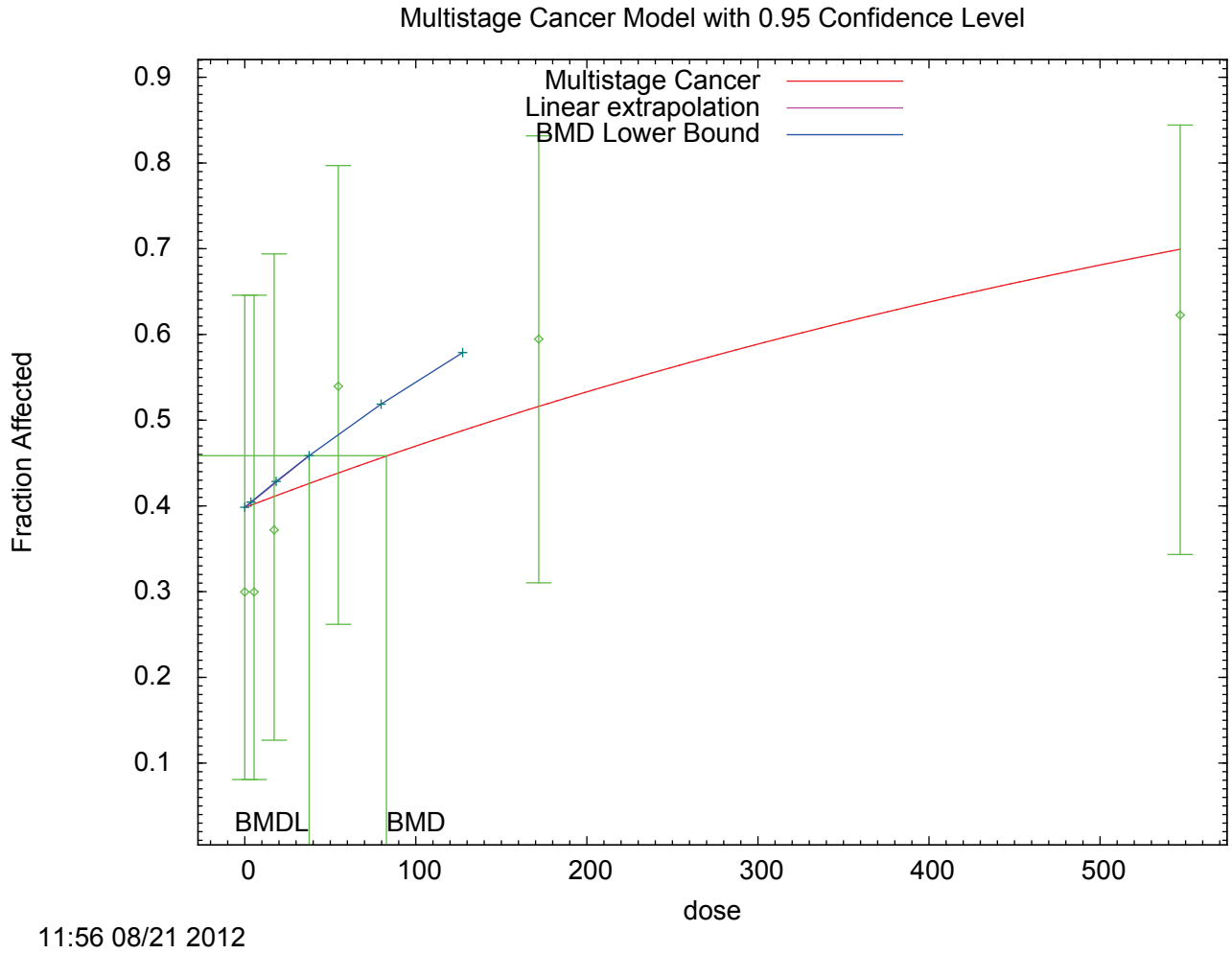
Run 10
Species: Rat
Gender: Female
Organ: Mammary gland
Response: Fibroadenoma/Carcinoma
Study: NTP 1999 m = 1



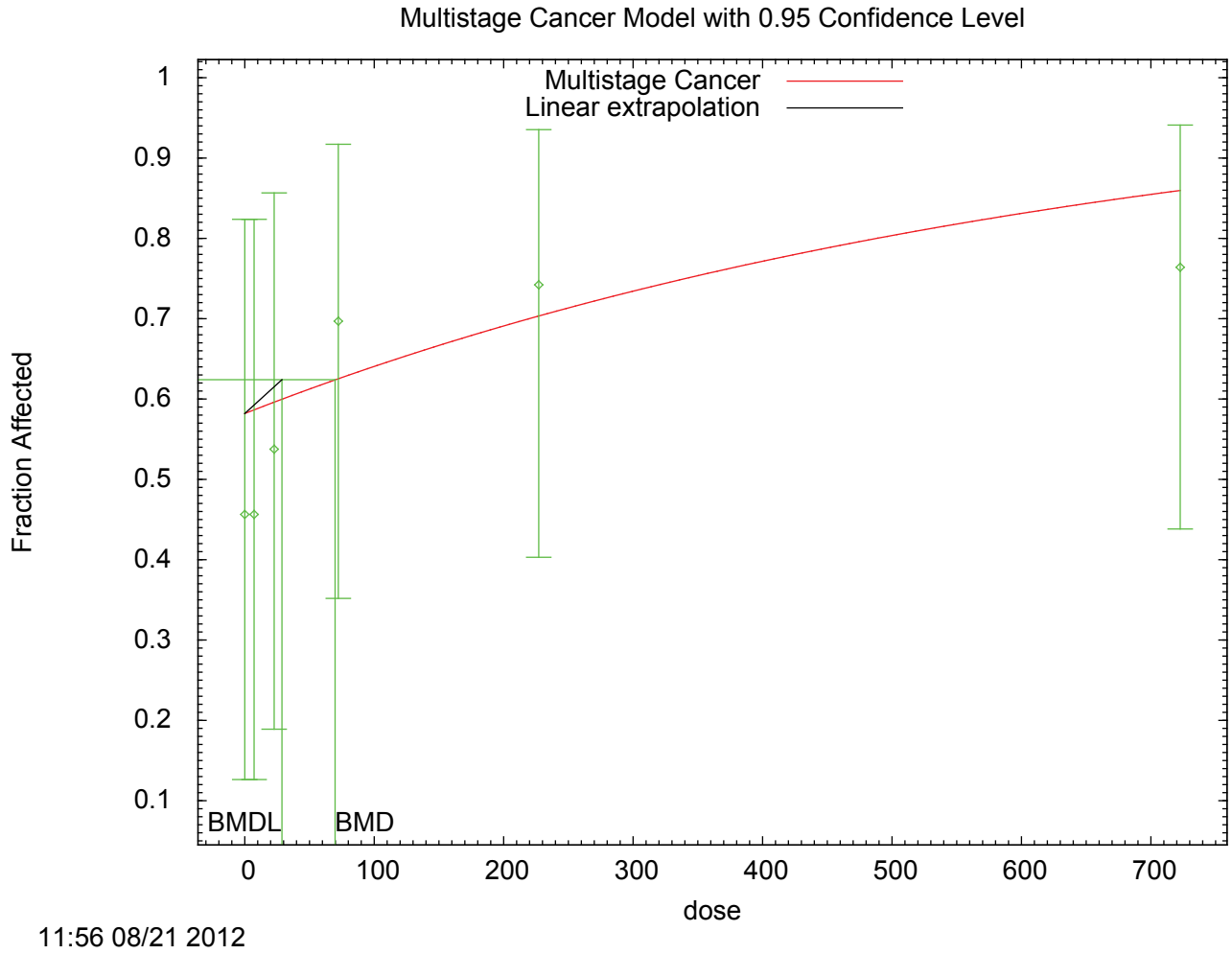
Run 11
Species: Rat
Gender: Male
Organ: Testis
Response: Adenoma
Study: NTP 1994 m = 1



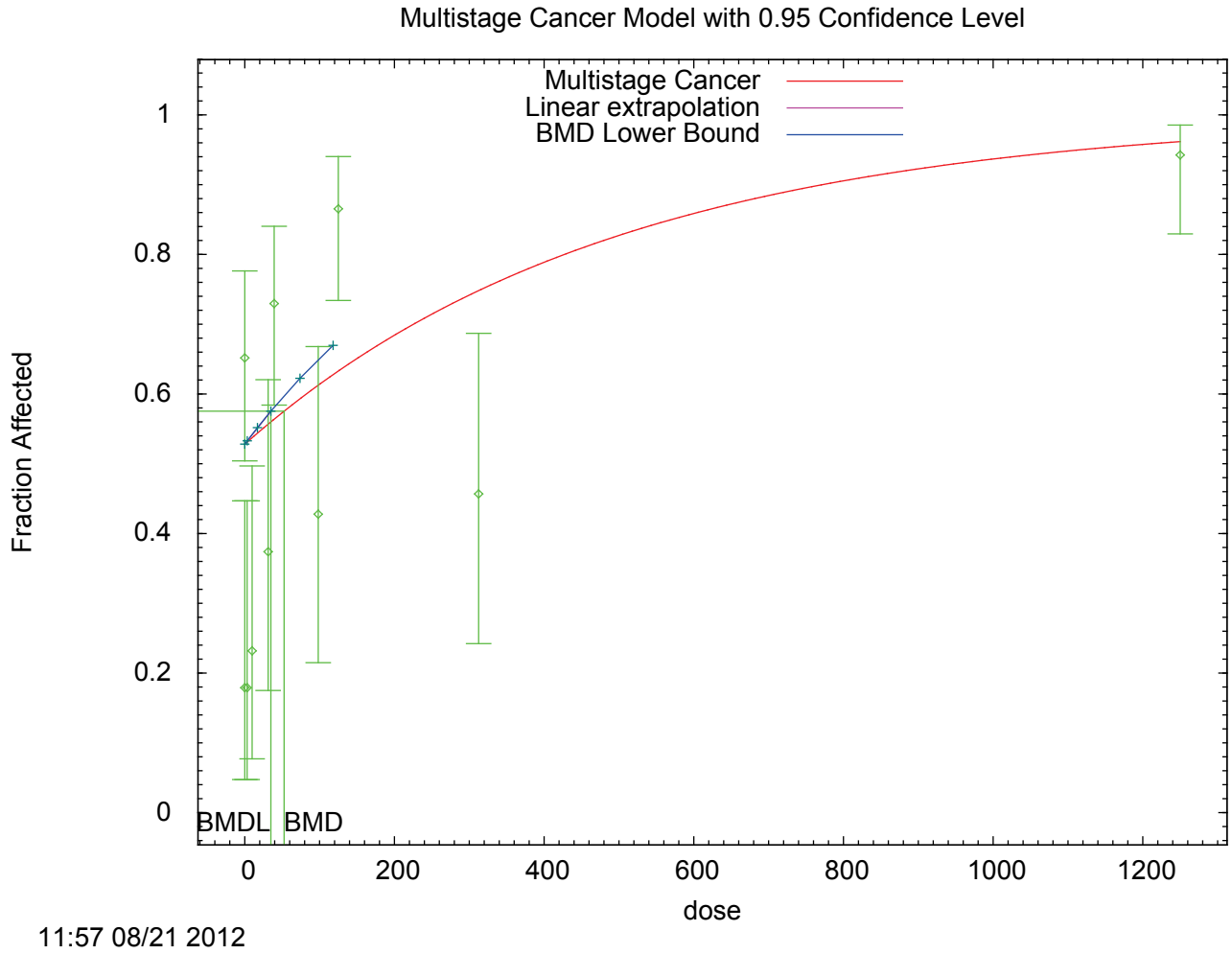
Run 12
Species: Rat
Gender: Male
Organ: Testis
Response: Adenoma
Study: NTP 1994 m = 2



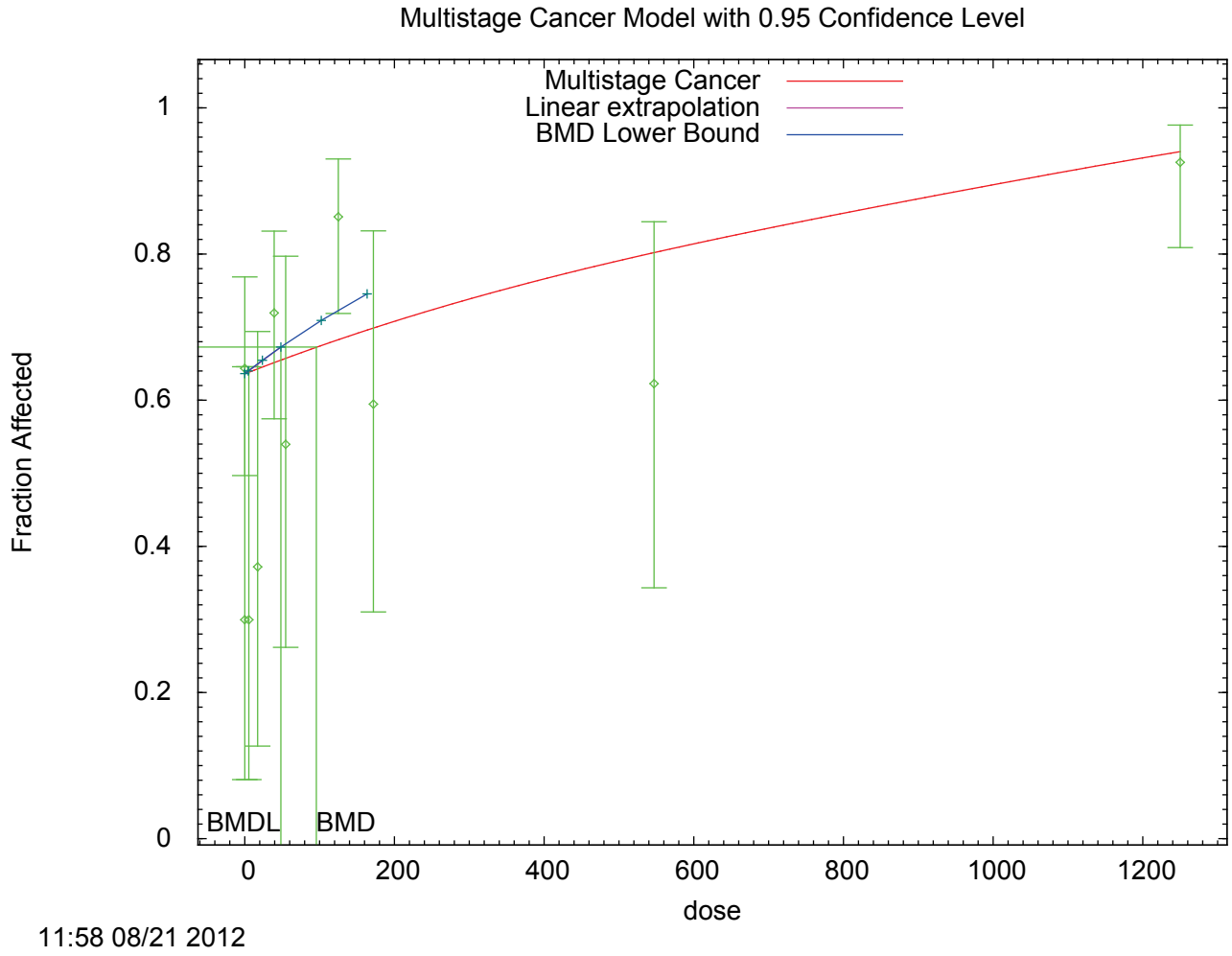
Run 13
Species: Rat
Gender: Male
Organ: Testis
Response: Adenoma
Study: NTP 1994 m = 3



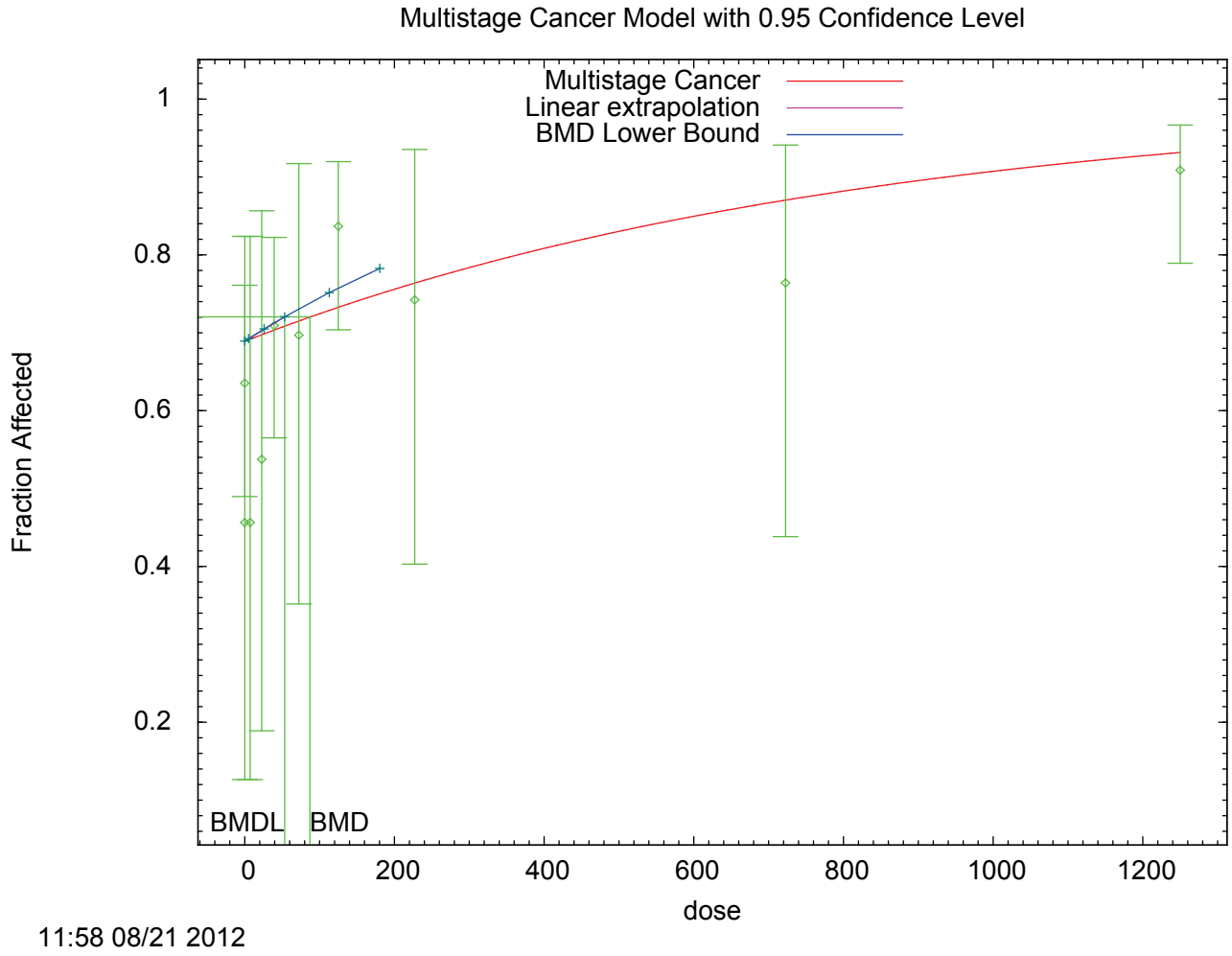
Run 14
Species: Rat
Gender: Male
Organ: Testis
Response: Adenoma
Study: NTP 1994 and NTP 1999 Combined m = 1



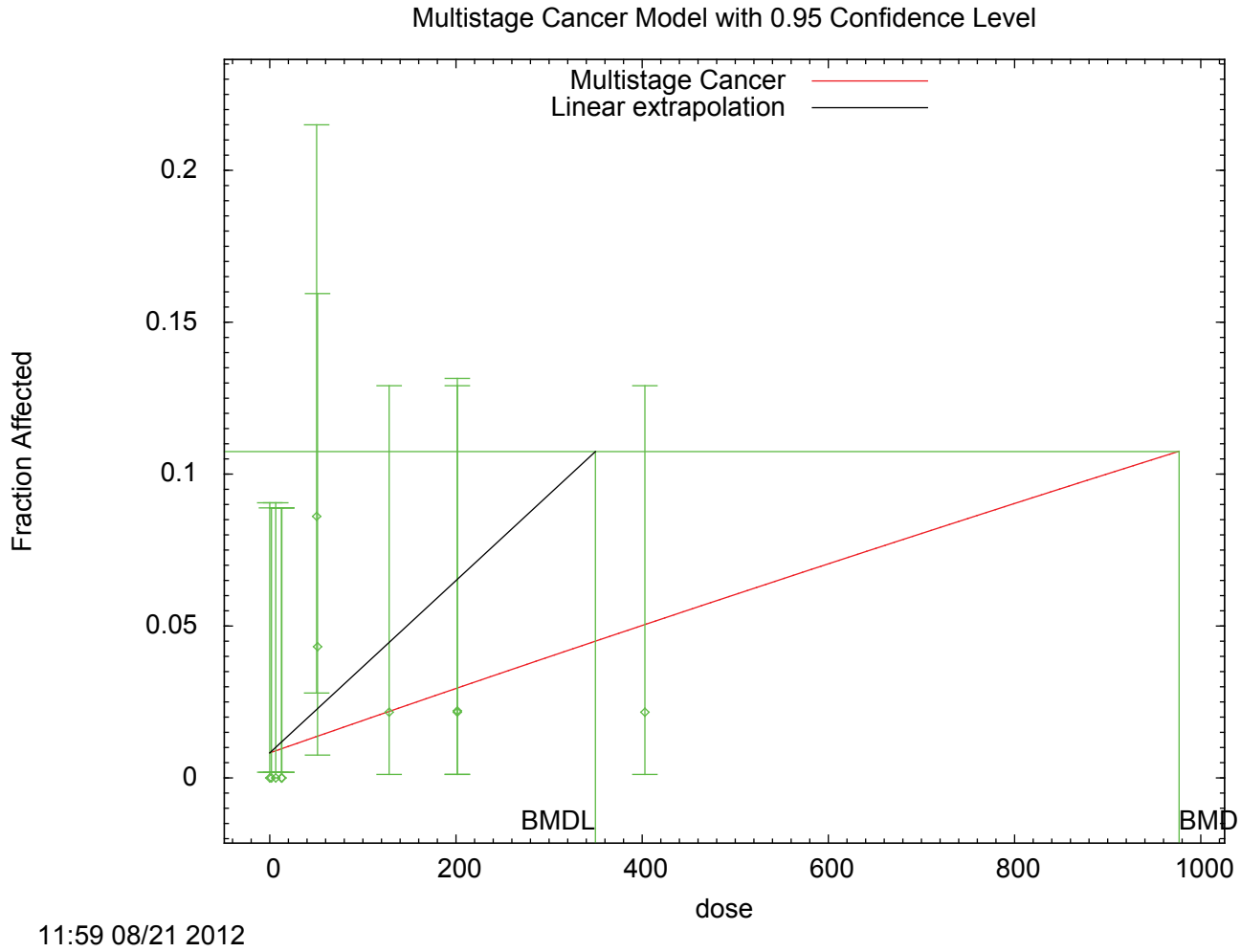
Run 15
Species: Rat
Gender: Male
Organ: Testis
Response: Adenoma
Study: NTP 1994 and NTP 1999 Combined m = 2



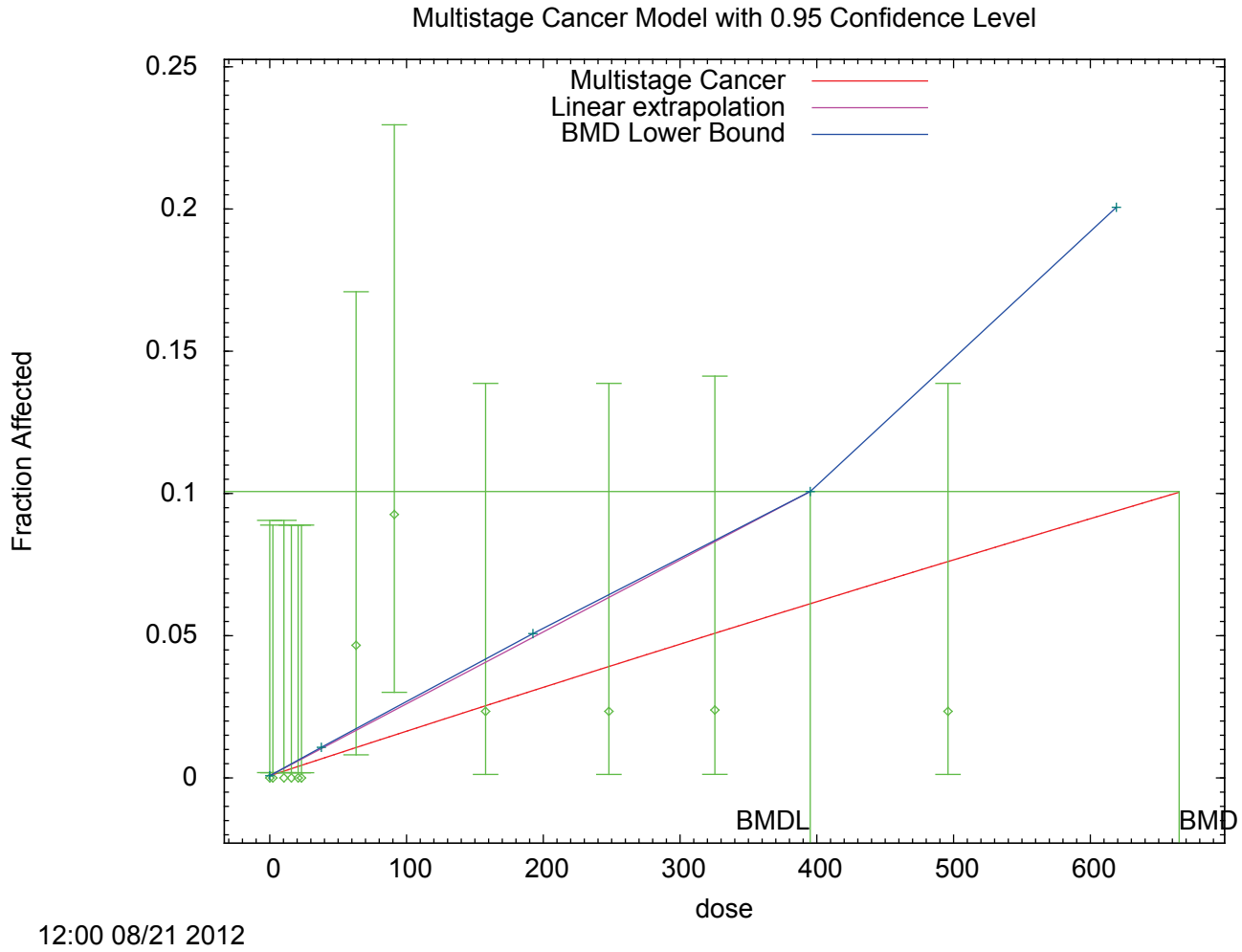
Run 16
Species: Rat
Gender: Male
Organ: Testis
Response: Adenoma
Study: NTP 1994 and NTP 1999 Combined m = 3



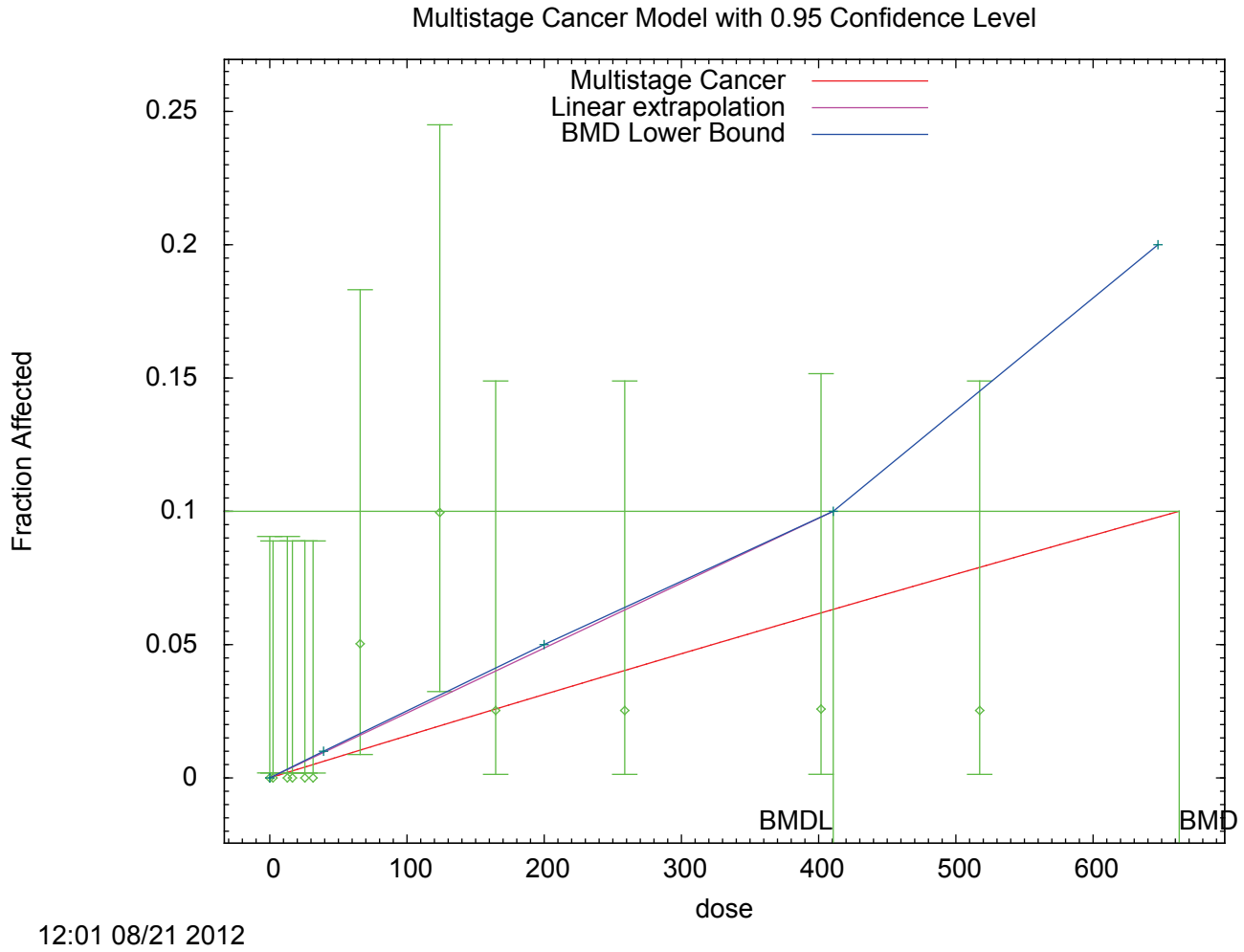
Run 17
Species: Mouse
Gender: Male
Organ: Heart
Response: Hemangiosarcoma
Study: Placke et al. 1996 m = 1



Run 18
Species: Mouse
Gender: Male
Organ: Heart
Response: Hemangiosarcoma
Study: Placke et al. 1996 m = 2

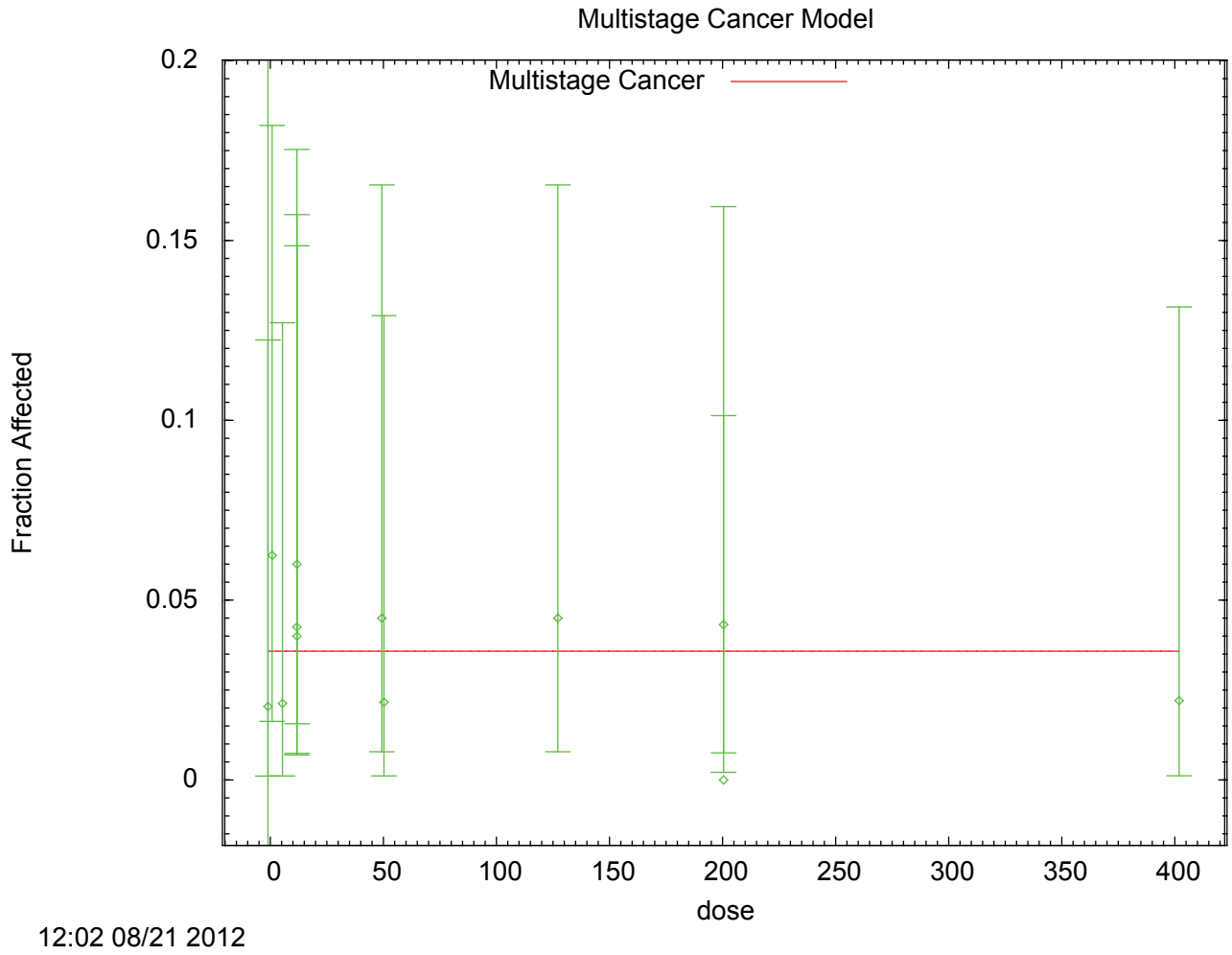


Run 19
Species: Mouse
Gender: Male
Organ: Heart
Response: Hemangiosarcoma
Study: Placke et al. 1996 m = 3



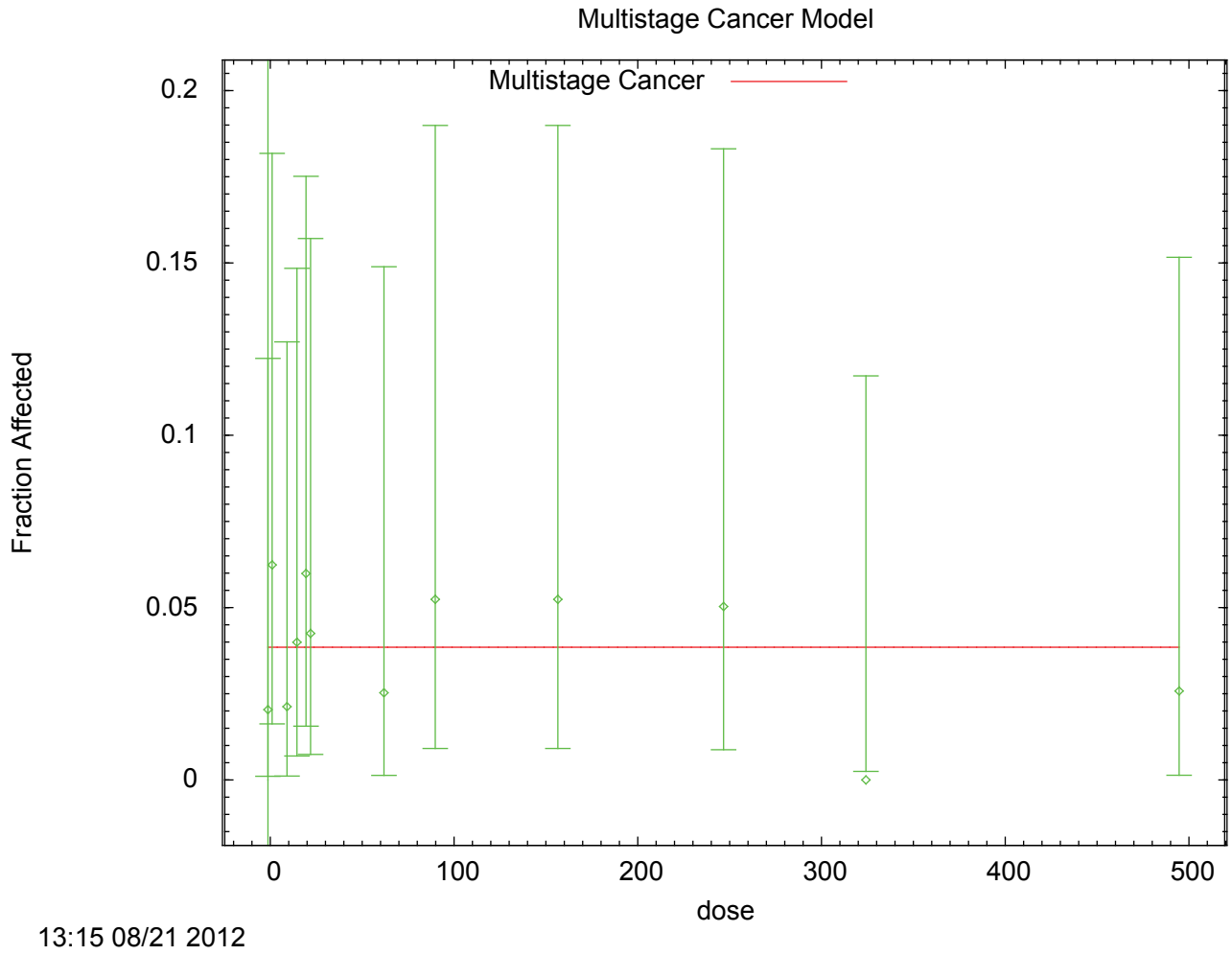
Run 20
Species: Mouse
Gender: Male
Organ: Spleen
Response: Hemangiosarcoma
Study: Placke et al. 1996 m = 1

```
BMD computation failed. BMD is larger than three times maximum input doses.
```



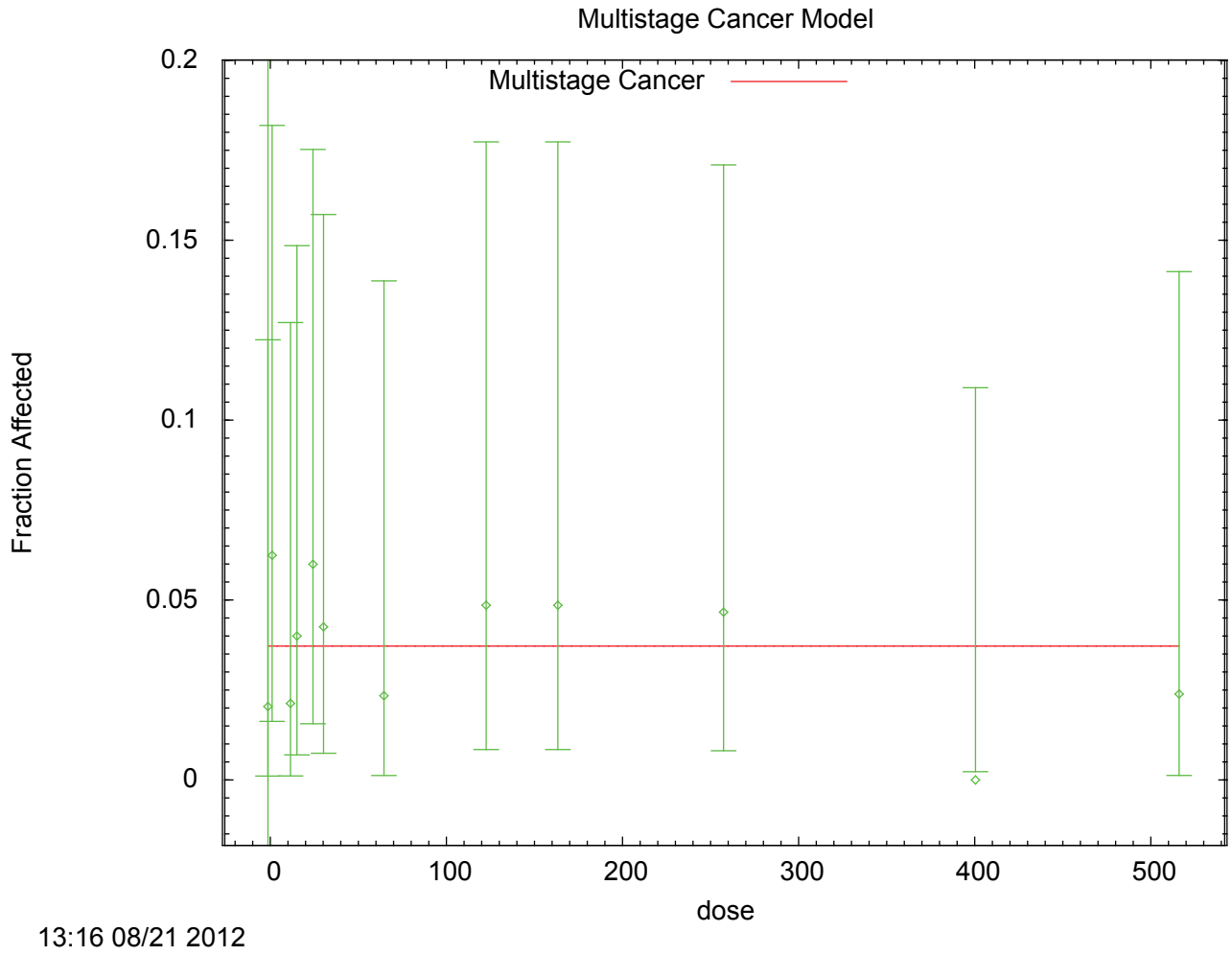
Run 21
Species: Mouse
Gender: Male
Organ: Spleen
Response: Hemangiosarcoma
Study: Placke et al. 1996 m = 2

```
BMD computation failed. BMD is larger than three times maximum input doses.
```

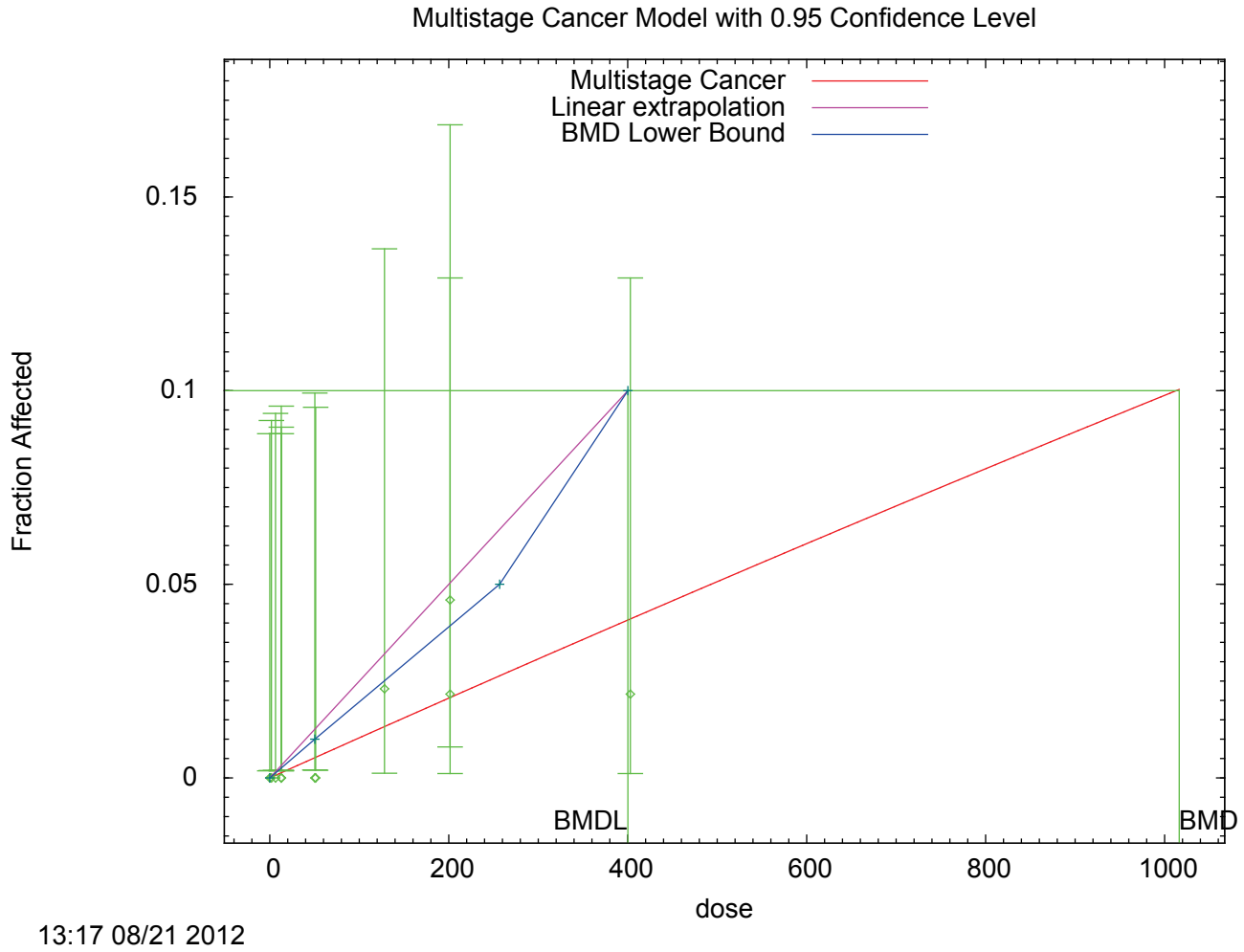


Run 22
Species: Mouse
Gender: Male
Organ: Spleen
Response: Hemangiosarcoma
Study: Placke et al. 1996 m = 3

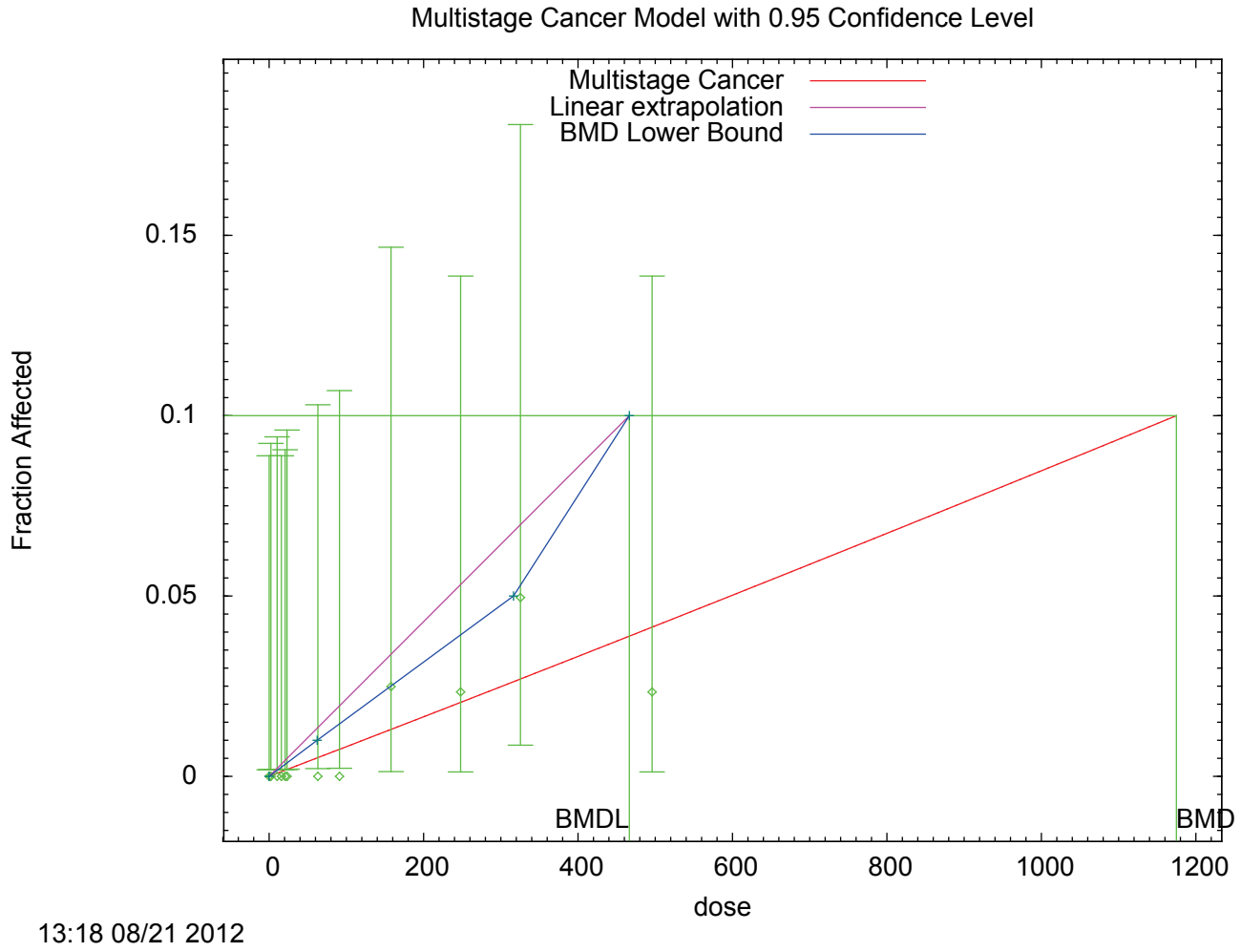
```
BMD computation failed. BMD is larger than three times maximum input doses.
```



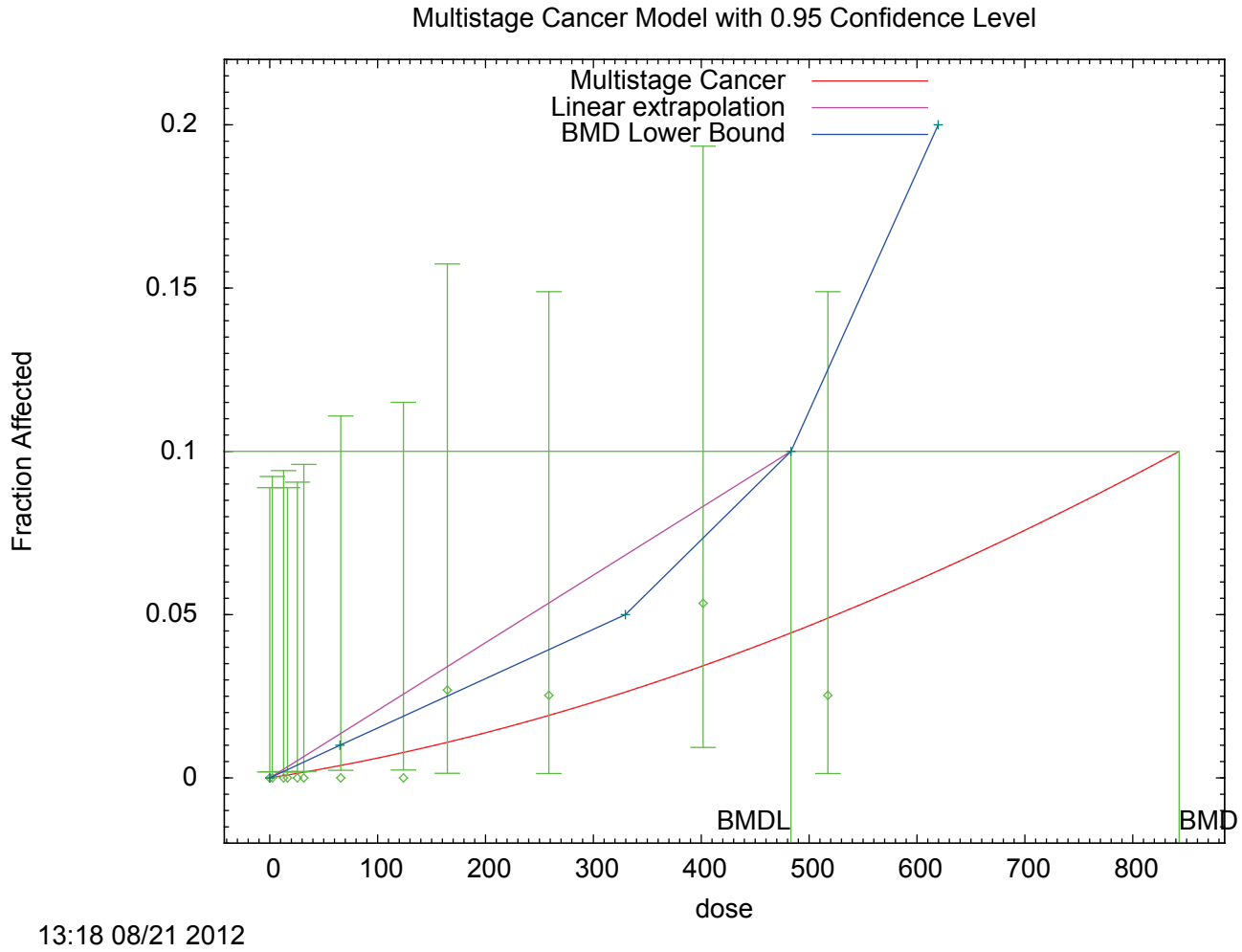
Run 23
Species: Mouse
Gender: Male
Organ: Forestomach
Response: Papilloma
Study: Placke et al. 1996 m = 1



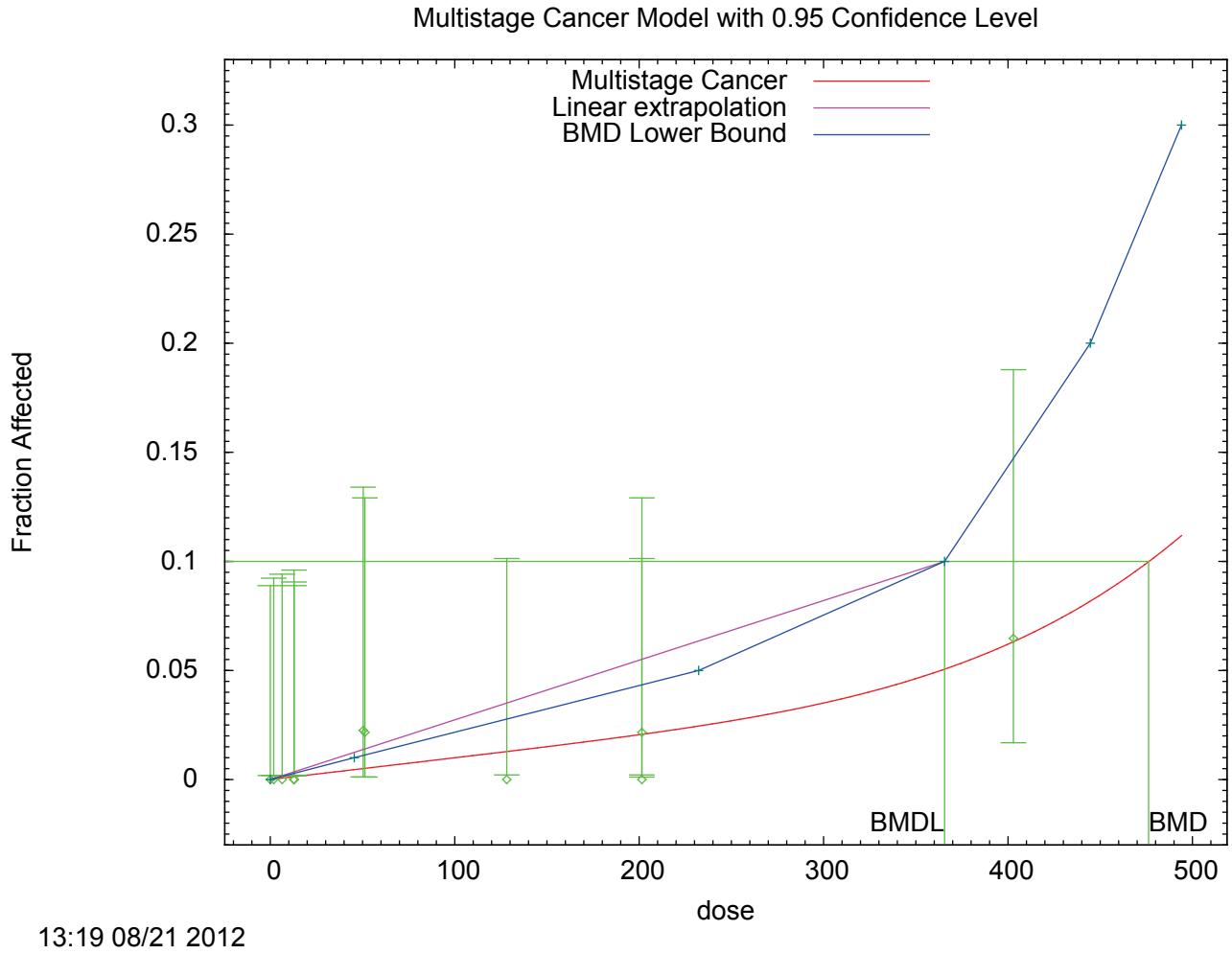
Run 24
Species: Mouse
Gender: Male
Organ: Forestomach
Response: Papilloma
Study: Placke et al. 1996 m = 2



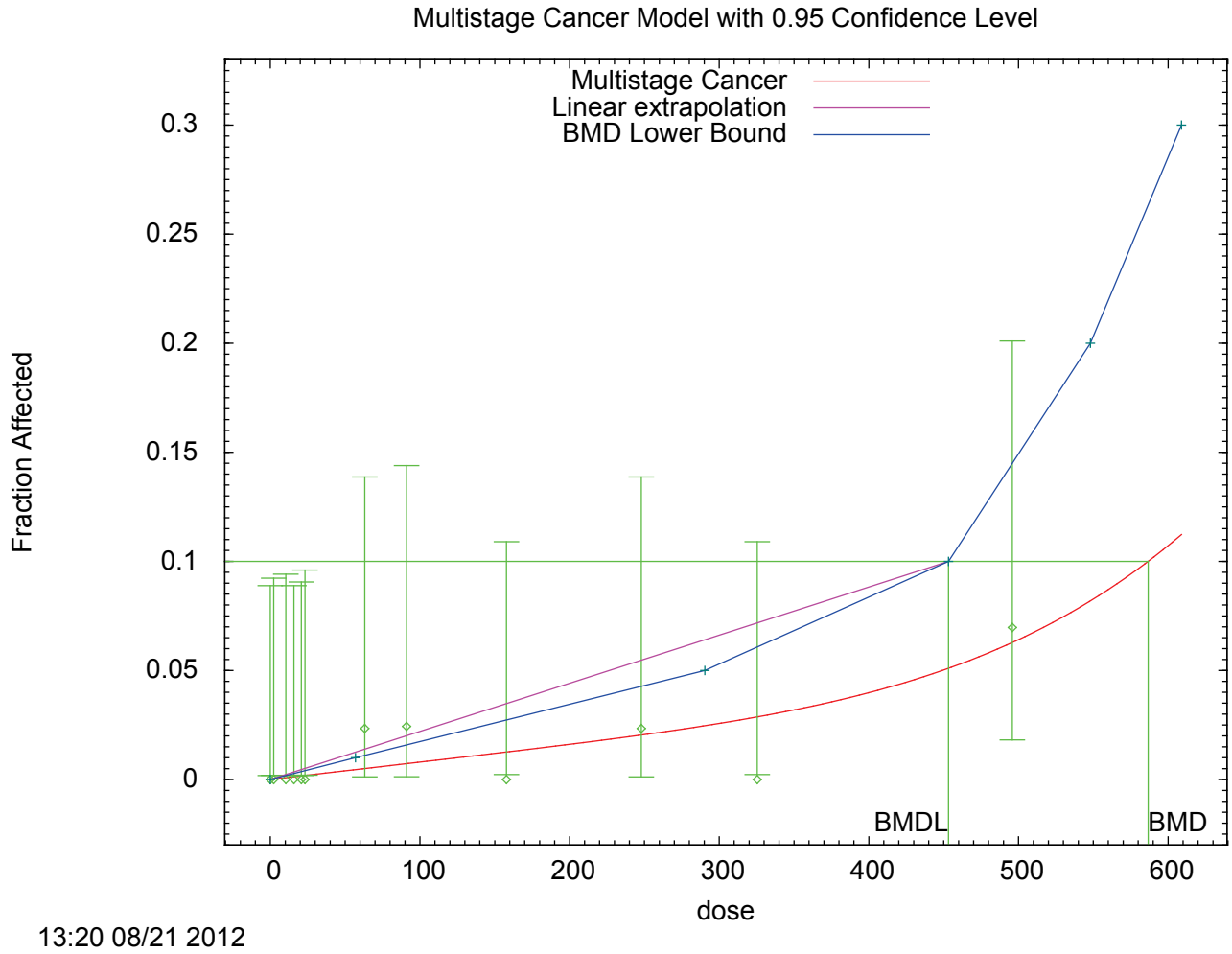
Run 25
Species: Mouse
Gender: Male
Organ: Forestomach
Response: Papilloma
Study: Placke et al. 1996 m = 3



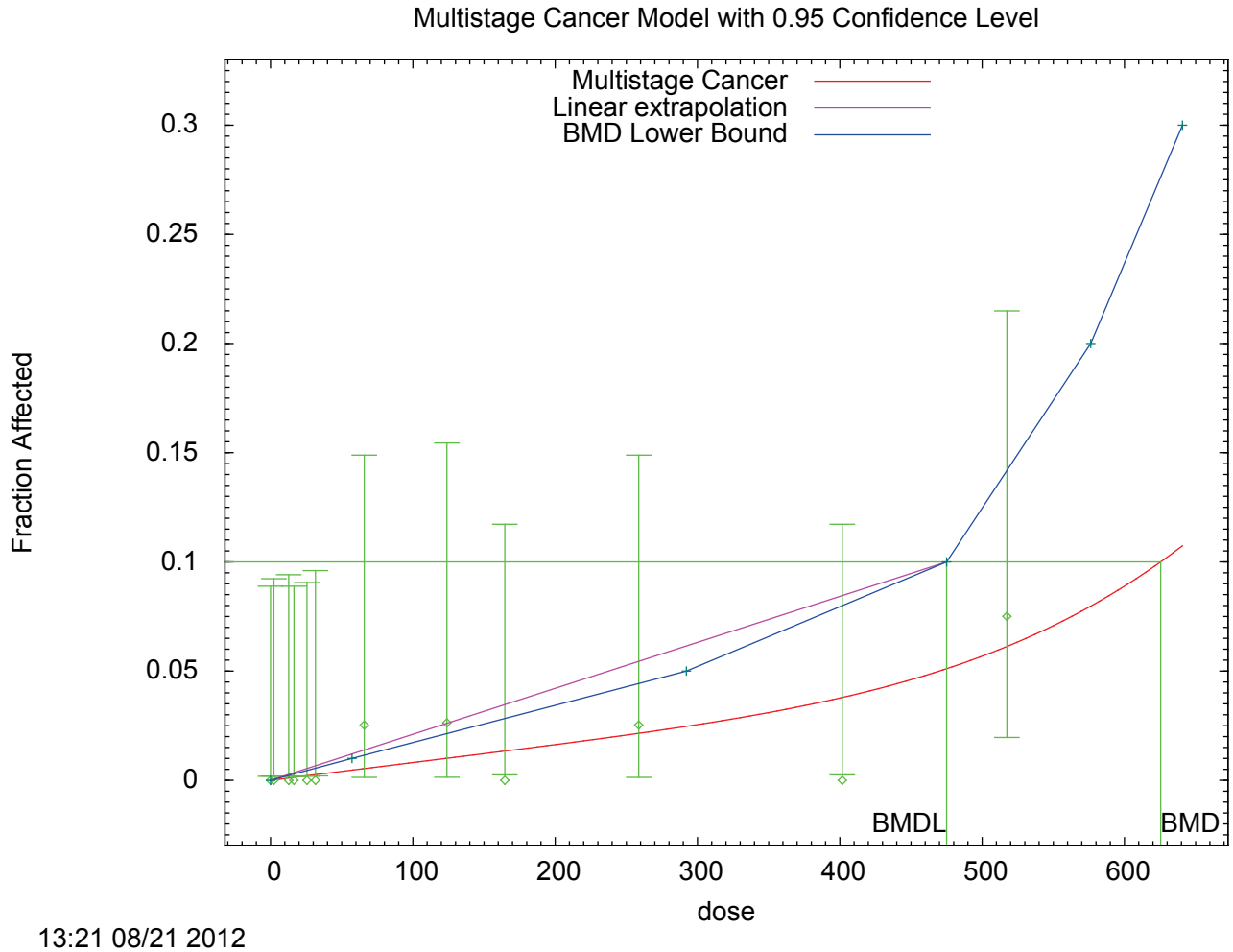
Run 26
Species: Mouse
Gender: Male
Organ: Forestomach
Response: Carcinoma
Study: Placke et al. 1996 m = 1



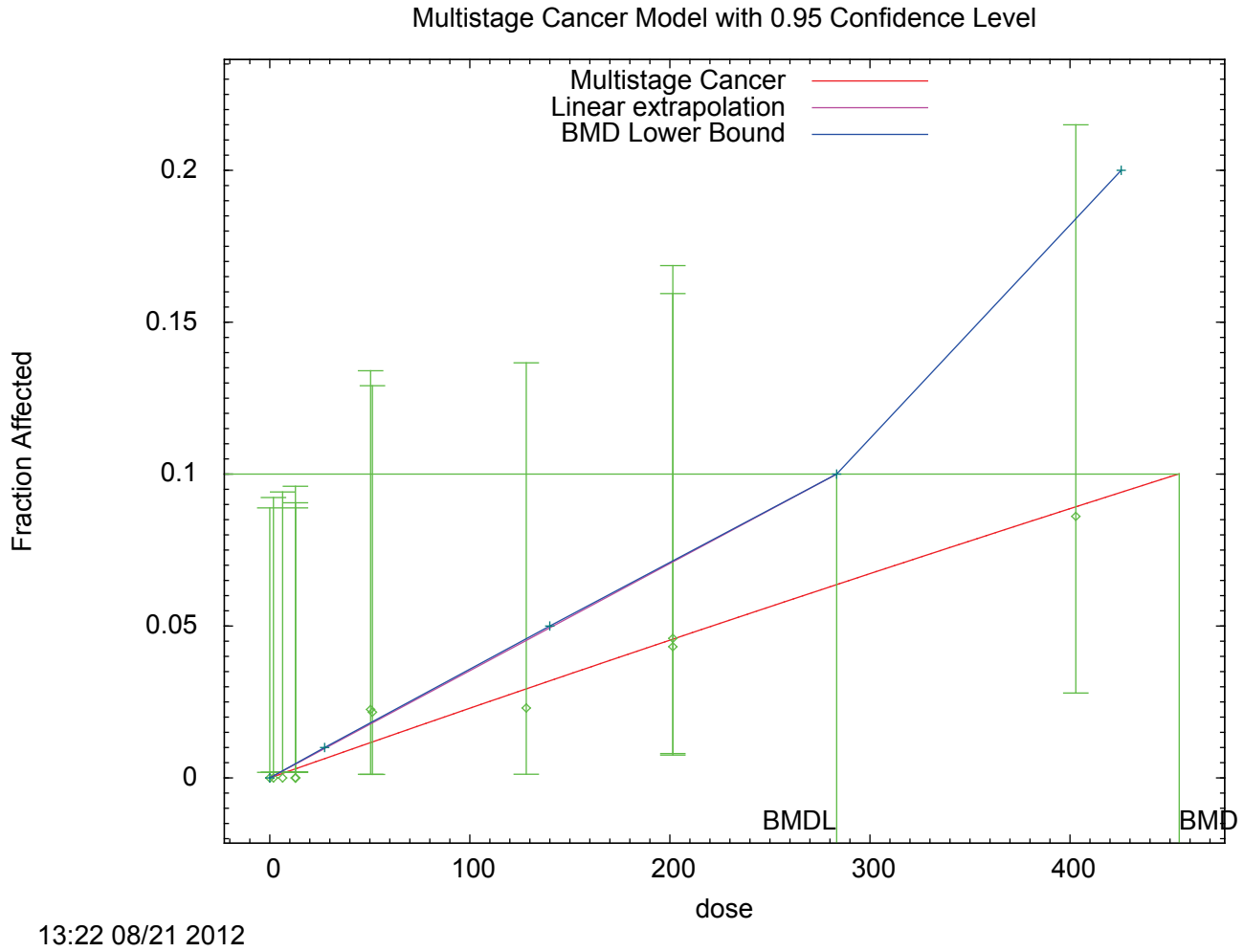
Run 27
Species: Mouse
Gender: Male
Organ: Forestomach
Response: Carcinoma
Study: Placke et al. 1996 m = 2



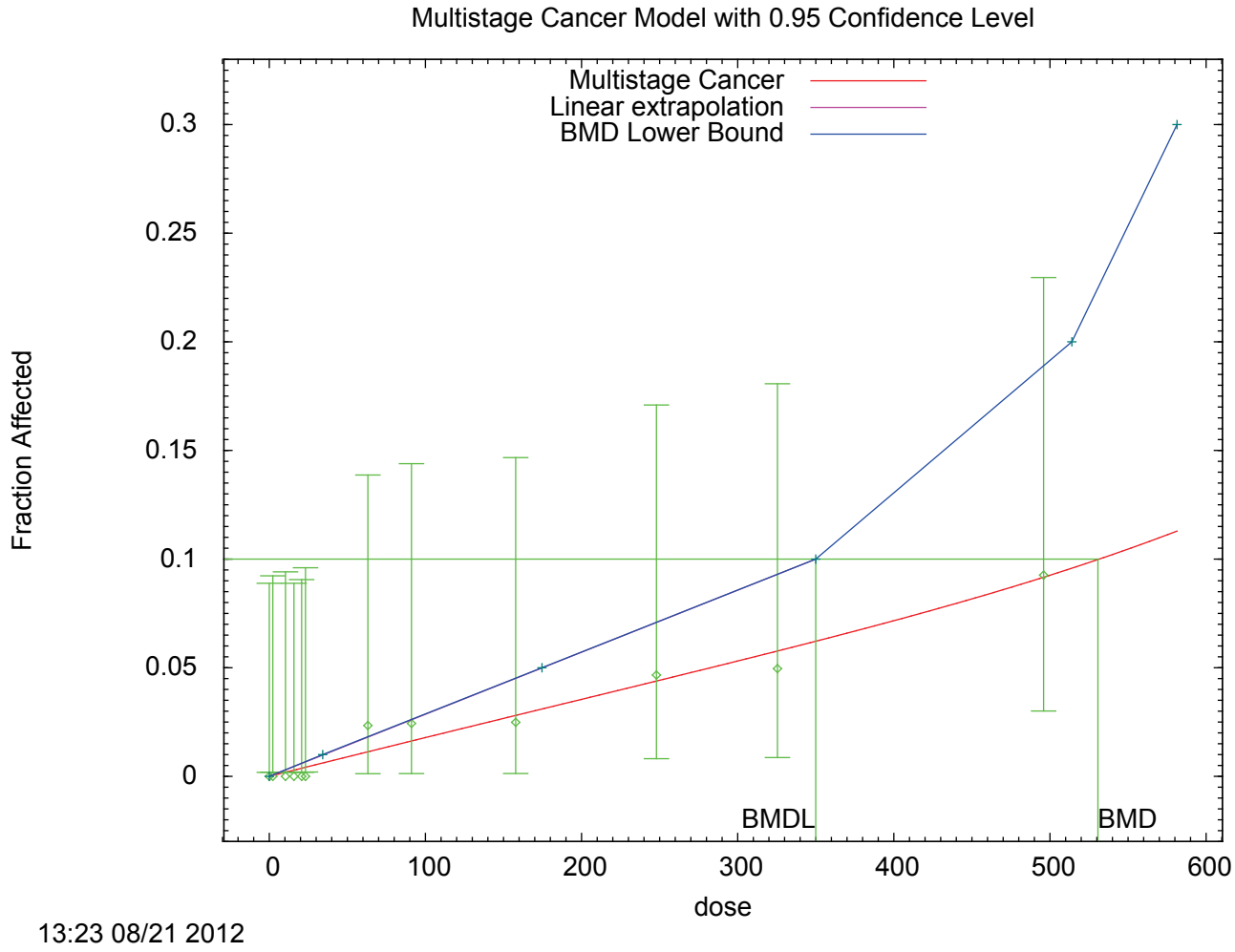
Run 28
Species: Mouse
Gender: Male
Organ: Forestomach
Response: Carcinoma
Study: Placke et al. 1996 m = 3



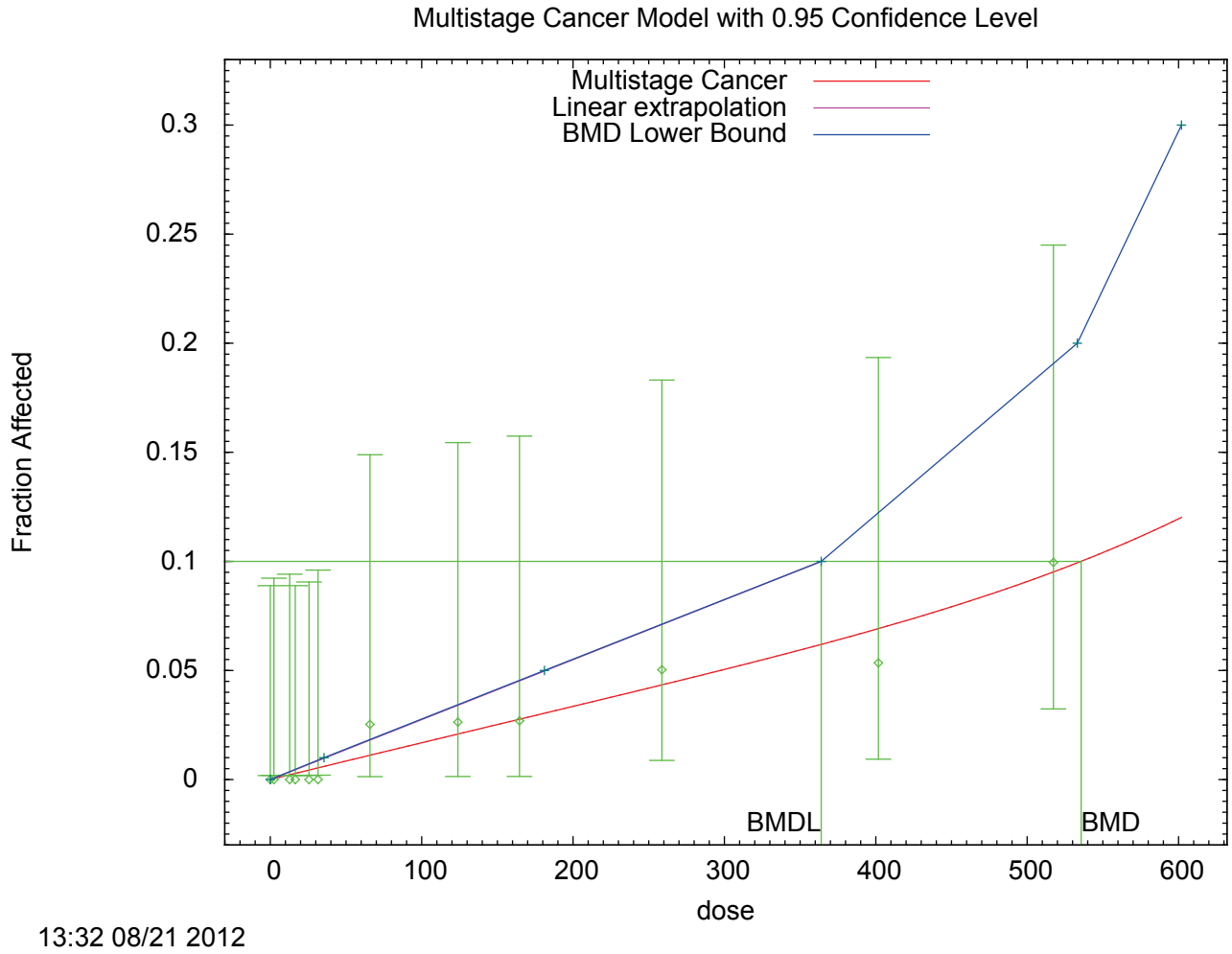
Run 29
Species: Mouse
Gender: Male
Organ: Forestomach
Response: Papilloma/Carcinoma
Study: Placke et al. 1996 m = 1



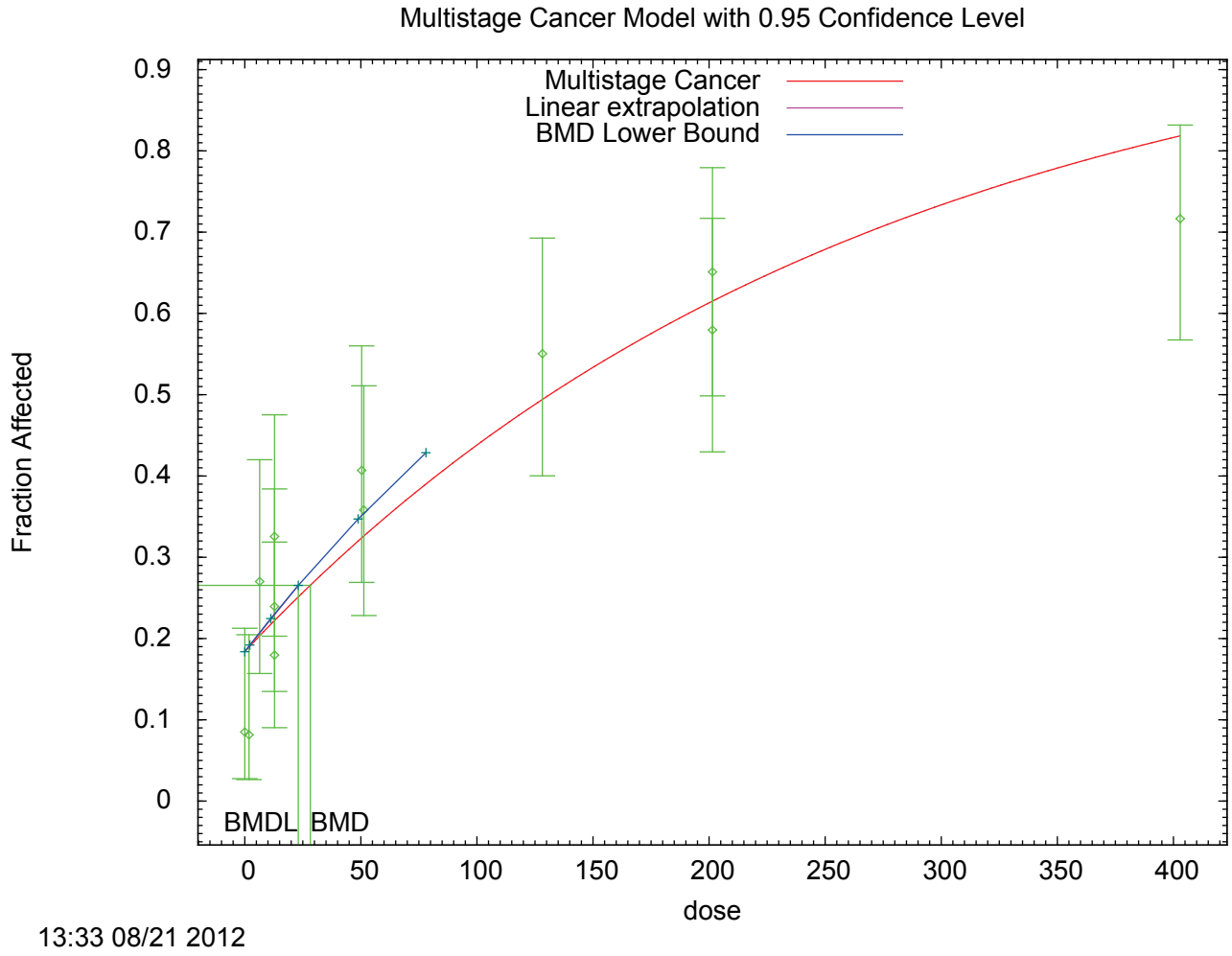
Run 30
Species: Mouse
Gender: Male
Organ: Forestomach
Response: Papilloma/Carcinoma
Study: Placke et al. 1996 m = 2



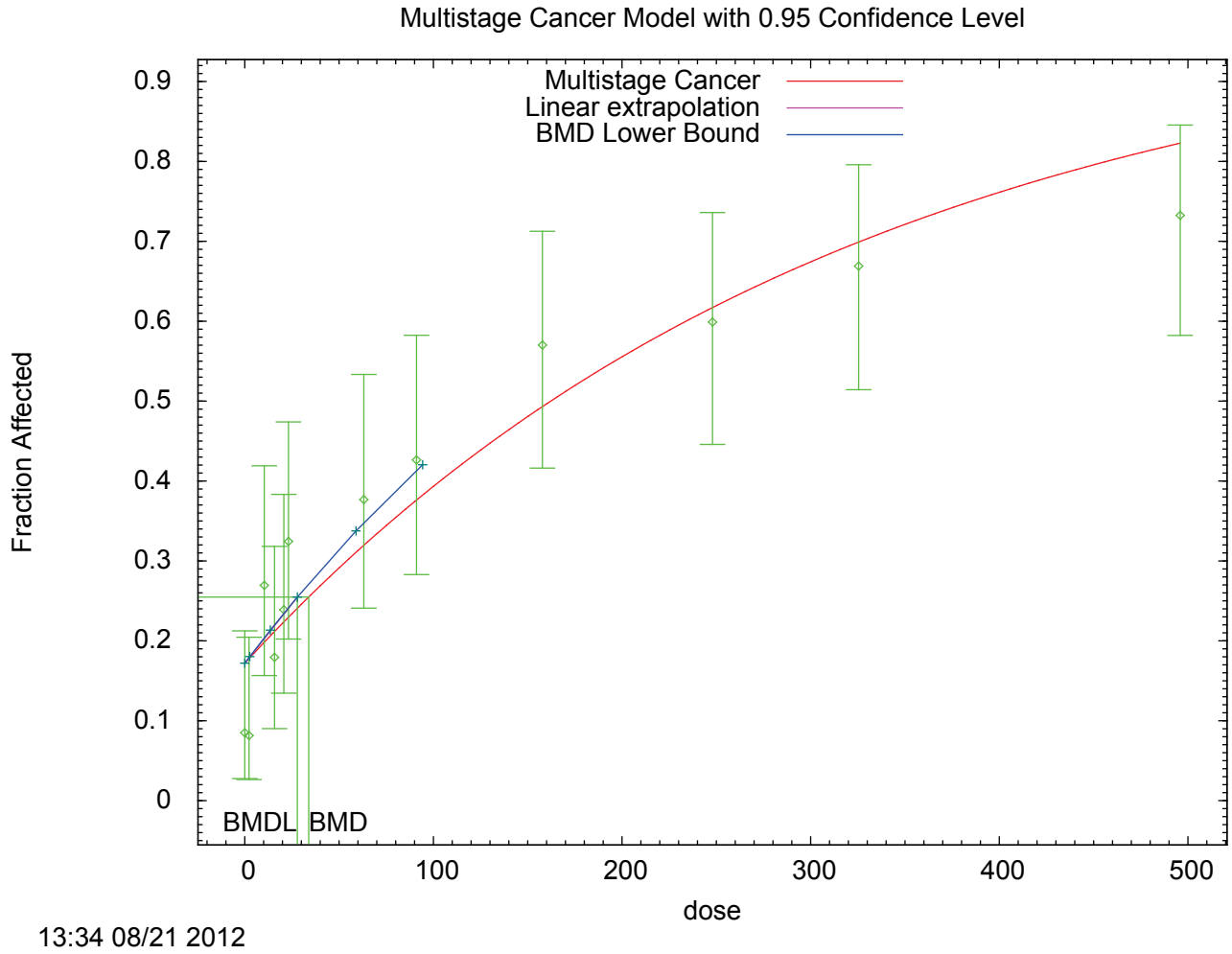
Run 31
Species: Mouse
Gender: Male
Organ: Forestomach
Response: Papilloma/Carcinoma
Study: Placke et al. 1996 m = 3



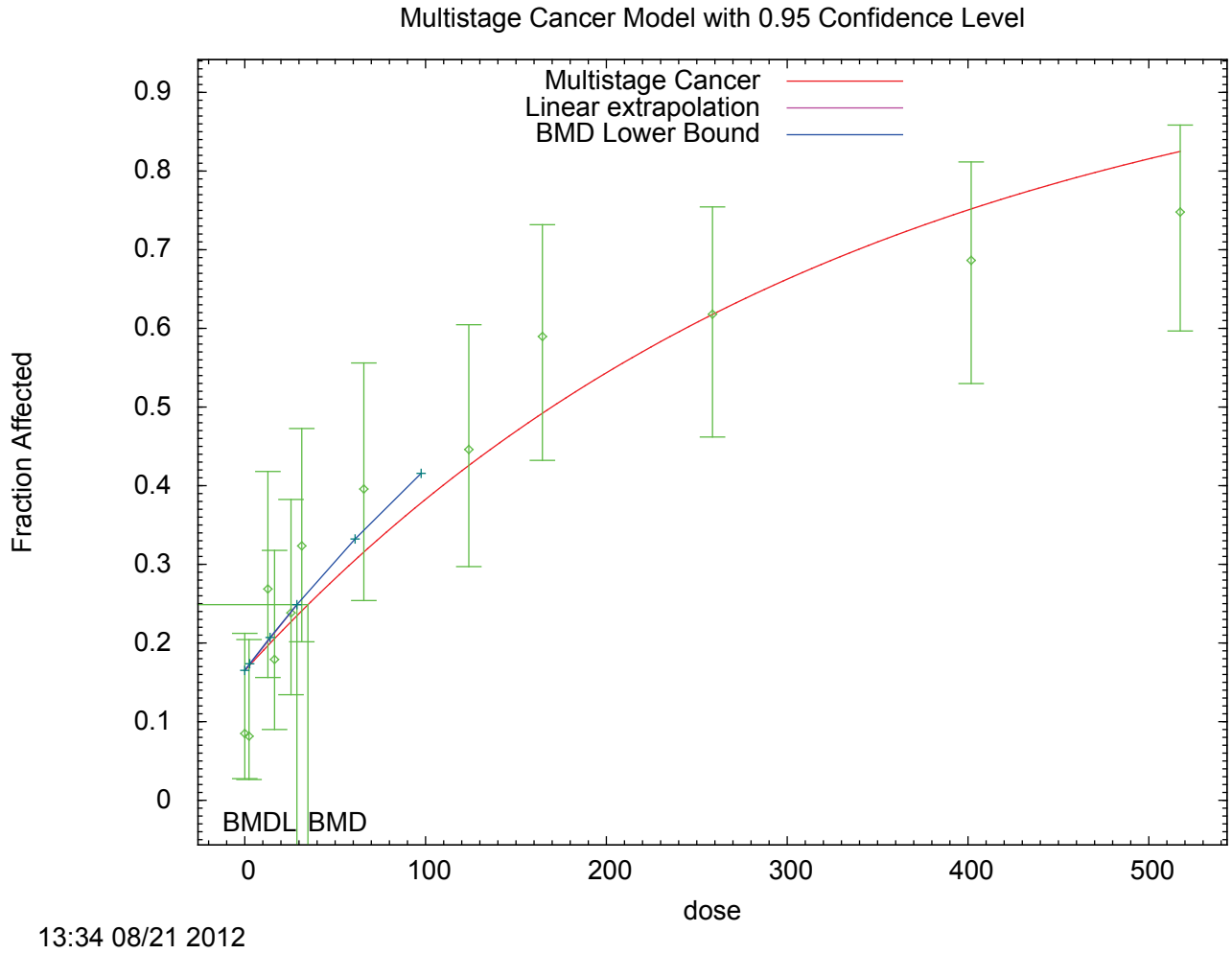
Run 32
Species: Mouse
Gender: Male
Organ: Harderian gland
Response: Adenoma
Study: Placke et al. 1996 m = 1



Run 33
Species: Mouse
Gender: Male
Organ: Harderian gland
Response: Adenoma
Study: Placke et al. 1996 m = 2

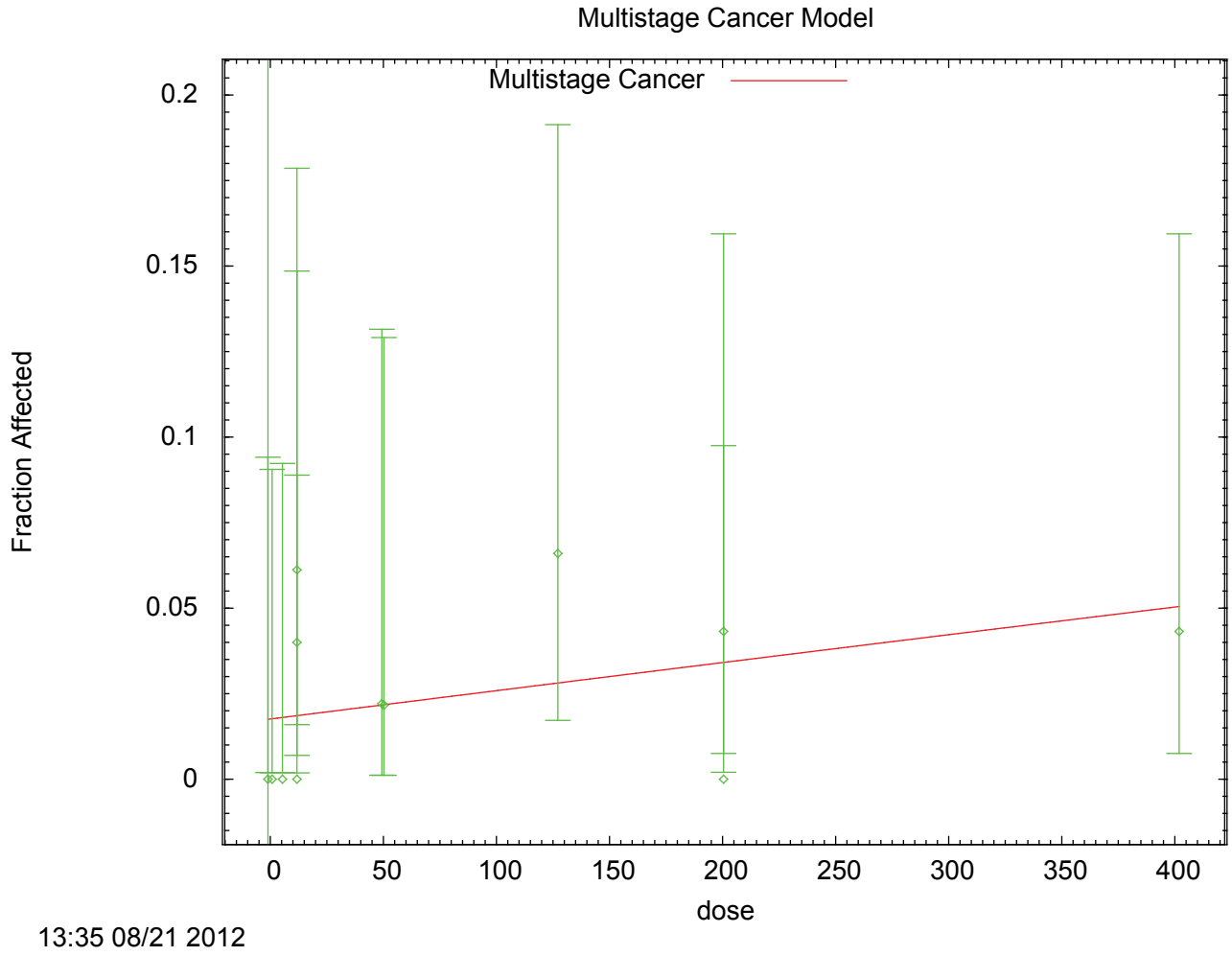


Run 34
Species: Mouse
Gender: Male
Organ: Harderian gland
Response: Adenoma
Study: Placke et al. 1996 m = 3

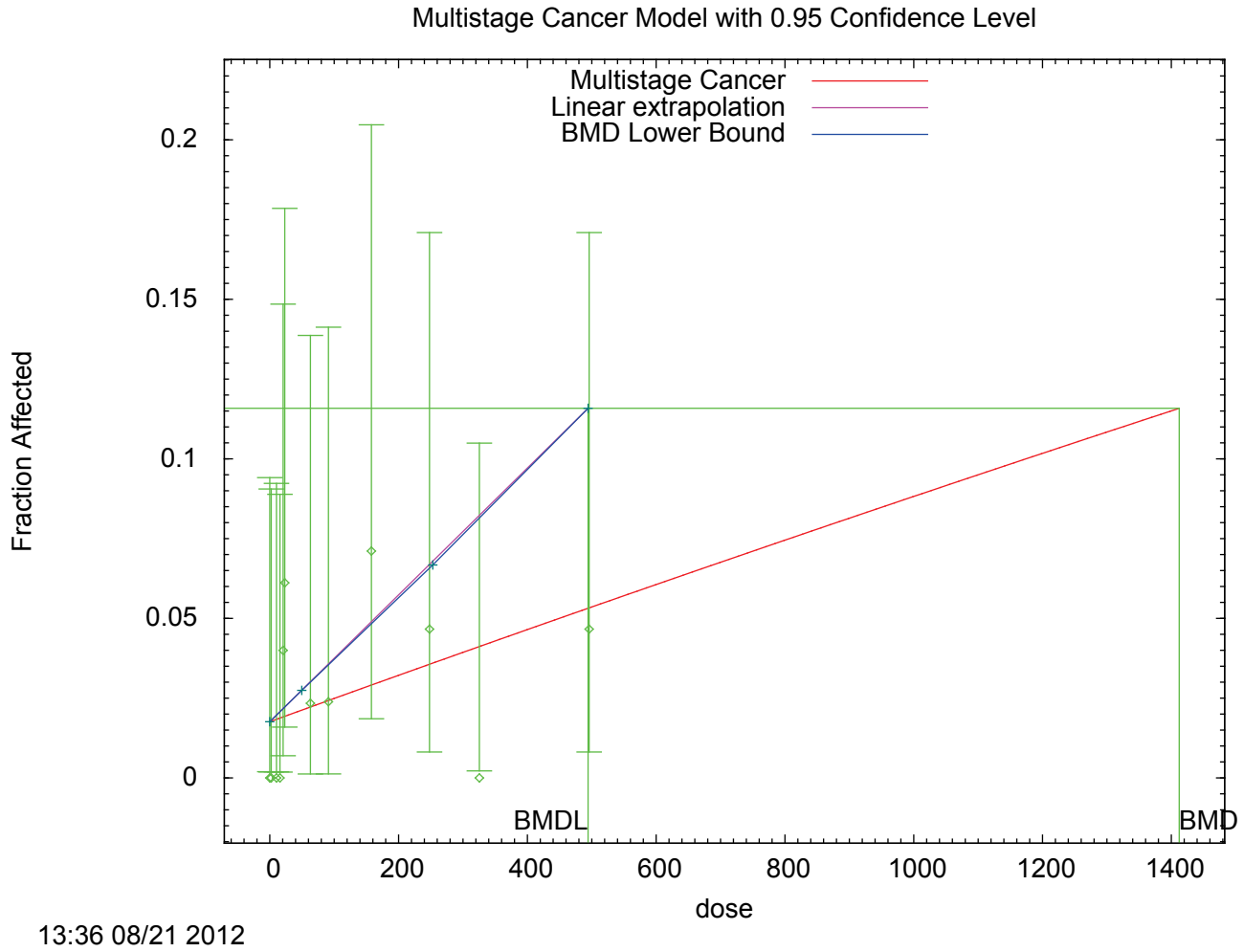


Run 35
Species: Mouse
Gender: Male
Organ: Harderian gland
Response: Carcinoma
Study: Placke et al. 1996 m = 1

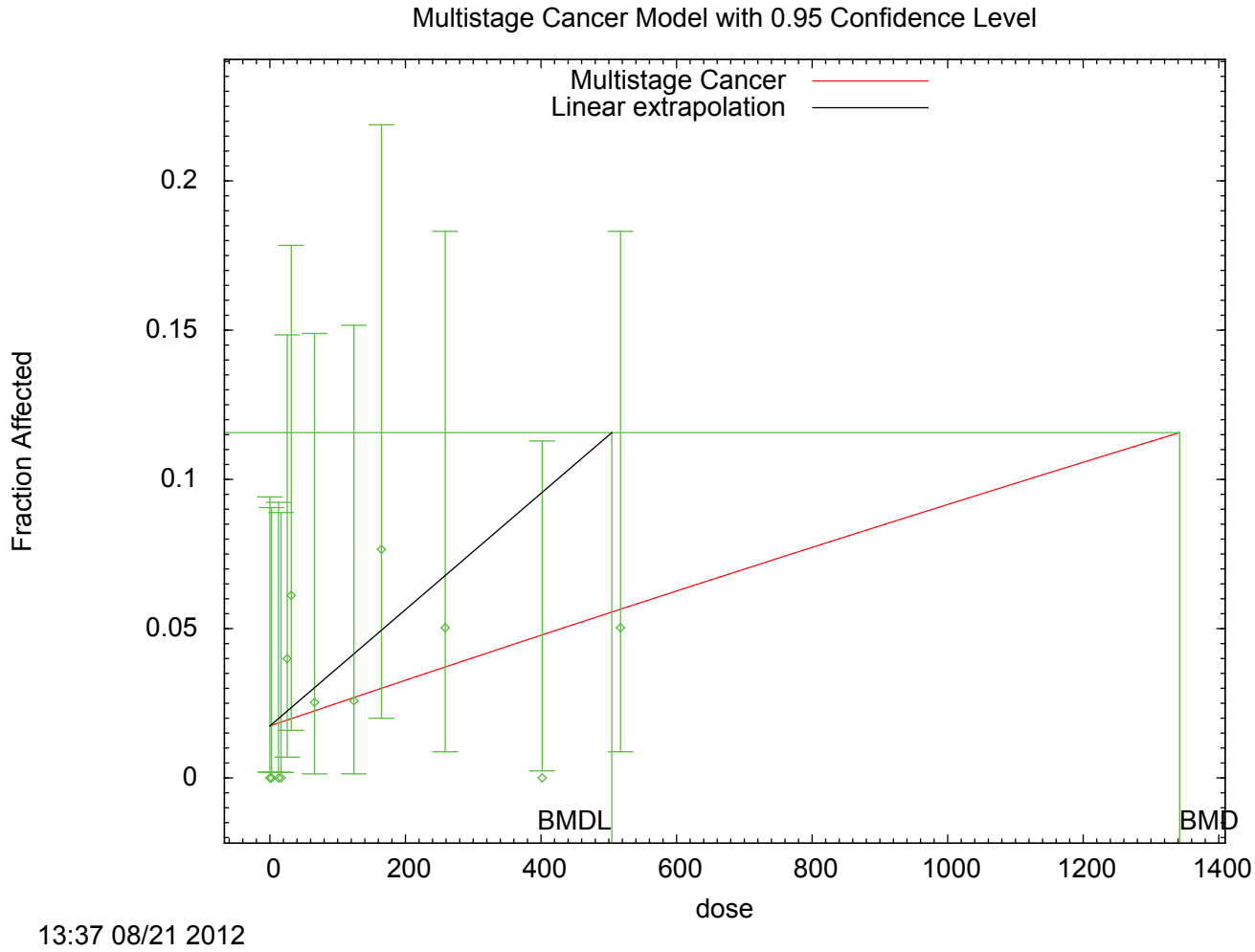
```
BMD computation failed. BMD is larger than three times maximum input doses.
```



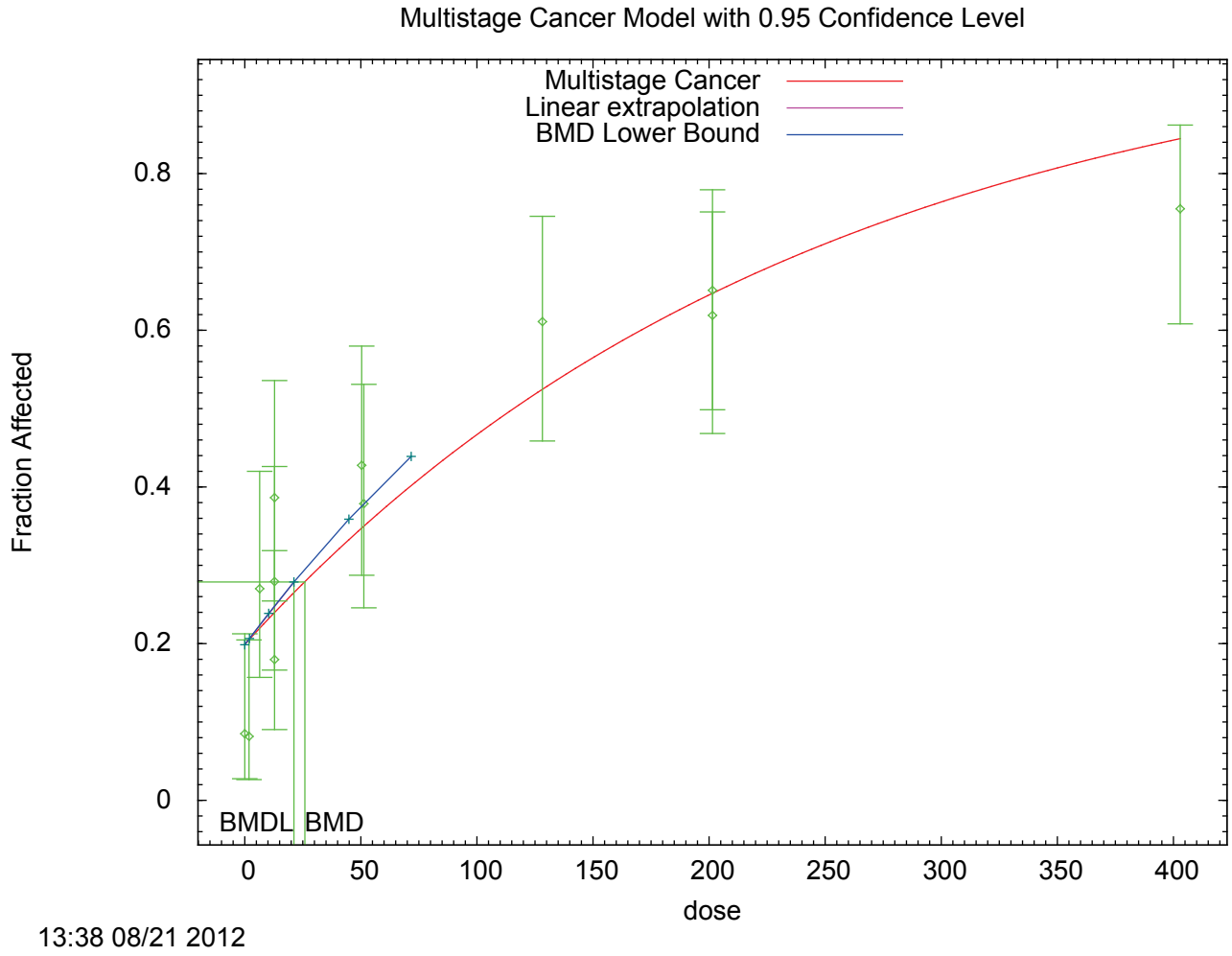
Run 36
Species: Mouse
Gender: Male
Organ: Harderian gland
Response: Carcinoma
Study: Placke et al. 1996 m = 2



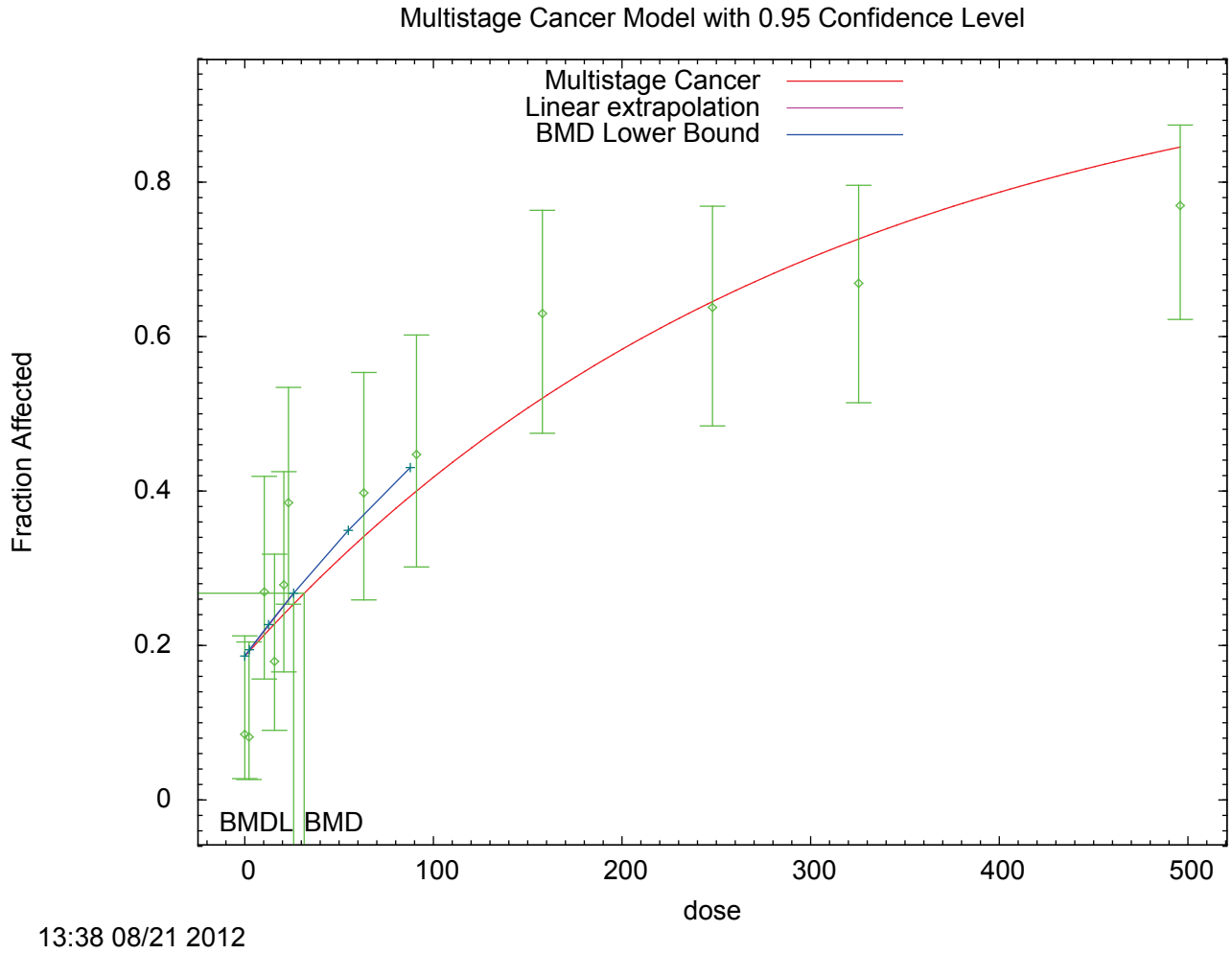
Run 37
Species: Mouse
Gender: Male
Organ: Harderian gland
Response: Carcinoma
Study: Placke et al. 1996 m = 3



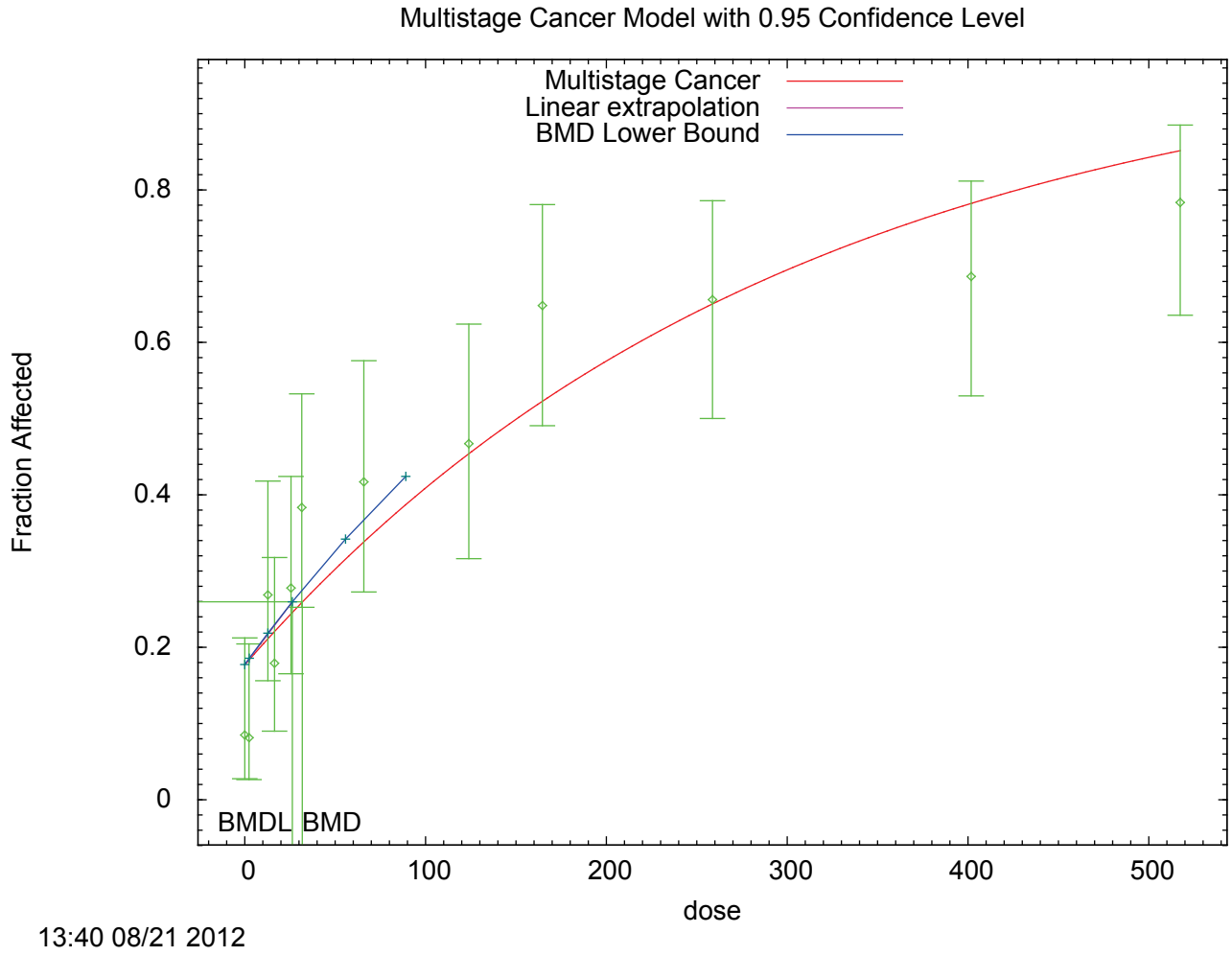
Run 38
Species: Mouse
Gender: Male
Organ: Harderian gland
Response: Adenoma/Carcinoma
Study: Placke et al. 1996 m = 1



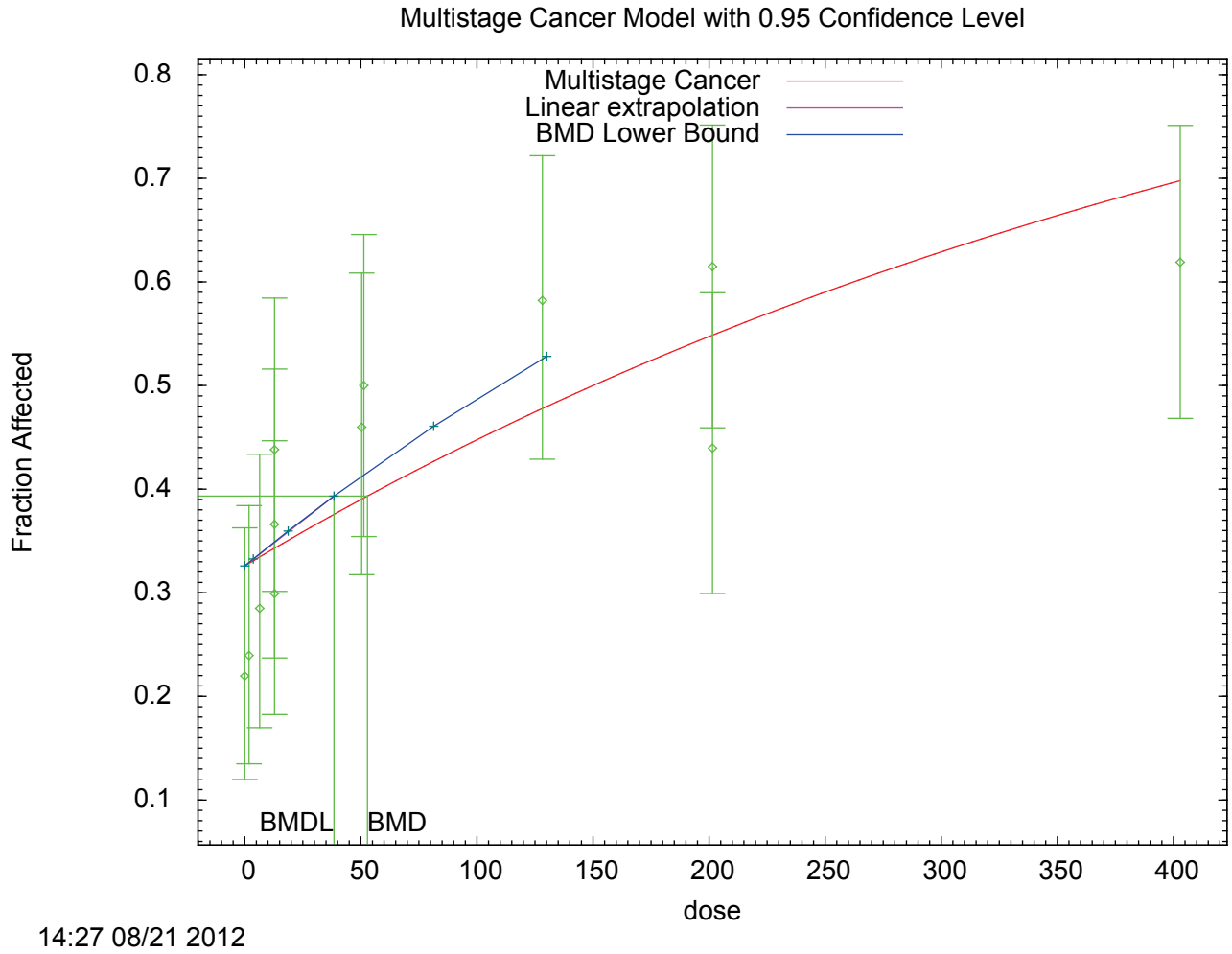
Run 39
Species: Mouse
Gender: Male
Organ: Harderian gland
Response: Adenoma/Carcinoma
Study: Placke et al. 1996 m = 2



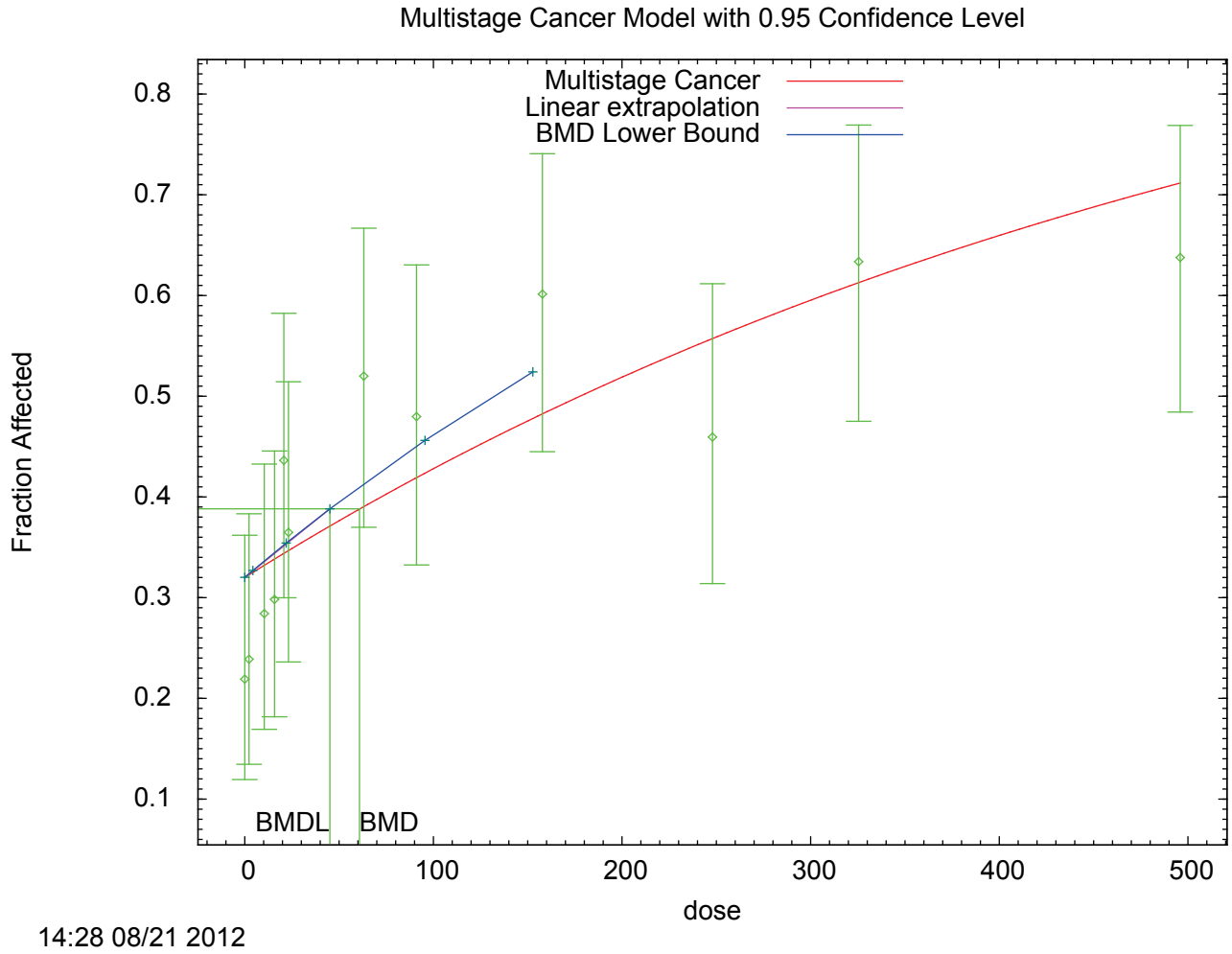
Run 40
Species: Mouse
Gender: Male
Organ: Harderian gland
Response: Adenoma/Carcinoma
Study: Placke et al. 1996 m = 3



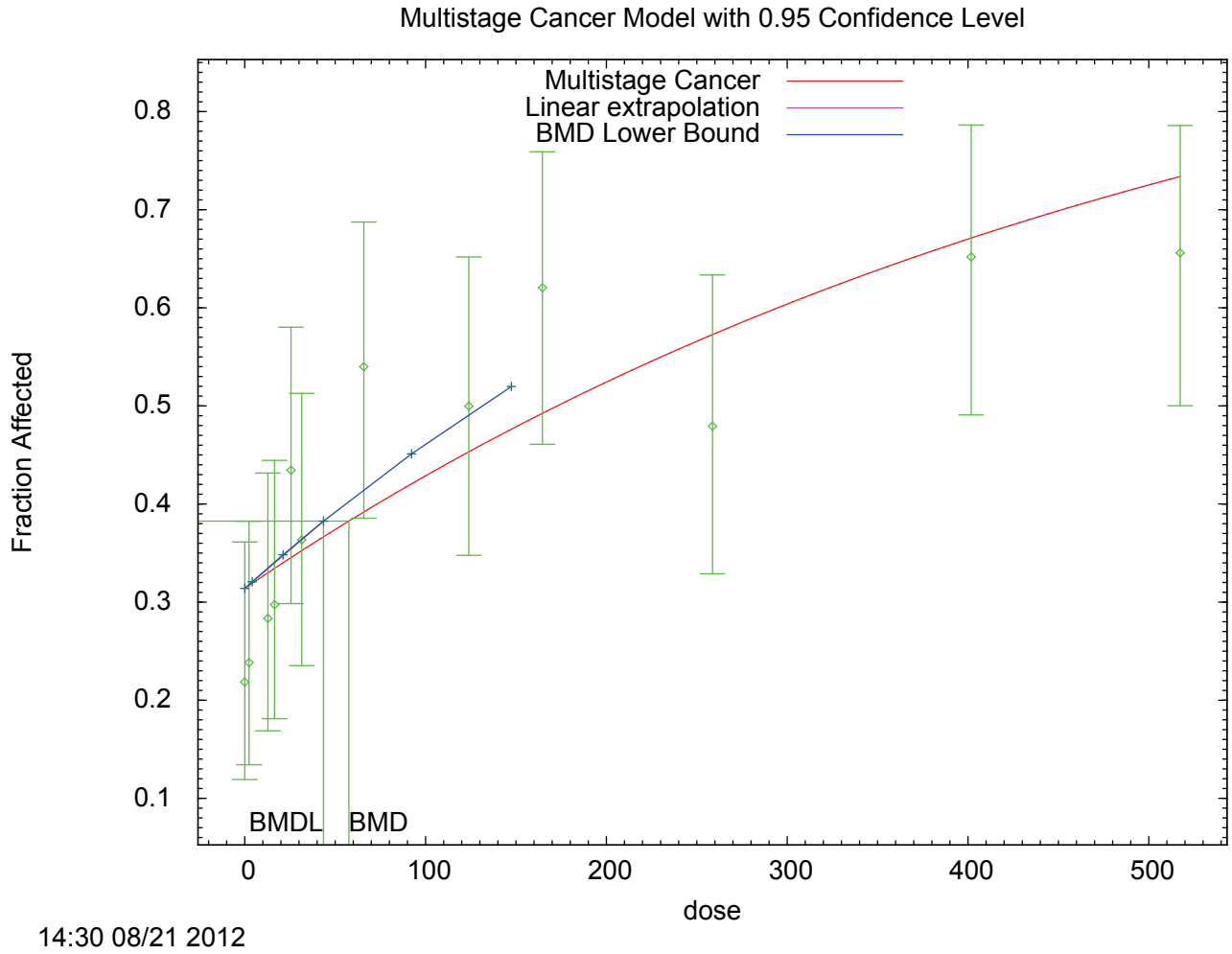
Run 41
Species: Mouse
Gender: Male
Organ: Liver
Response: Adenoma
Study: Placke et al. 1996 m = 1



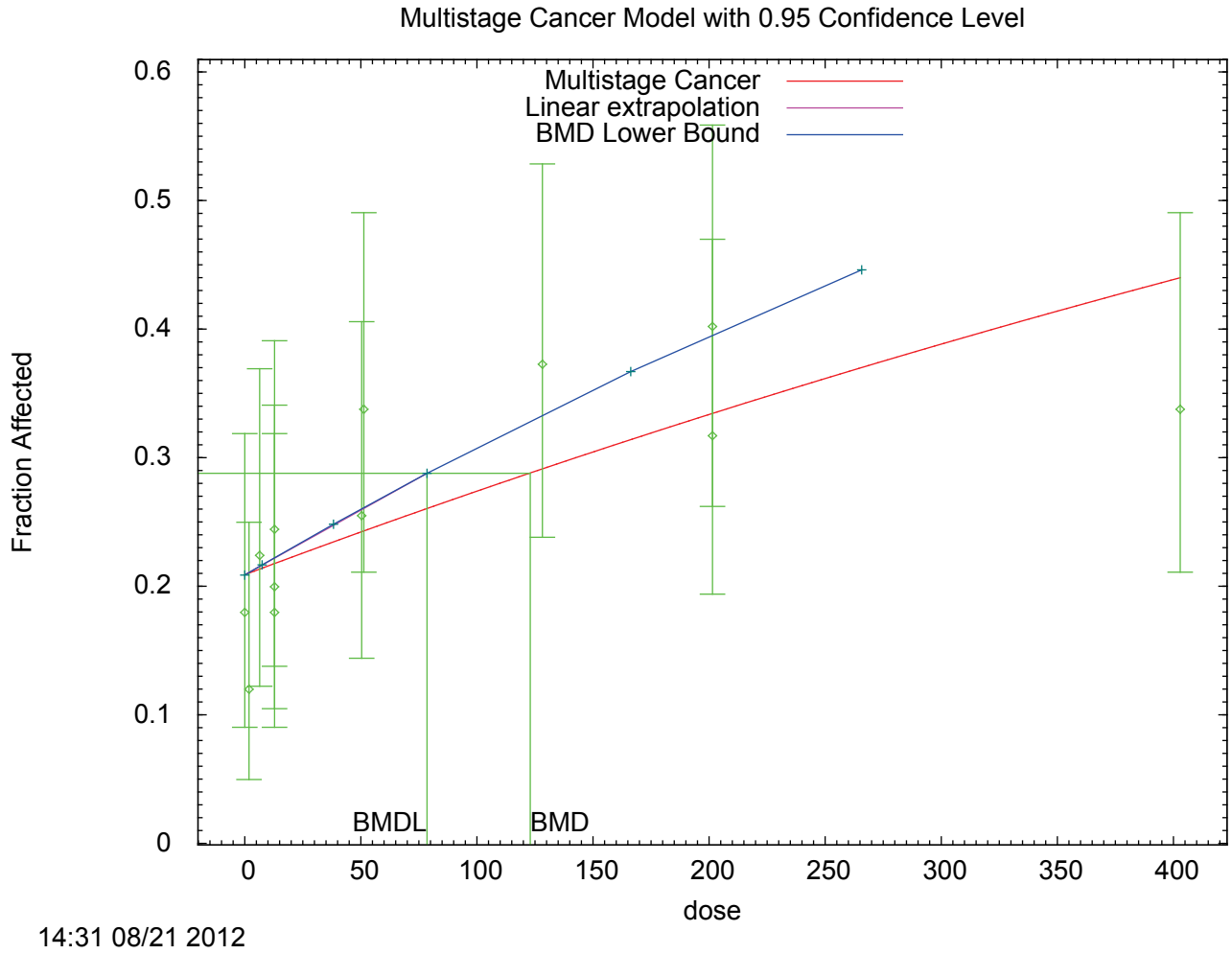
Run 42
Species: Mouse
Gender: Male
Organ: Liver
Response: Adenoma
Study: Placke et al. 1996 m = 2



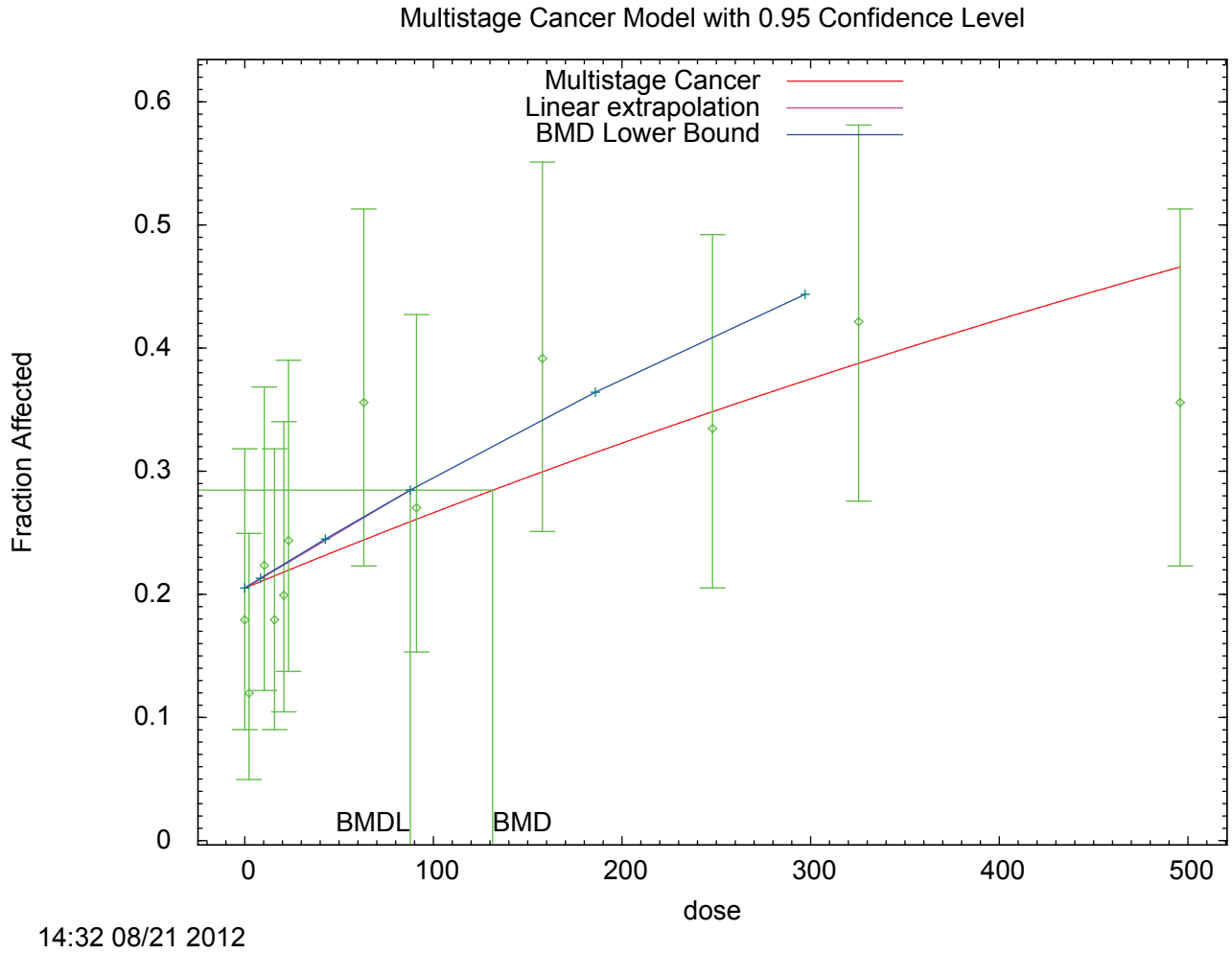
Run 43
Species: Mouse
Gender: Male
Organ: Liver
Response: Adenoma
Study: Placke et al. 1996 m = 3



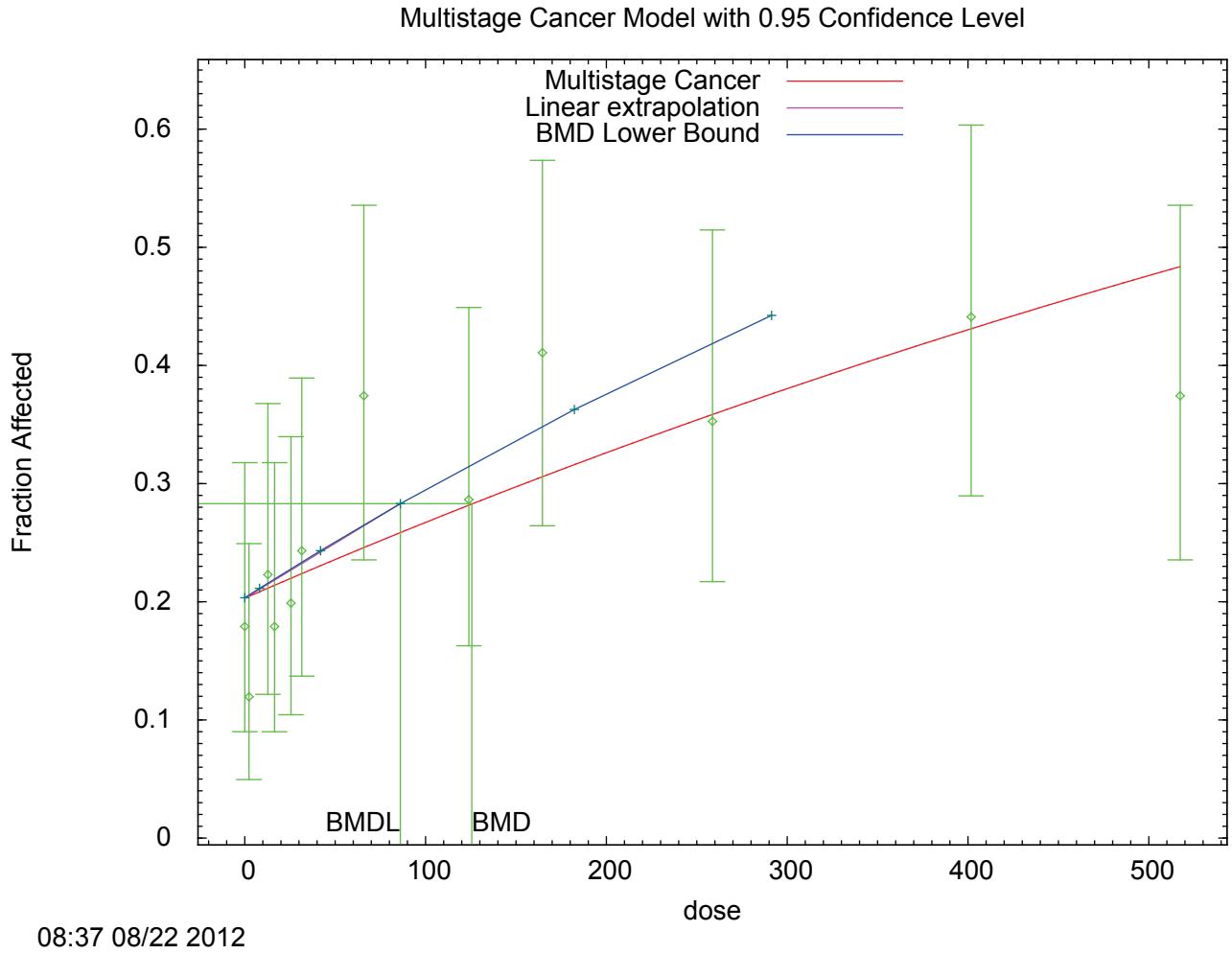
Run 44
Species: Mouse
Gender: Male
Organ: Liver
Response: Carcinoma
Study: Placke et al. 1996 m = 1



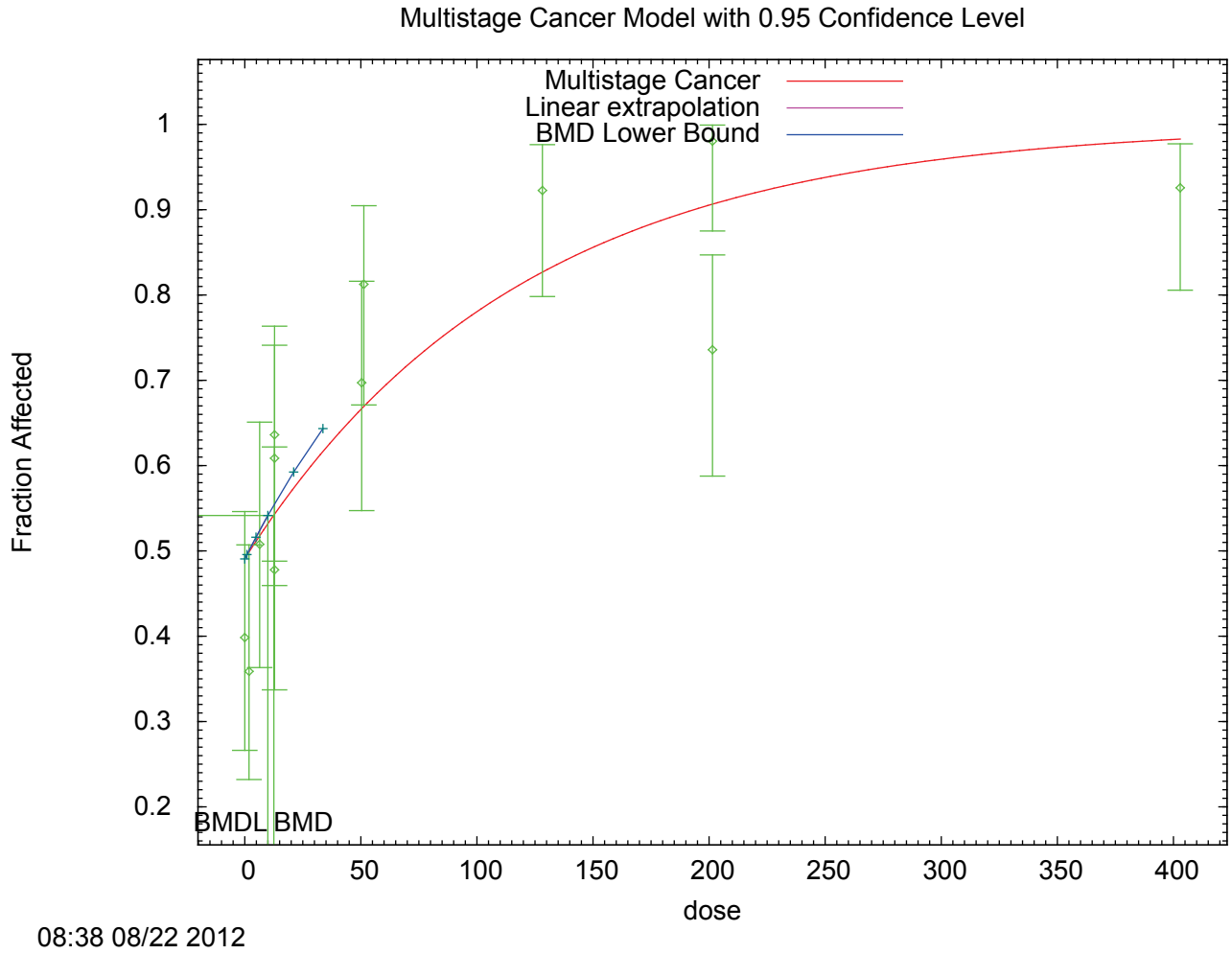
Run 45
Species: Mouse
Gender: Male
Organ: Liver
Response: Carcinoma
Study: Placke et al. 1996 m = 2



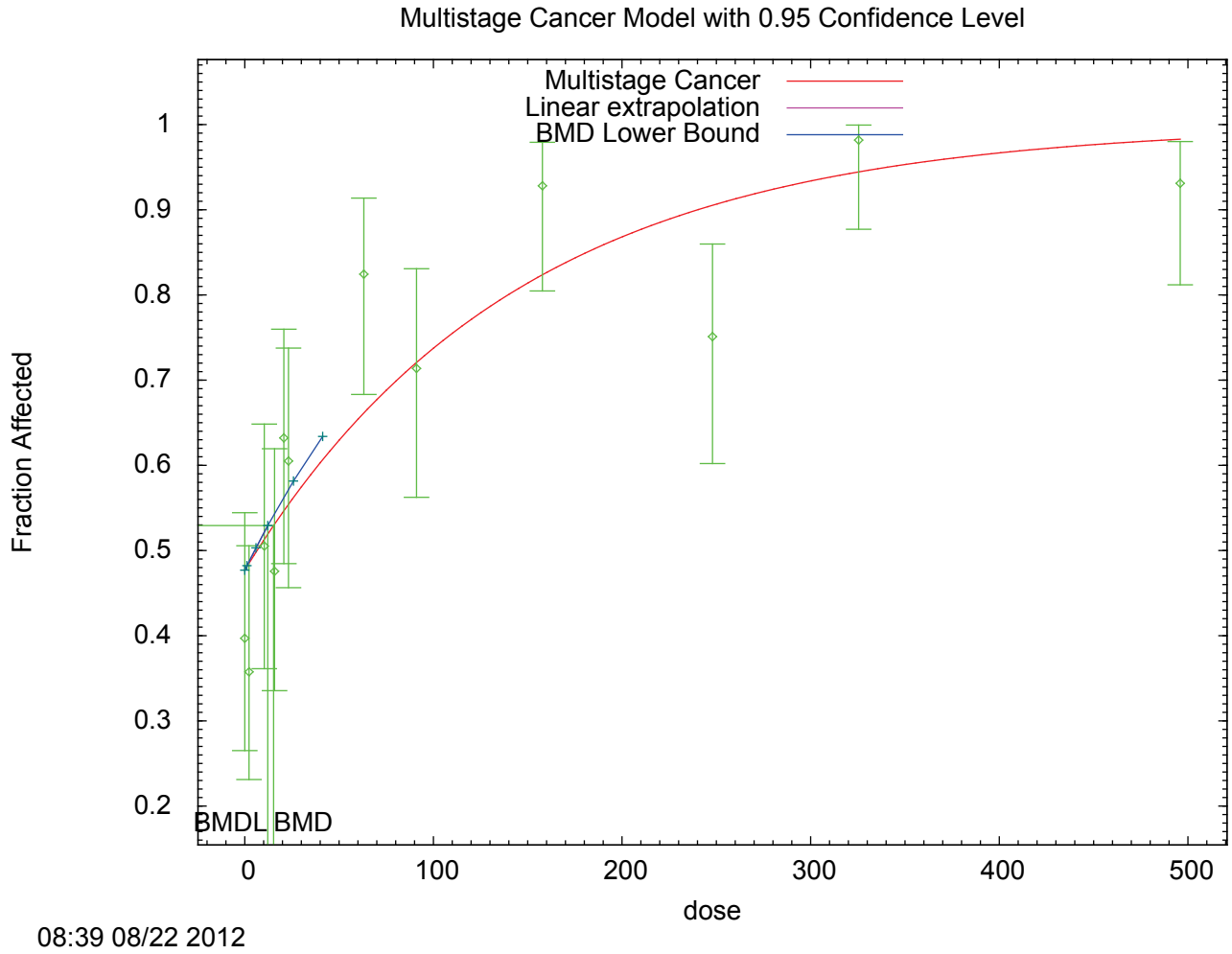
Run 46
Species: Mouse
Gender: Male
Organ: Liver
Response: Carcinoma
Study: Placke et al. 1996 m = 3



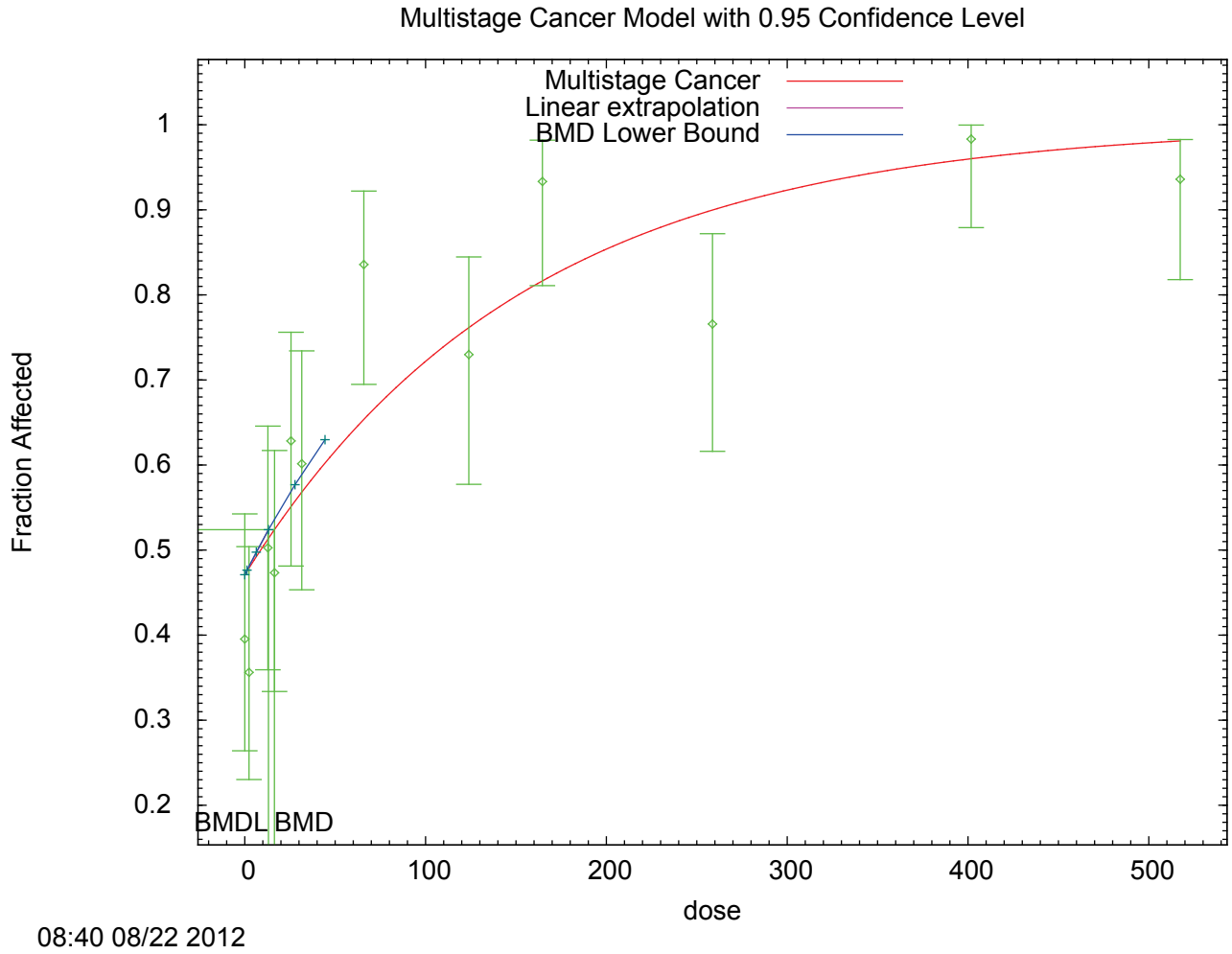
Run 47
Species: Mouse
Gender: Male
Organ: Liver
Response: Adenoma/Carcinoma
Study: Placke et al. 1996 m = 1



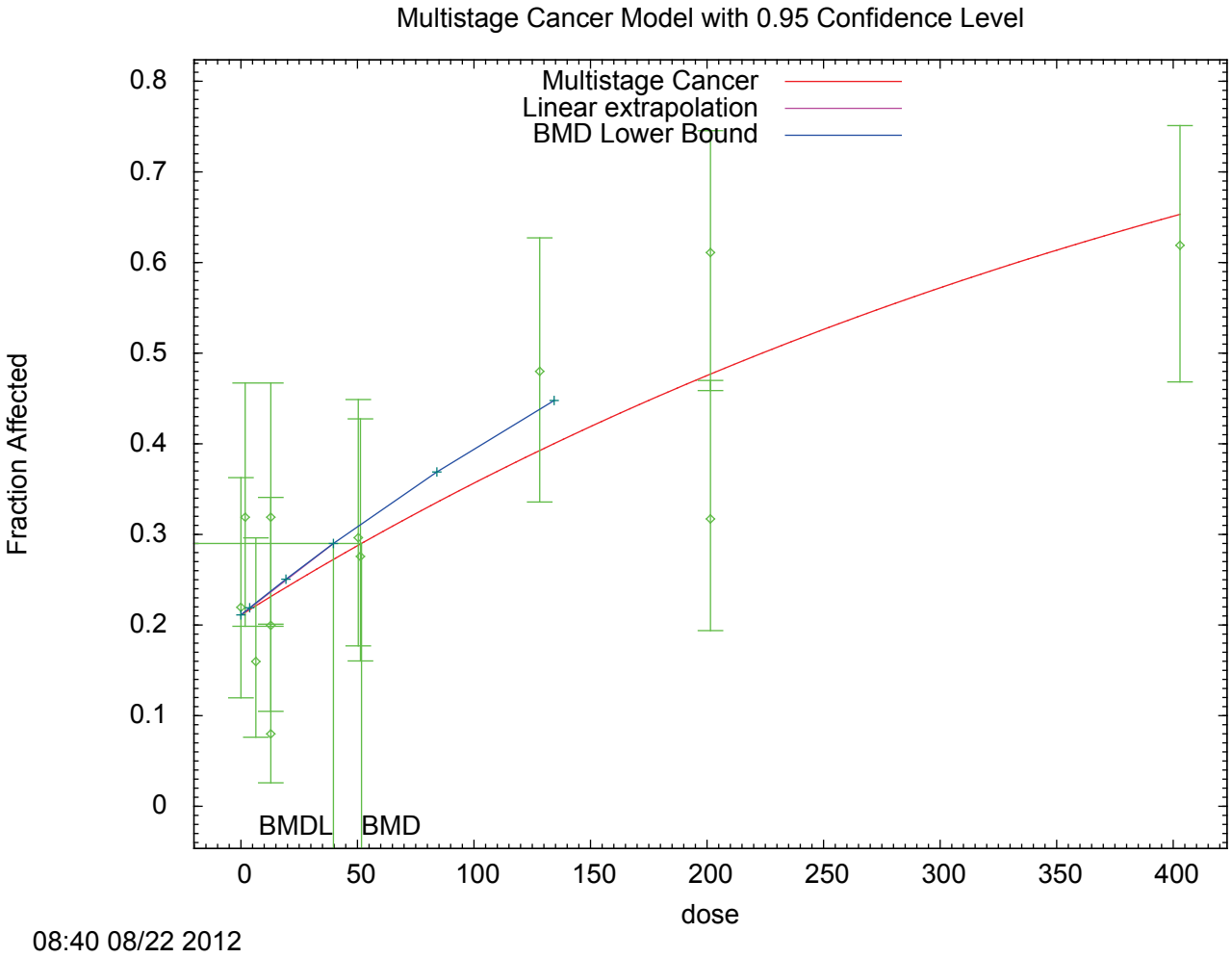
Run 48
Species: Mouse
Gender: Male
Organ: Liver
Response: Adenoma/Carcinoma
Study: Placke et al. 1996 m = 2



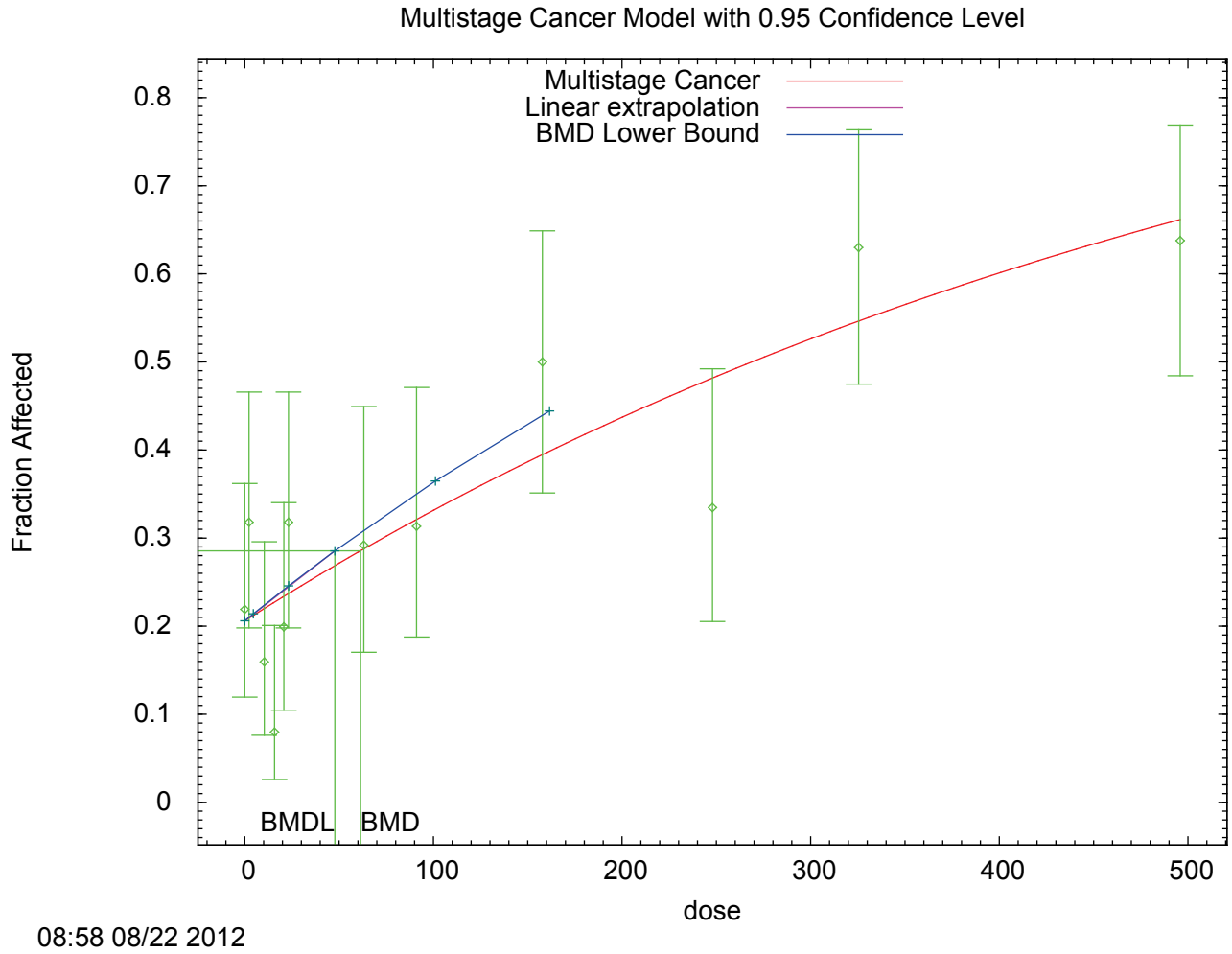
Run 49
Species: Mouse
Gender: Male
Organ: Liver
Response: Adenoma/Carcinoma
Study: Placke et al. 1996 m = 3



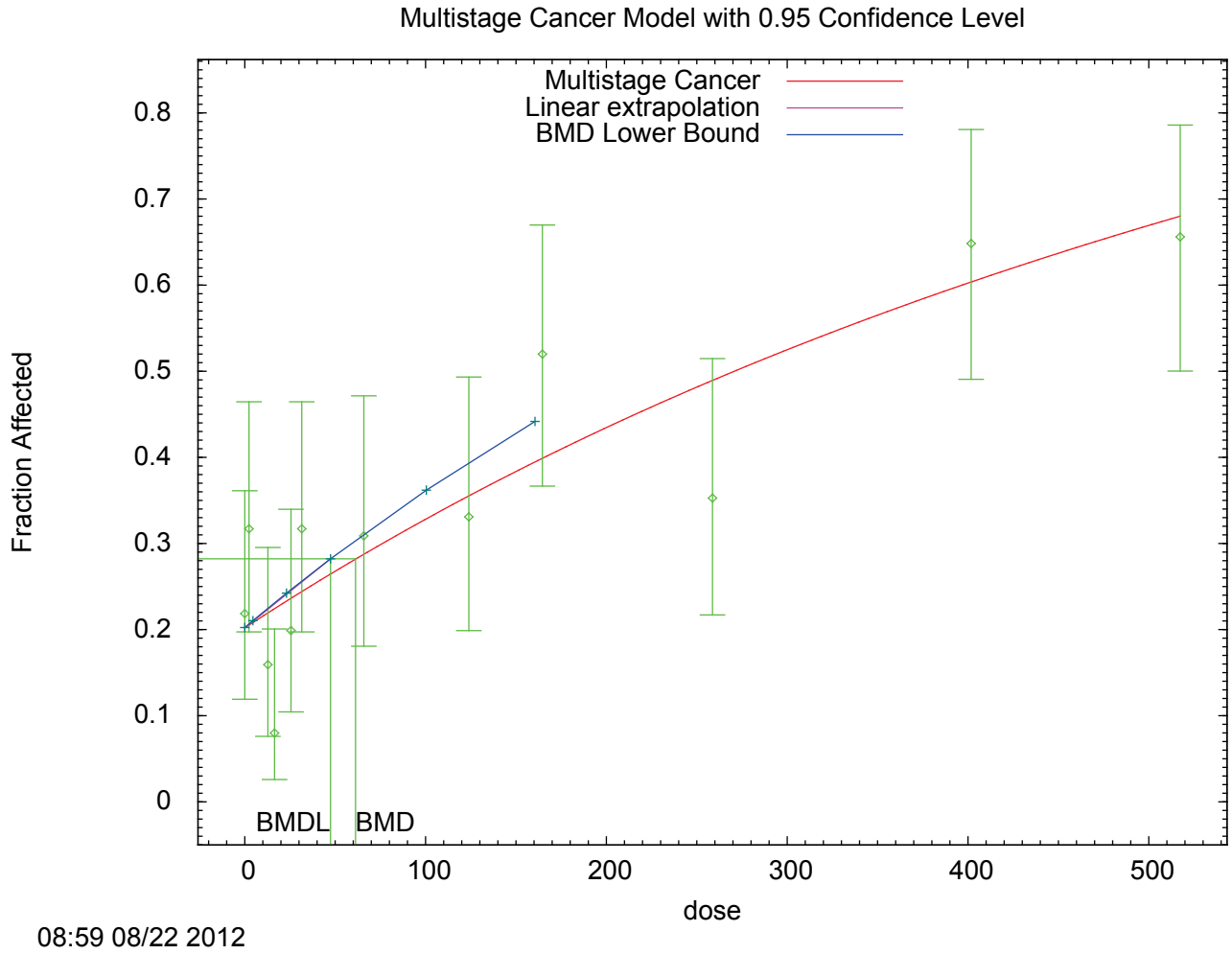
Run 50
Species: Mouse
Gender: Male
Organ: Lung
Response: Adenoma
Study: Placke et al. 1996 m = 1



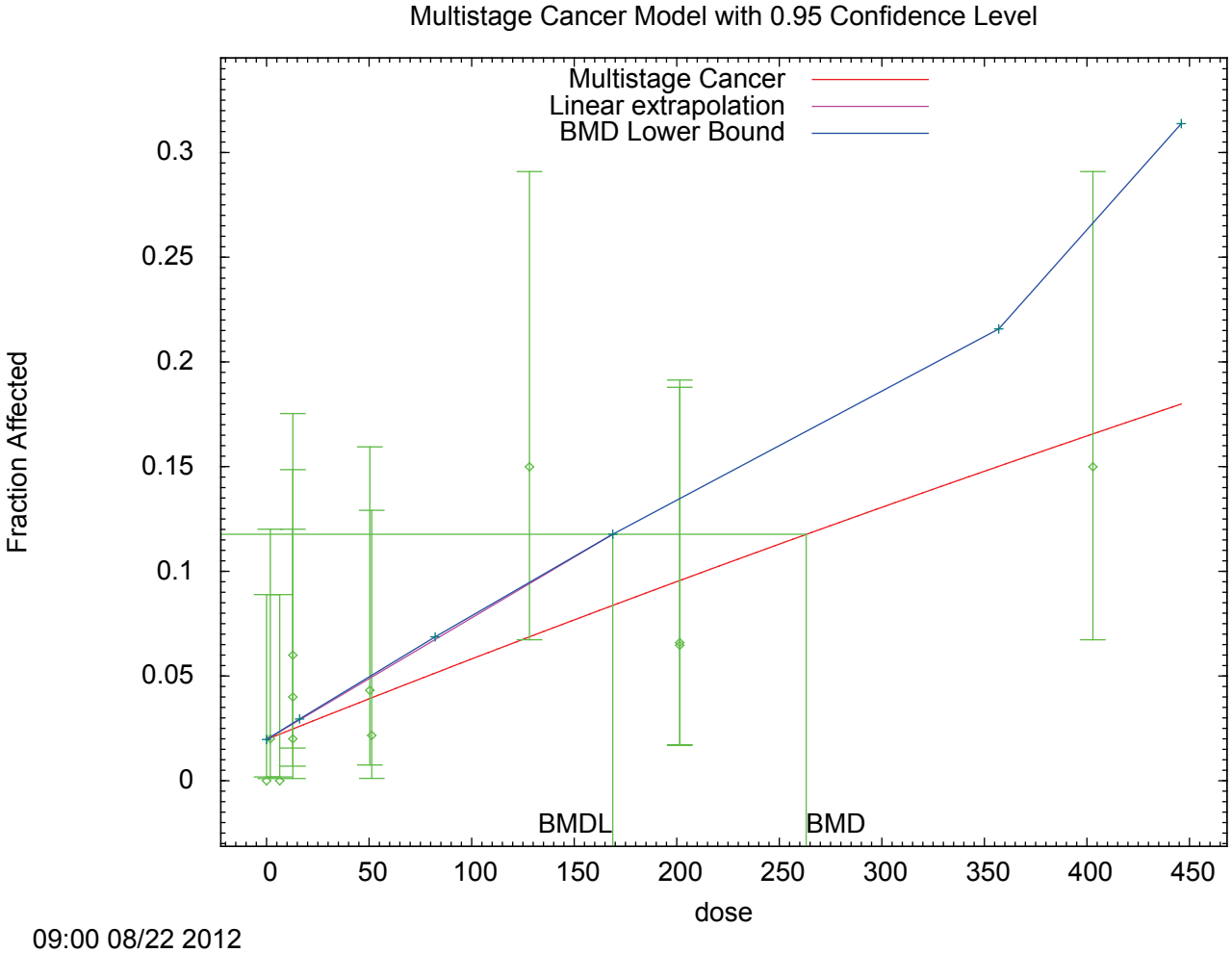
Run 51
Species: Mouse
Gender: Male
Organ: Lung
Response: Adenoma
Study: Placke et al. 1996 m = 2



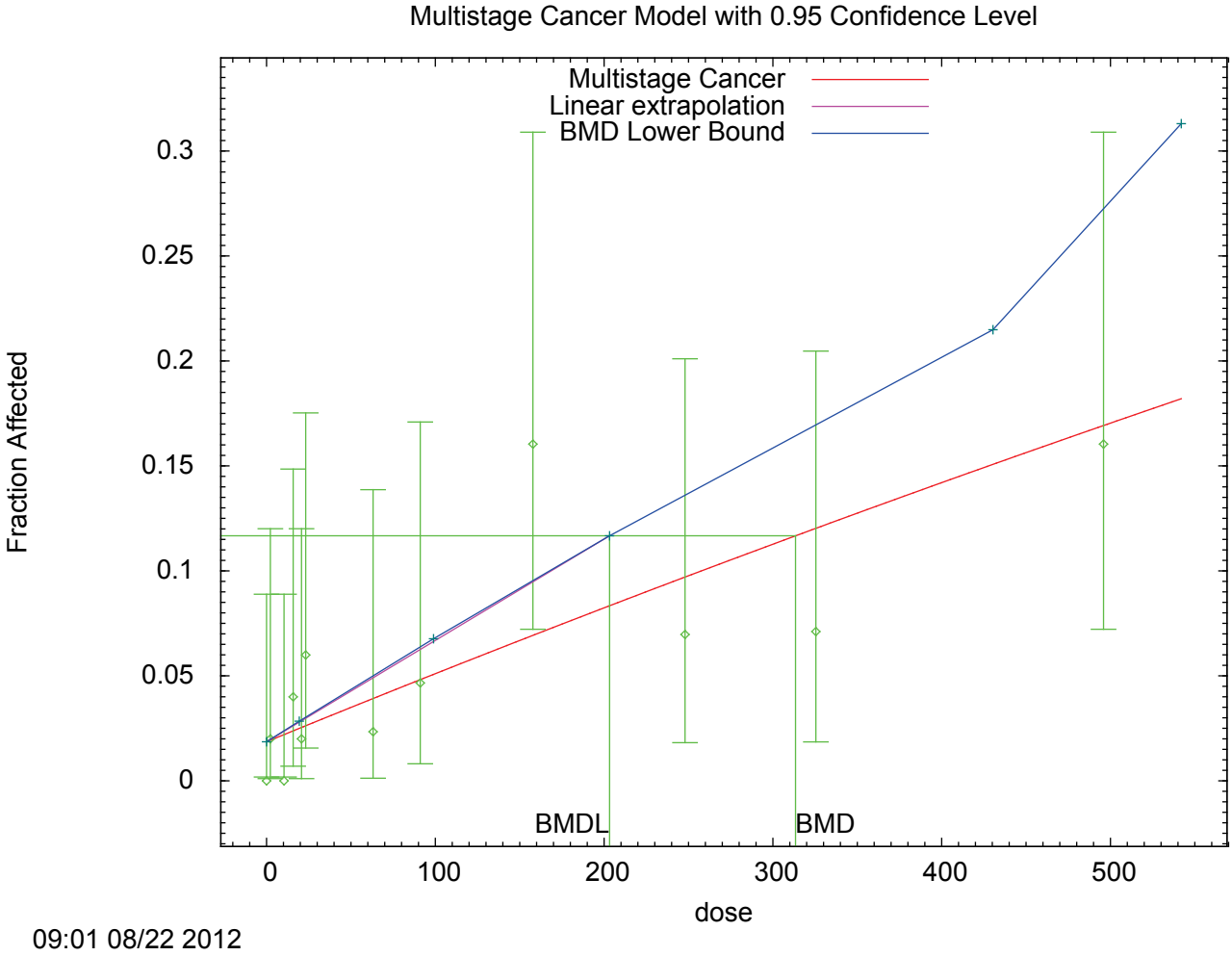
Run 52
Species: Mouse
Gender: Male
Organ: Lung
Response: Adenoma
Study: Placke et al. 1996 m = 3



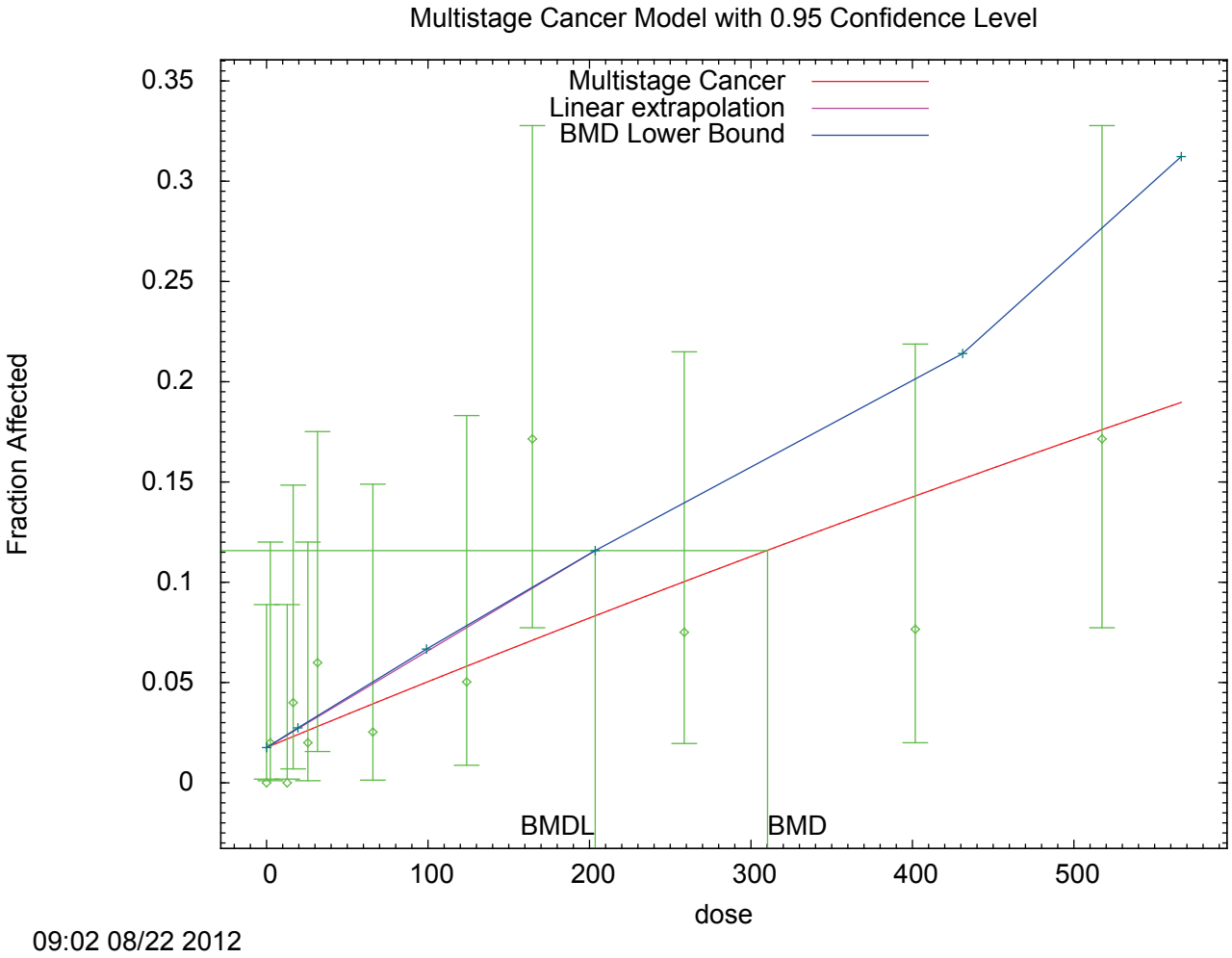
Run 53
Species: Mouse
Gender: Male
Organ: Lung
Response: Carcinoma
Study: Placke et al. 1996 m = 1



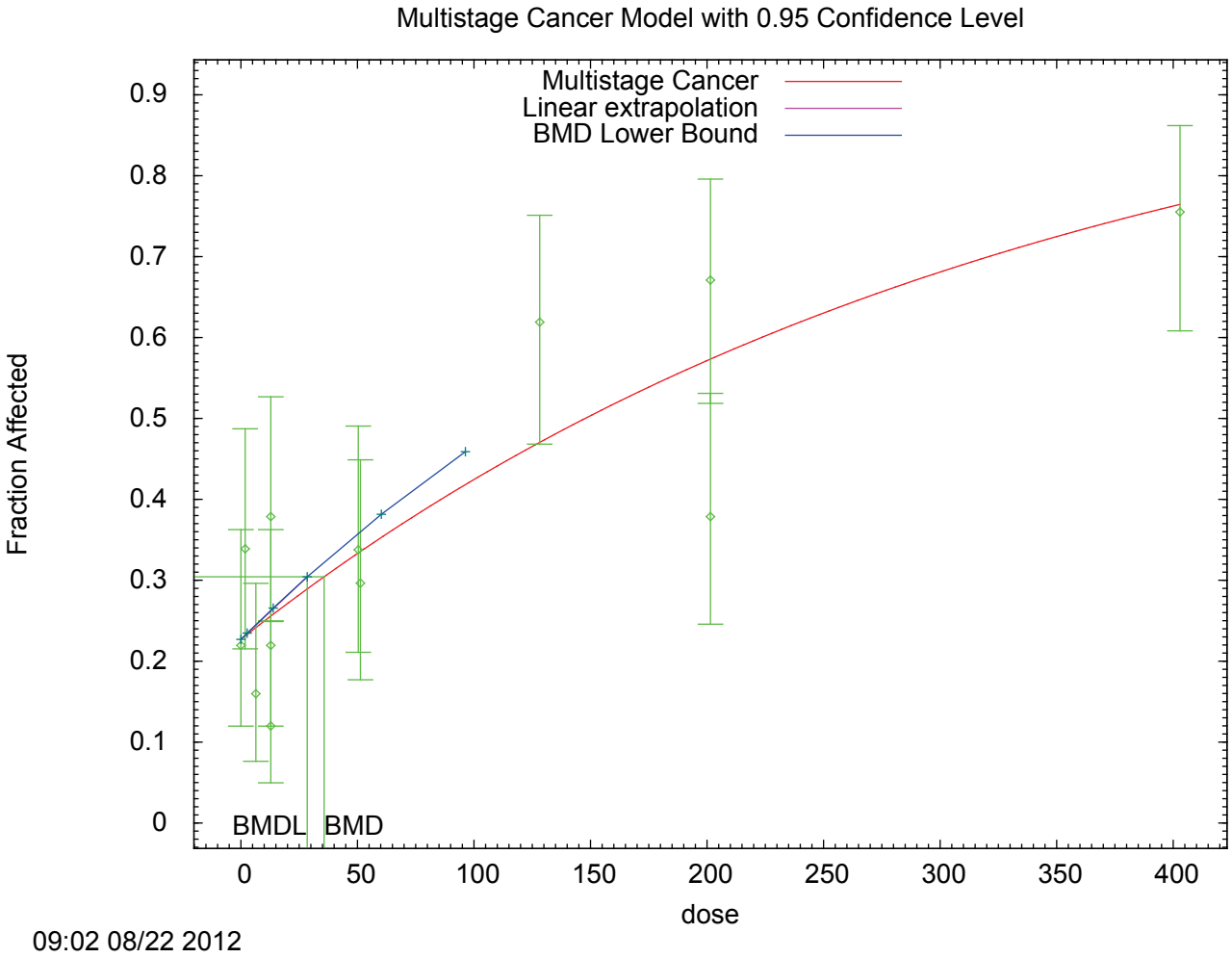
Run 54
Species: Mouse
Gender: Male
Organ: Lung
Response: Carcinoma
Study: Placke et al. 1996 m = 2



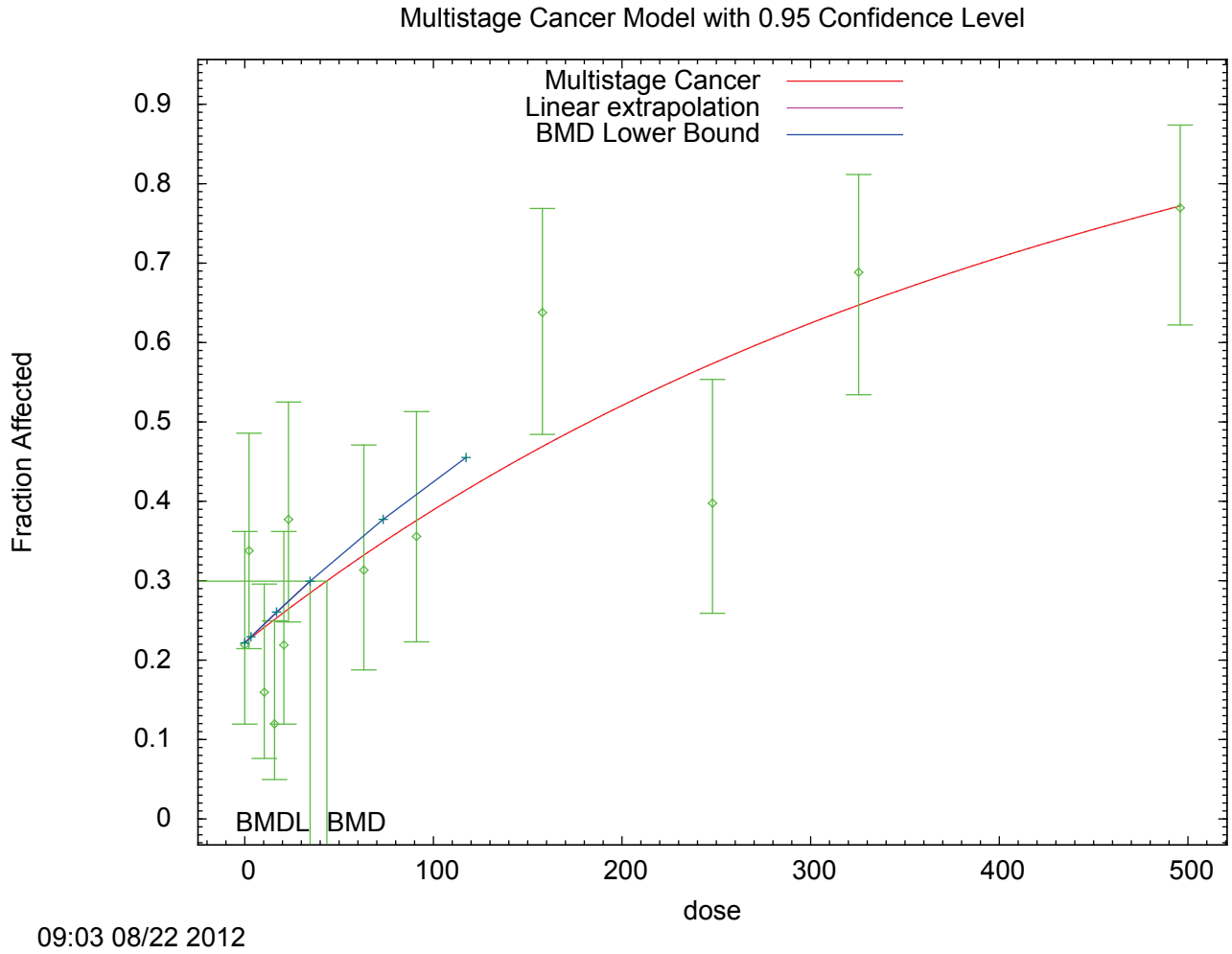
Run 55
Species: Mouse
Gender: Male
Organ: Lung
Response: Carcinoma
Study: Placke et al. 1996 m = 3



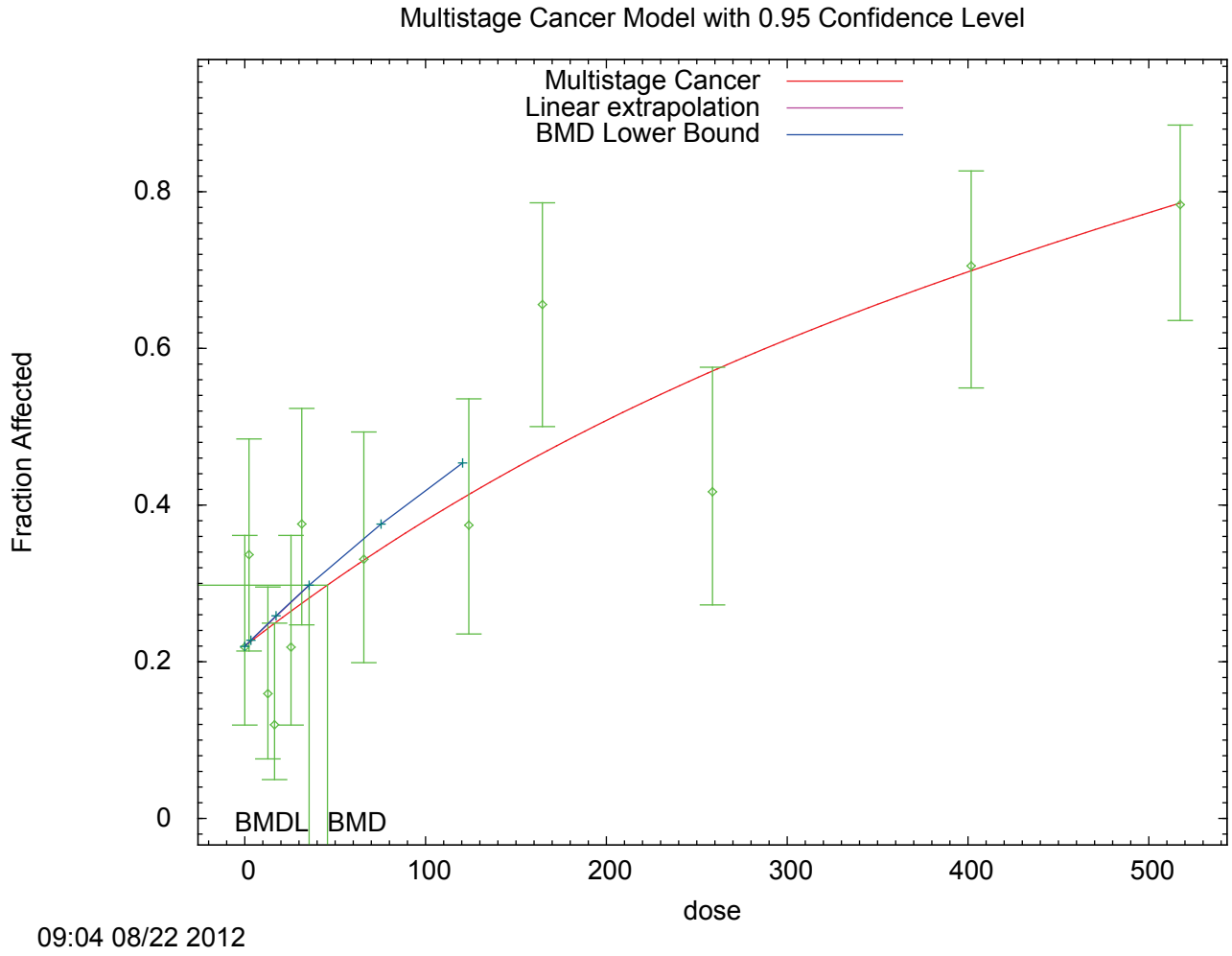
Run 56
Species: Mouse
Gender: Male
Organ: Lung
Response: Adenoma/Carcinoma
Study: Placke et al. 1996 m = 1



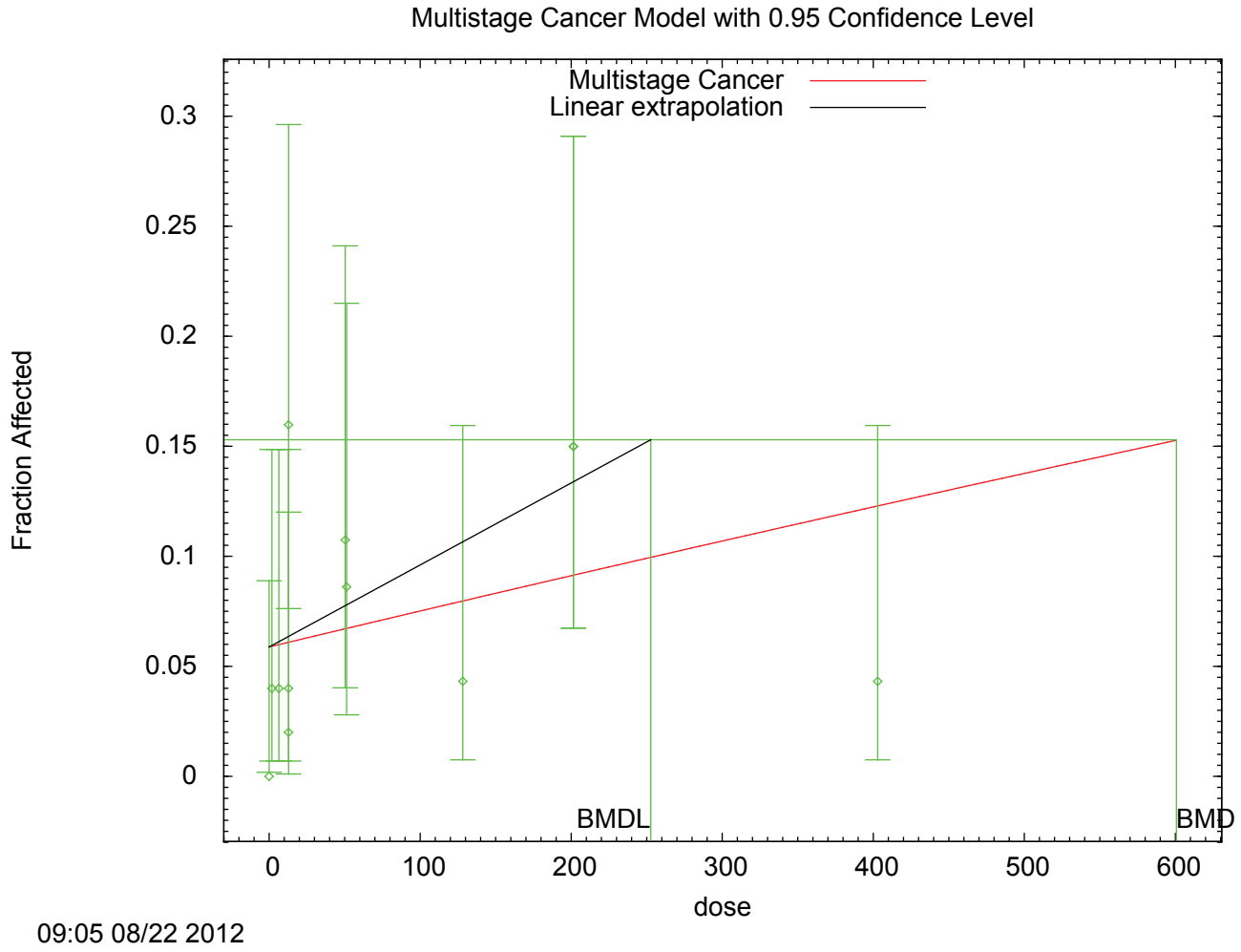
Run 57
Species: Mouse
Gender: Male
Organ: Lung
Response: Adenoma/Carcinoma
Study: Placke et al. 1996 m = 2



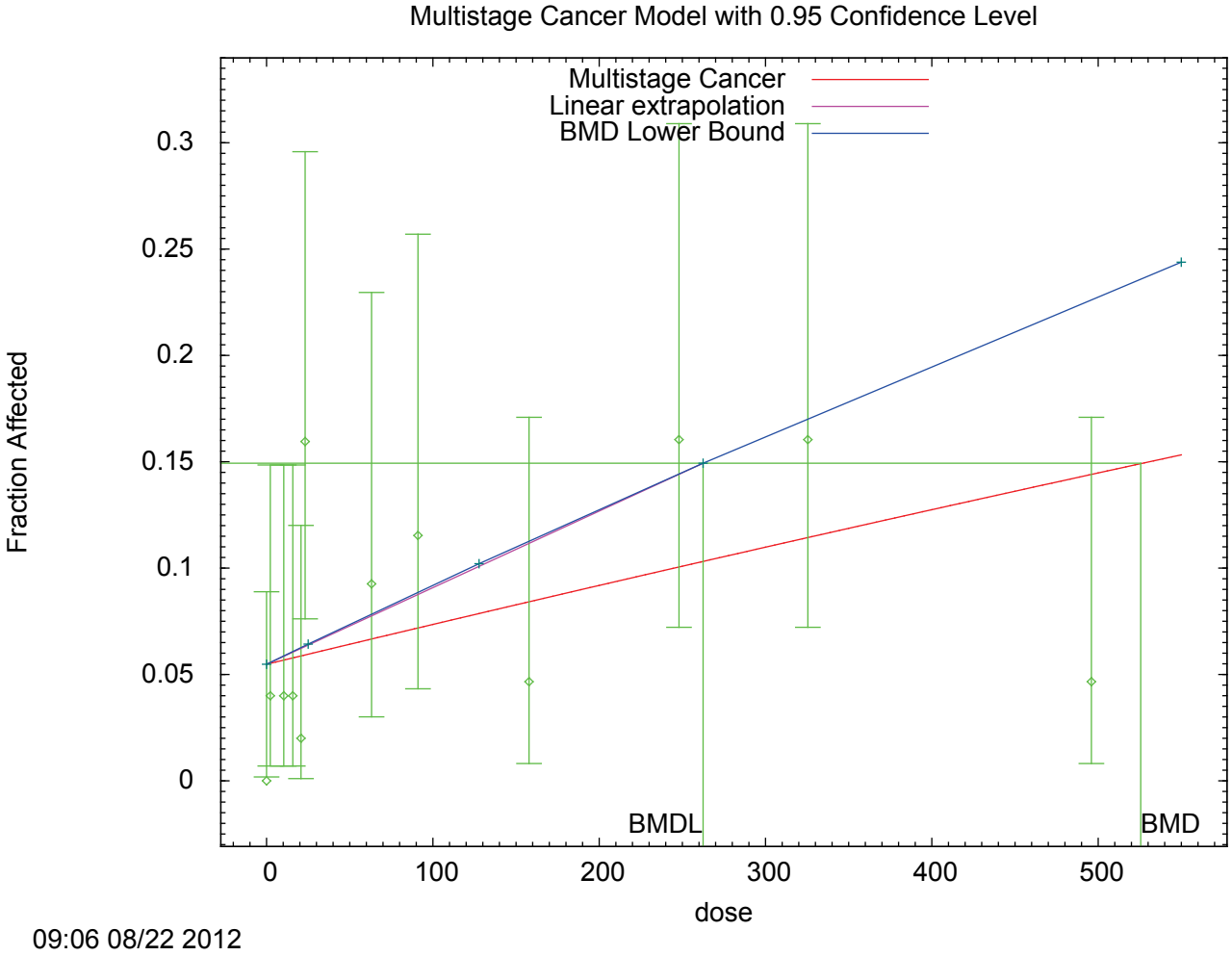
Run 58
Species: Mouse
Gender: Male
Organ: Lung
Response: Adenoma/Carcinoma
Study: Placke et al. 1996 m = 3



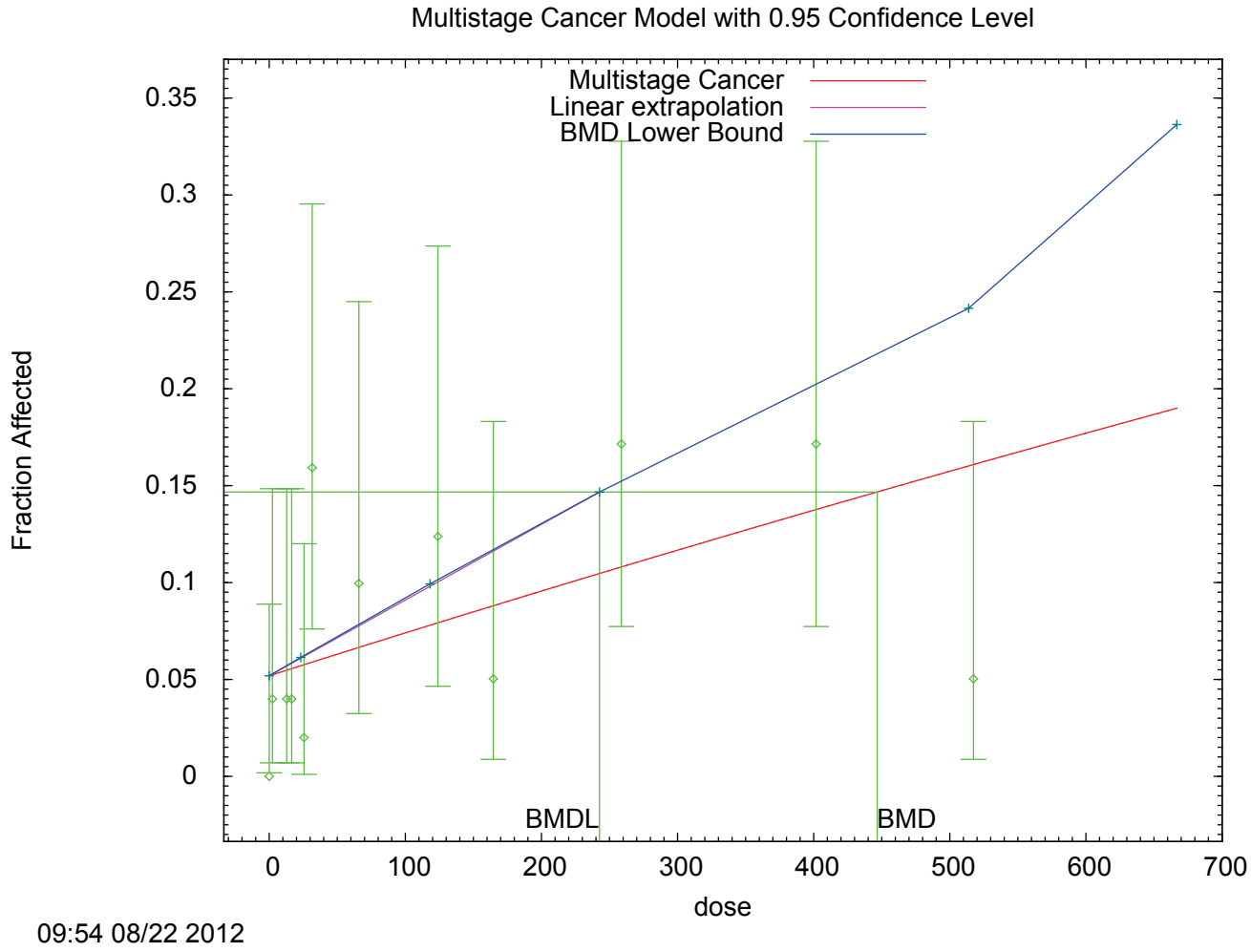
Run 59
Species: Mouse
Gender: Male
Organ: Hematopoietic system
Response: Histiocytic sarcoma
Study: Placke et al. 1996 m = 1



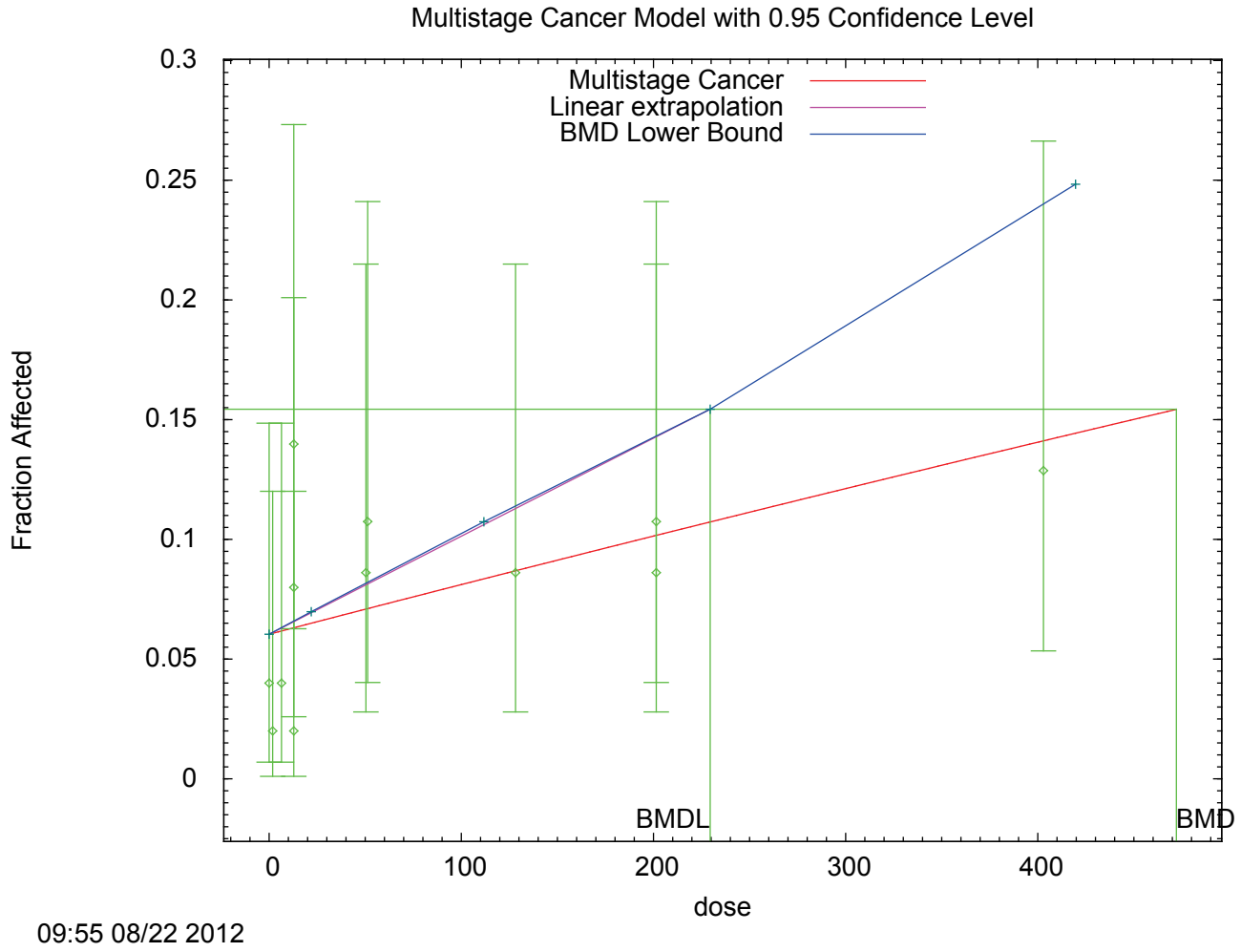
Run 60
Species: Mouse
Gender: Male
Organ: Hematopoietic system
Response: Histiocytic sarcoma
Study: Placke et al. 1996 m = 2



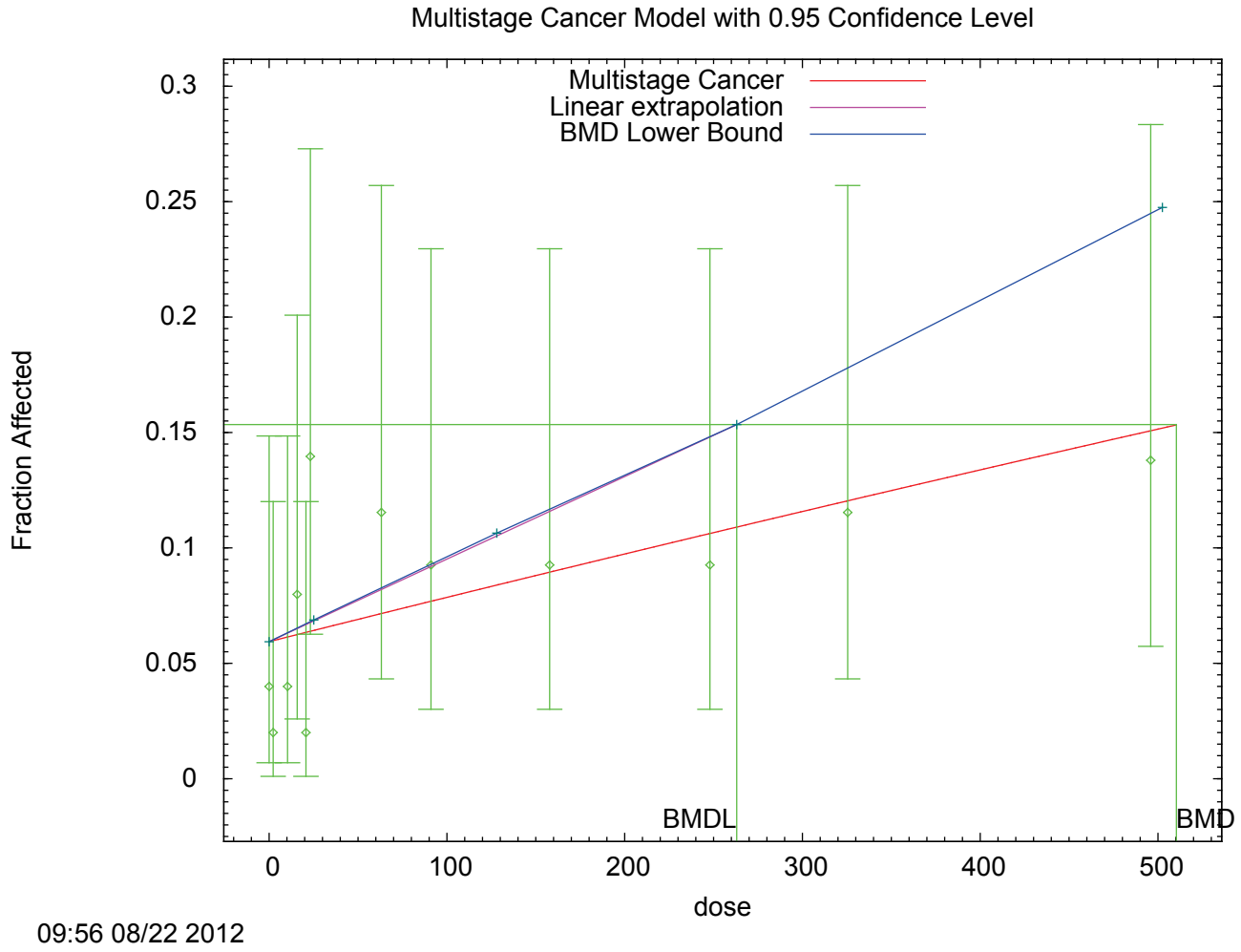
Run 61
Species: Mouse
Gender: Male
Organ: Hematopoietic system
Response: Histiocytic sarcoma
Study: Placke et al. 1996 m = 3



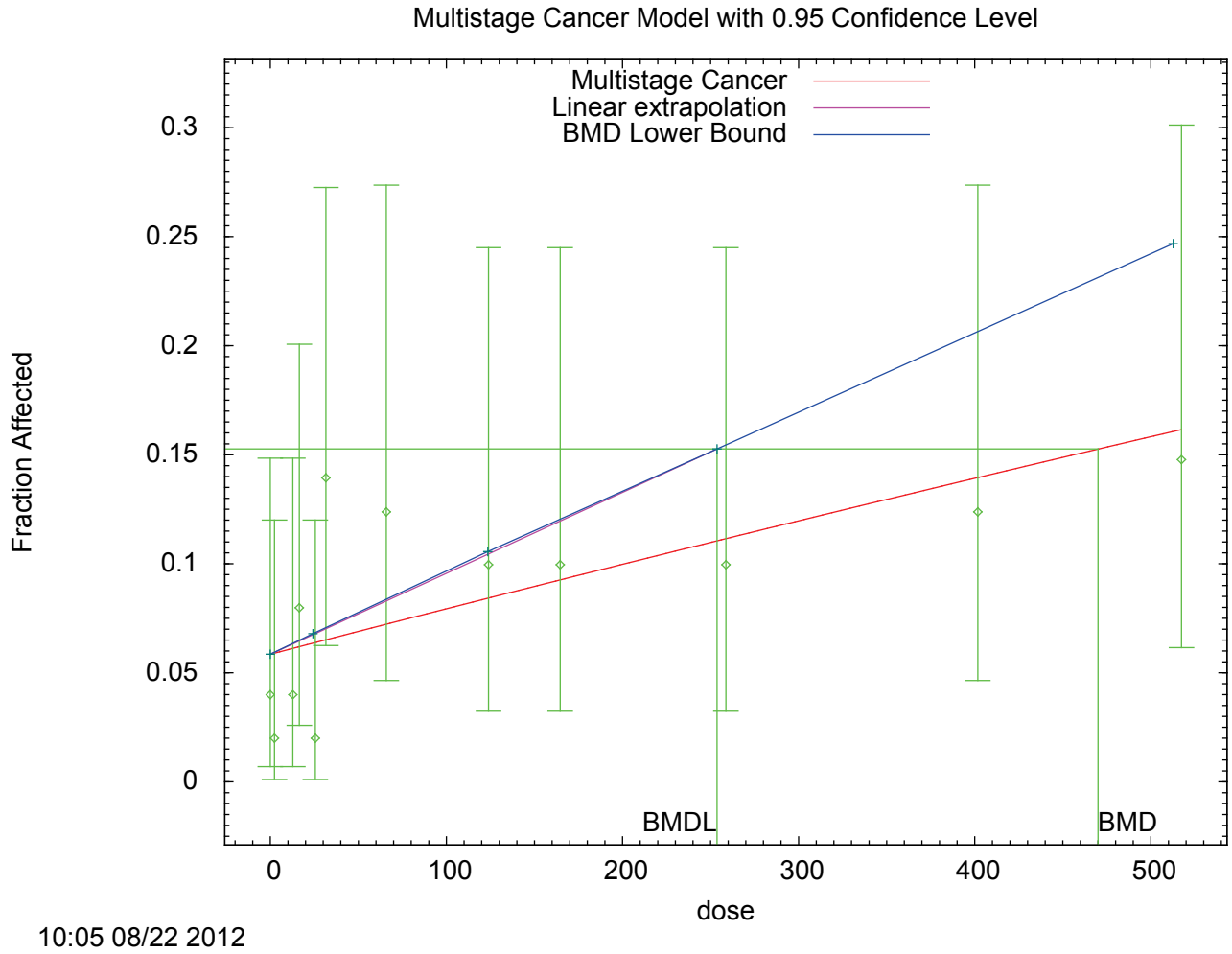
Run 62
Species: Mouse
Gender: Male
Organ: Hematopoietic system
Response: any lymphoma
Study: Placke et al. 1996 m = 1



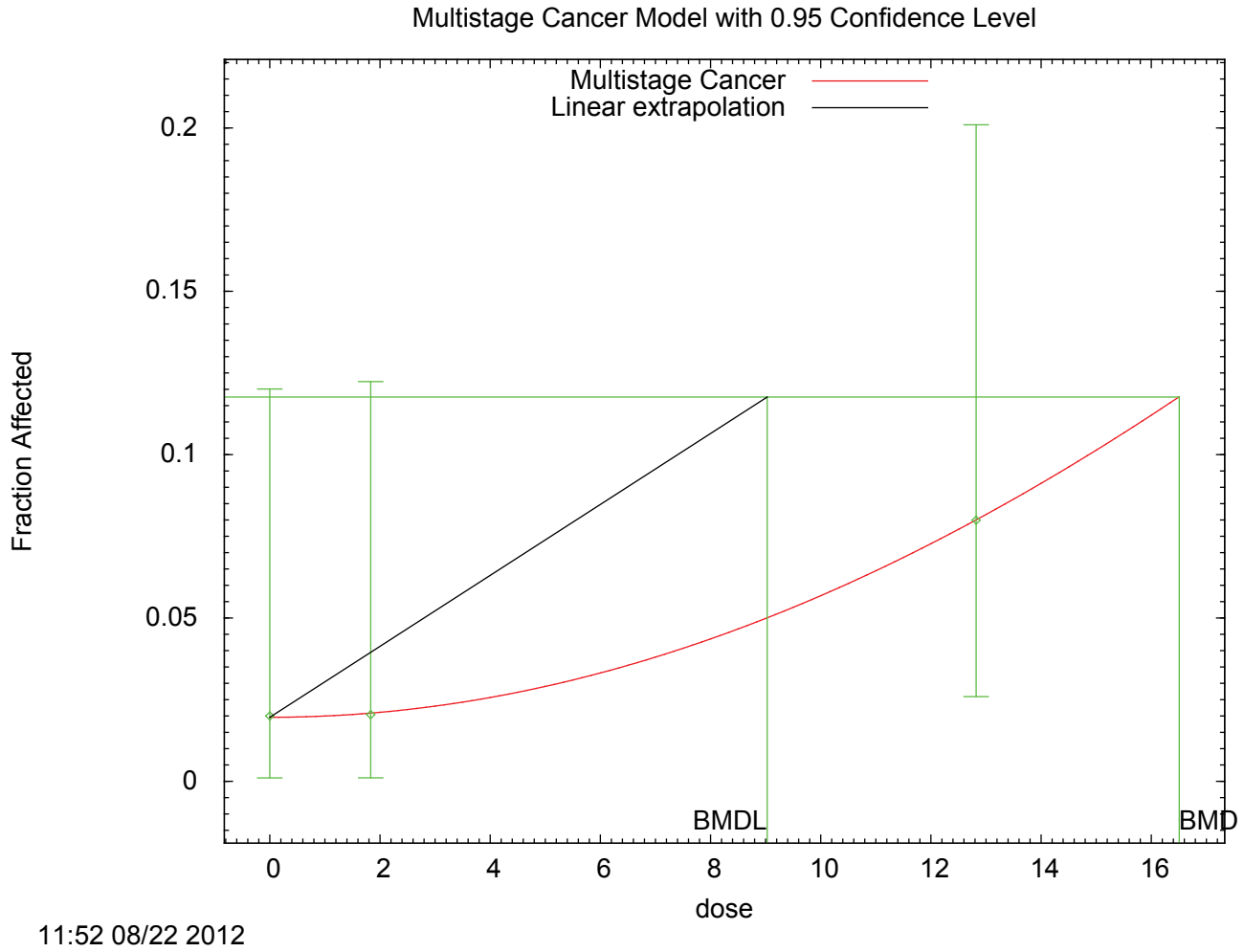
Run 63
Species: Mouse
Gender: Male
Organ: Hematopoietic system
Response: any lymphoma
Study: Placke et al. 1996 m = 2



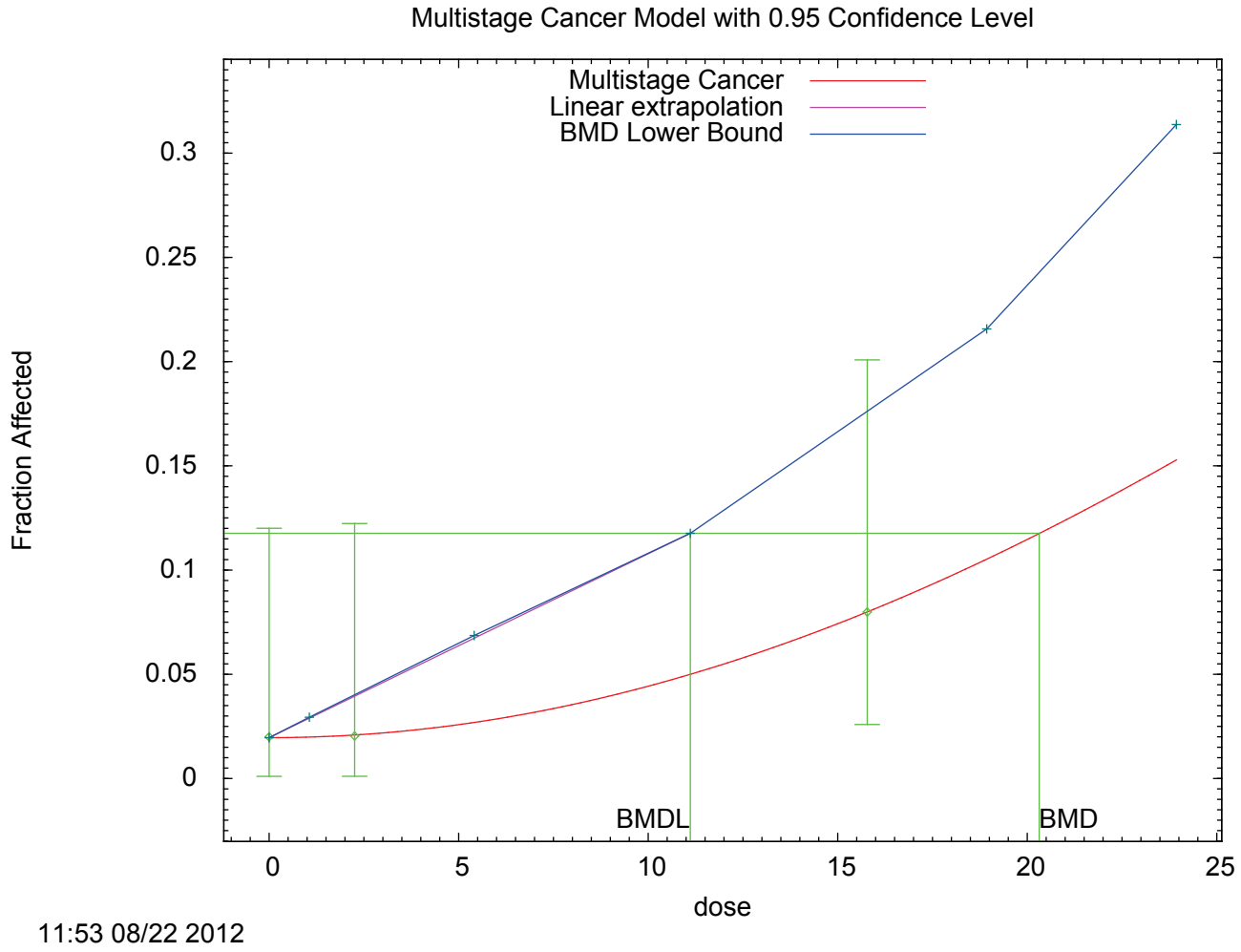
Run 64
Species: Mouse
Gender: Male
Organ: Hematopoietic system
Response: any lymphoma
Study: Placke et al. 1996 m = 3



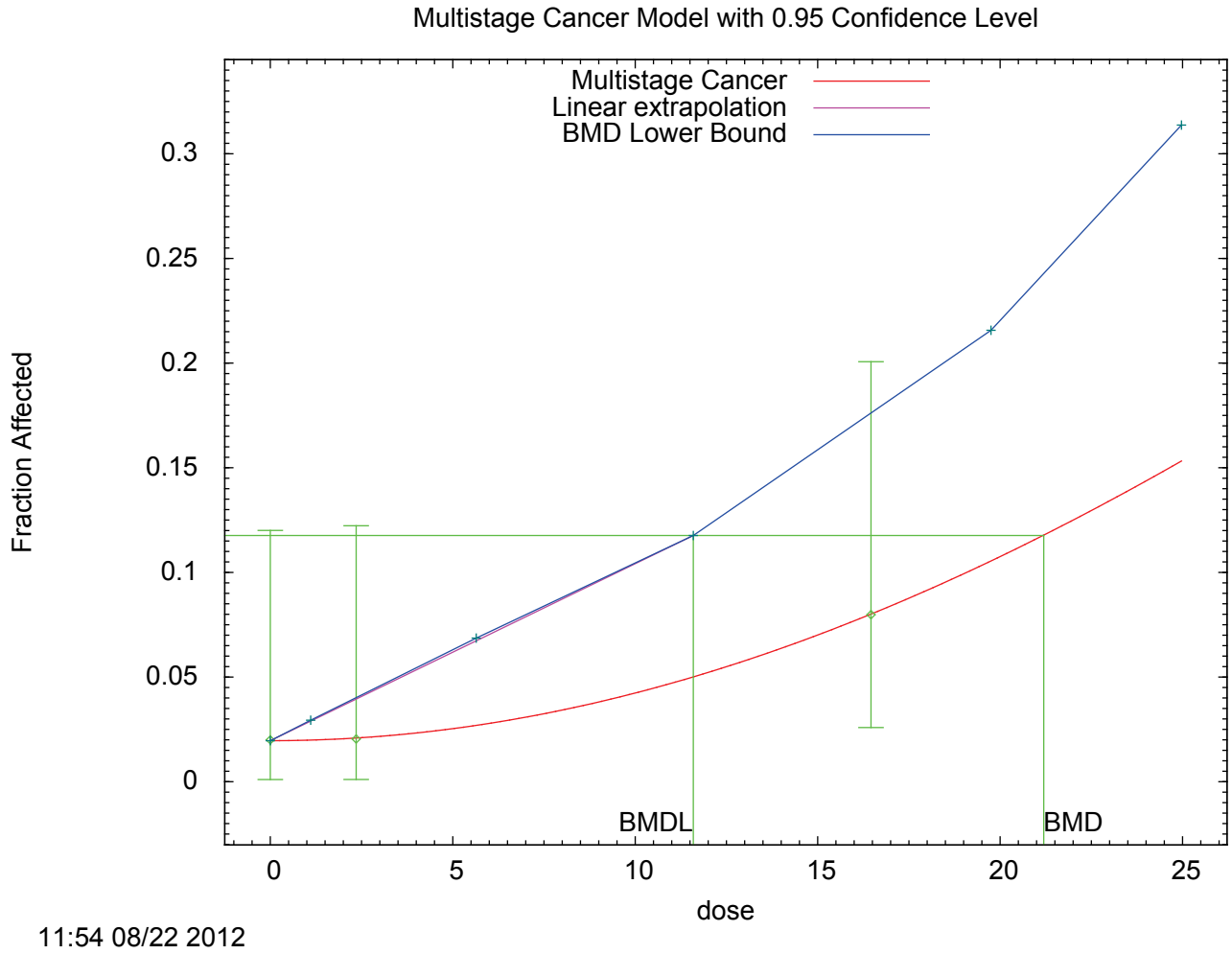
Run 65
Species: Mouse
Gender: Female
Organ: Spleen
Response: hemangiosarcoma
Study: Placke et al. 1996 m = 1



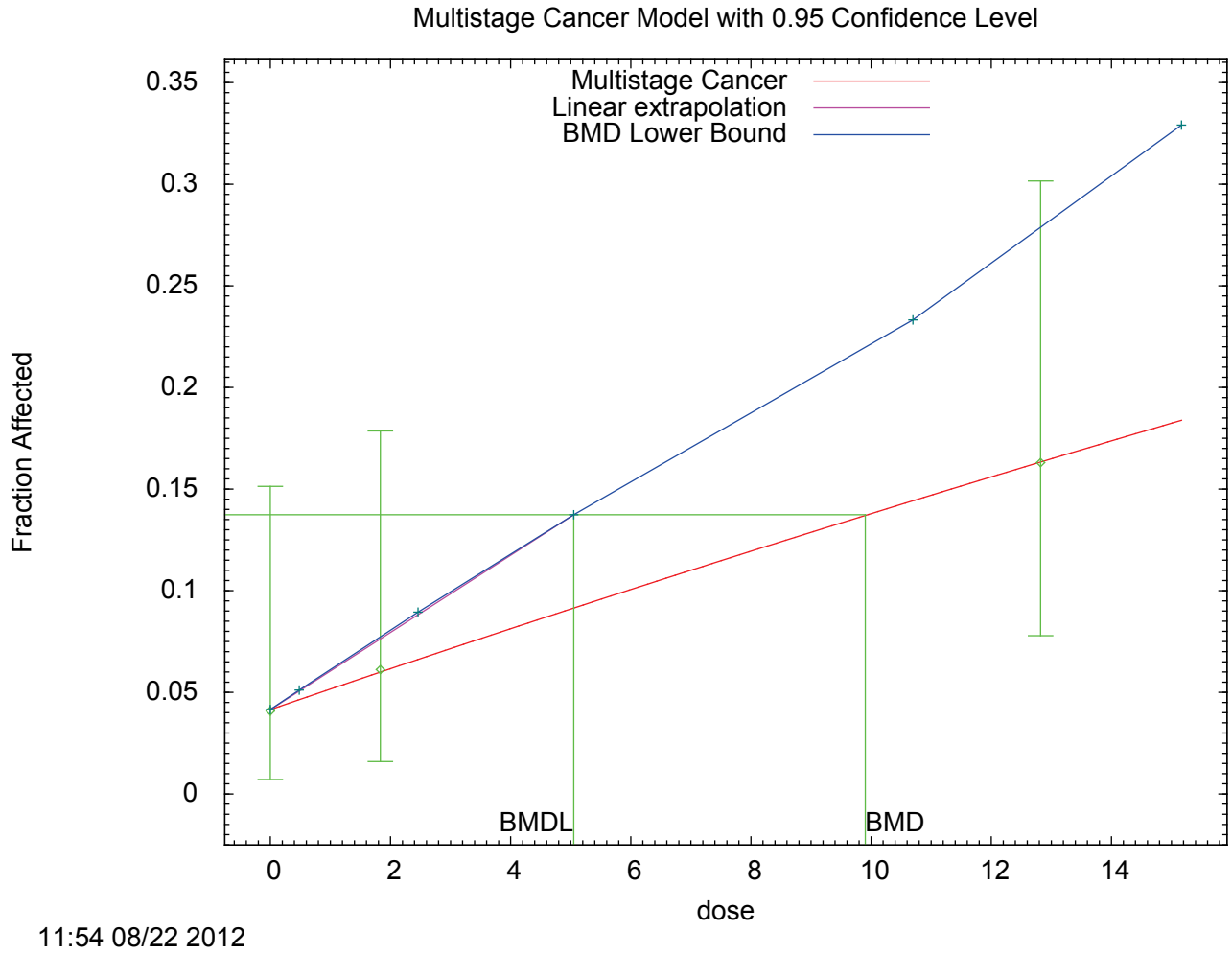
Run 66
Species: Mouse
Gender: Female
Organ: Spleen
Response: hemangiosarcoma
Study: Placke et al. 1996 m = 2



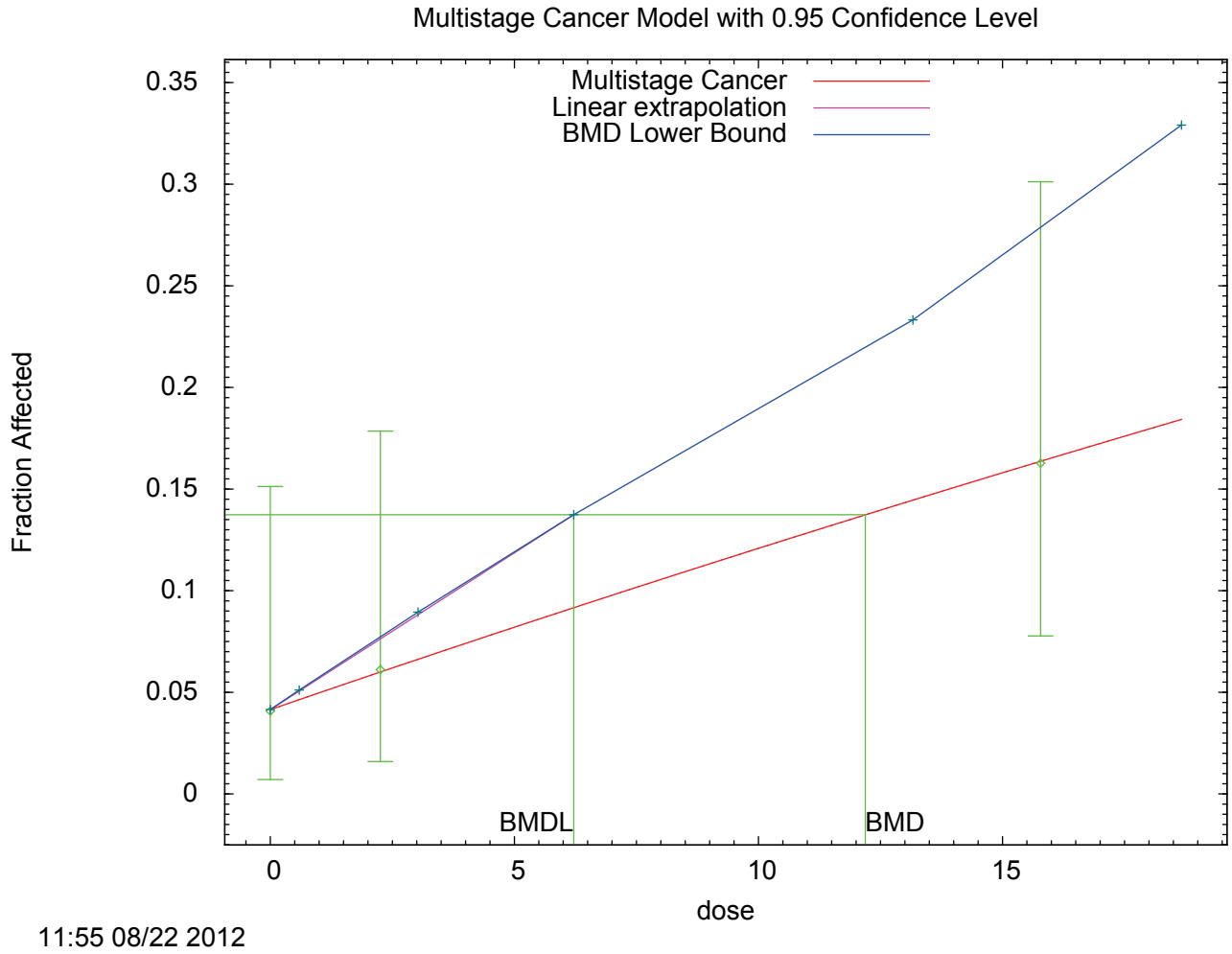
Run 67
Species: Mouse
Gender: Female
Organ: Spleen
Response: hemangiosarcoma
Study: Placke et al. 1996 m = 3



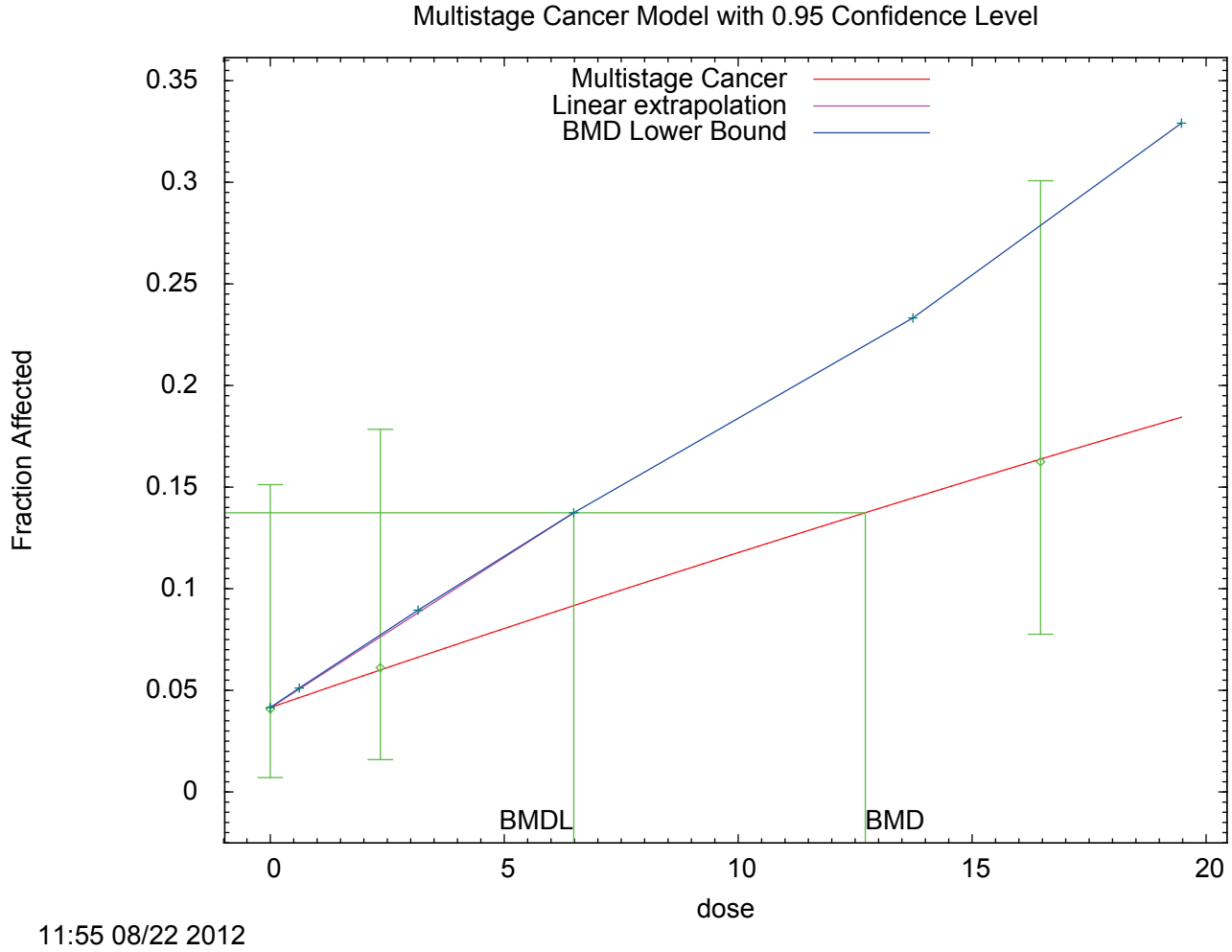
Run 68
Species: Mouse
Gender: Female
Organ: Harderian gland
Response: Adenoma
Study: Placke et al. 1996 m = 1



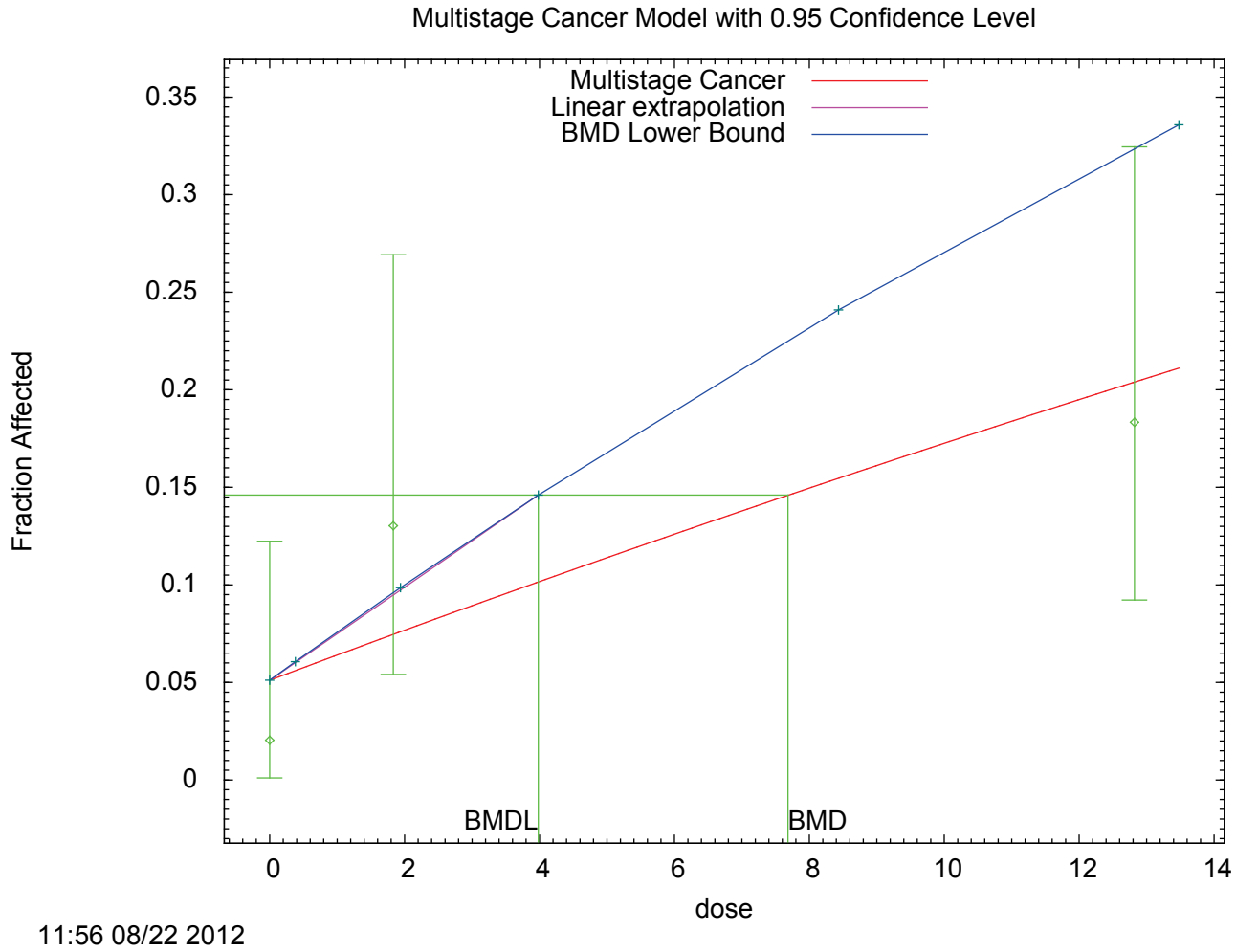
Run 69
Species: Mouse
Gender: Female
Organ: Harderian gland
Response: Adenoma
Study: Placke et al. 1996 m = 2



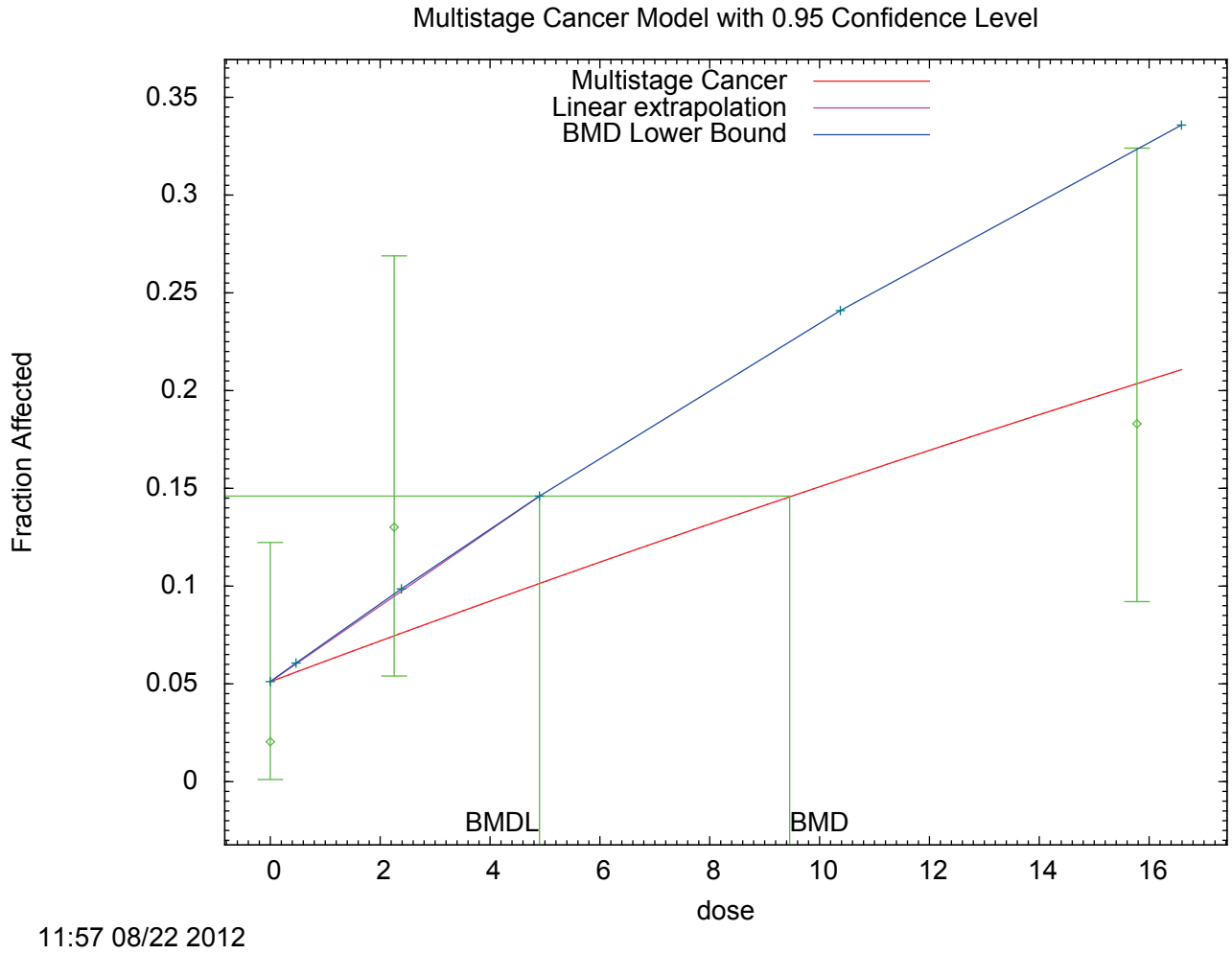
Run 70
Species: Mouse
Gender: Female
Organ: Harderian gland
Response: Adenoma
Study: Placke et al. 1996 m = 3



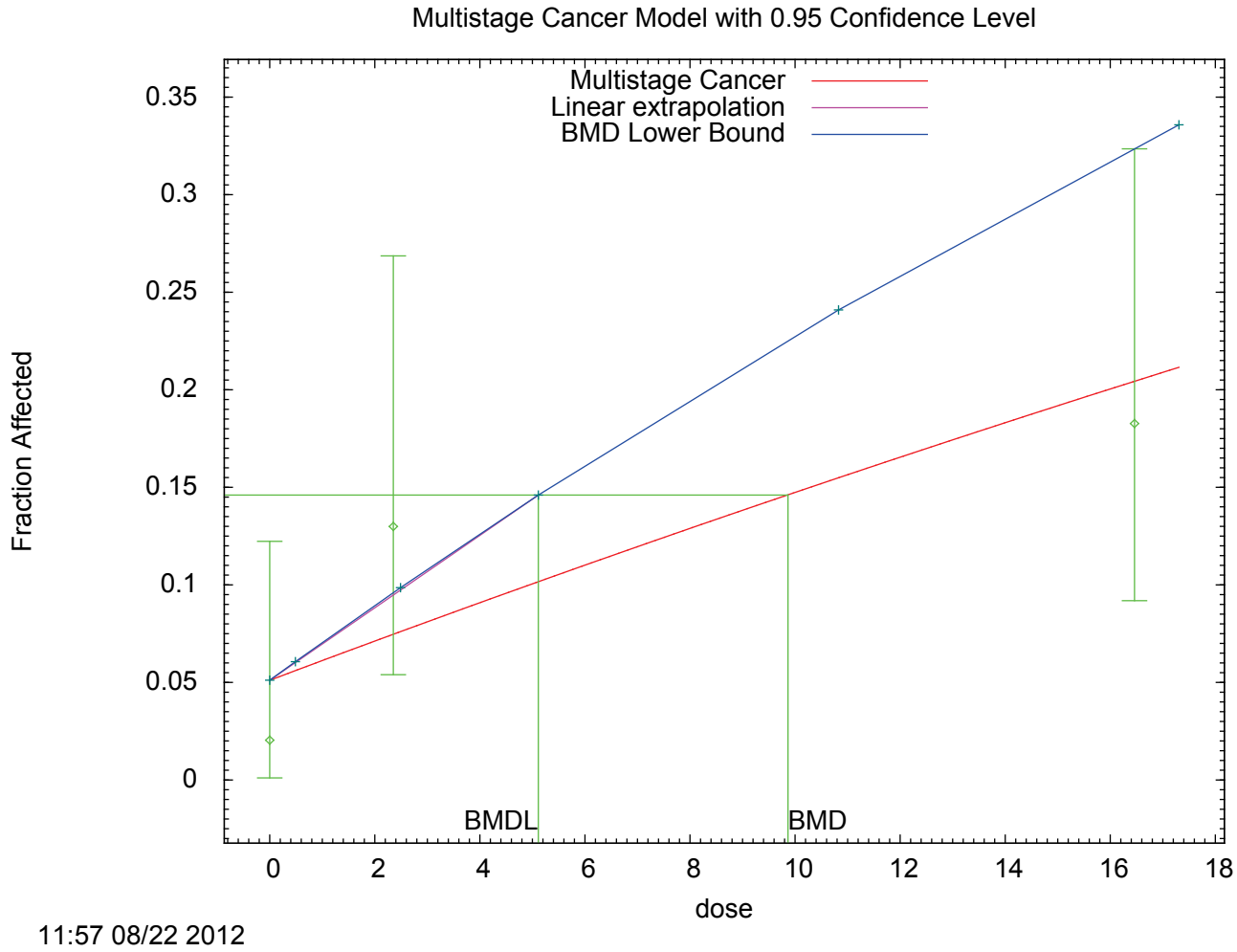
Run 71
Species: Mouse
Gender: Female
Organ: Pituitary gland
Response: Adenoma
Study: Placke et al. 1996 m = 1



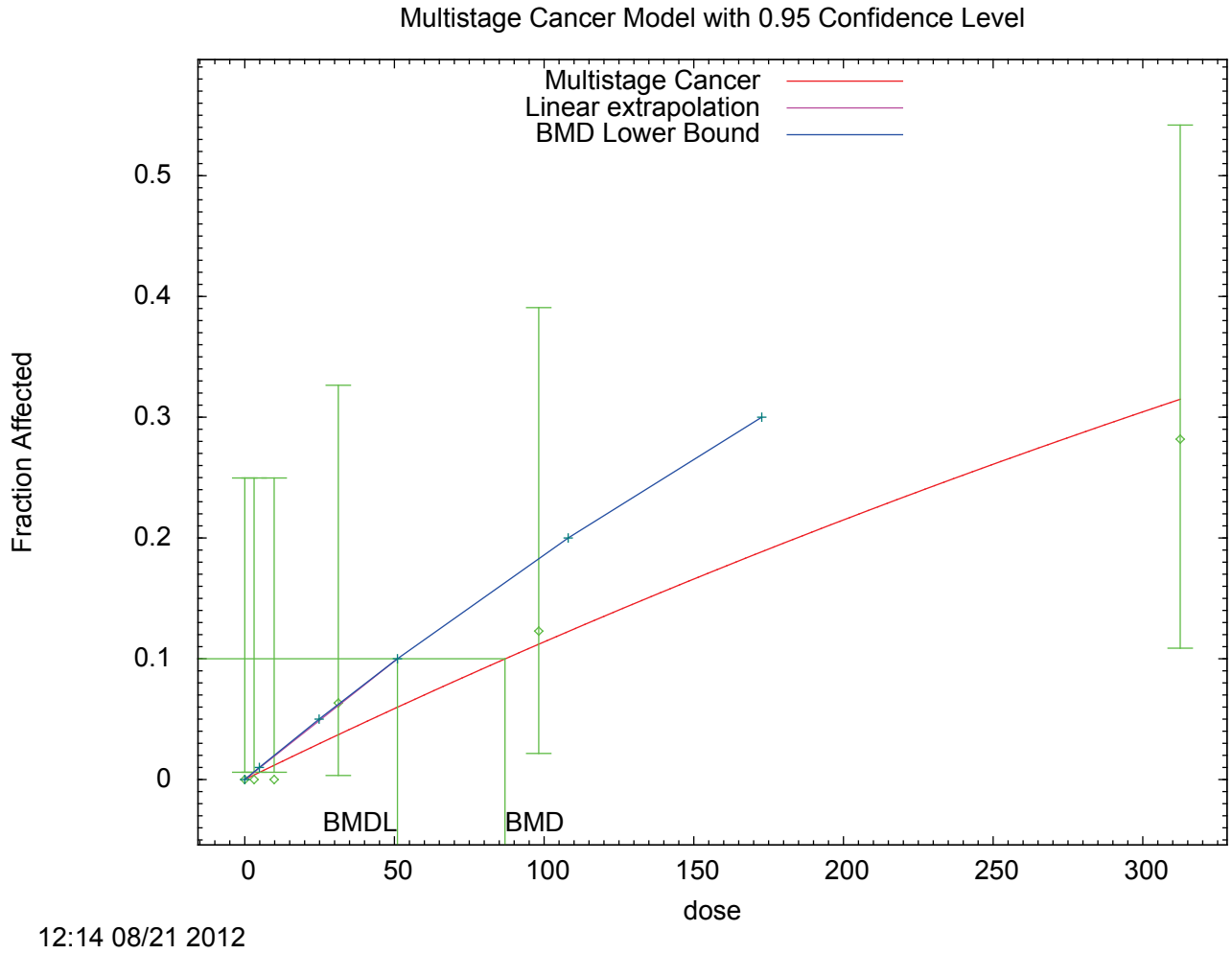
Run 72
Species: Mouse
Gender: Female
Organ: Pituitary gland
Response: Adenoma
Study: Placke et al. 1996 m = 2



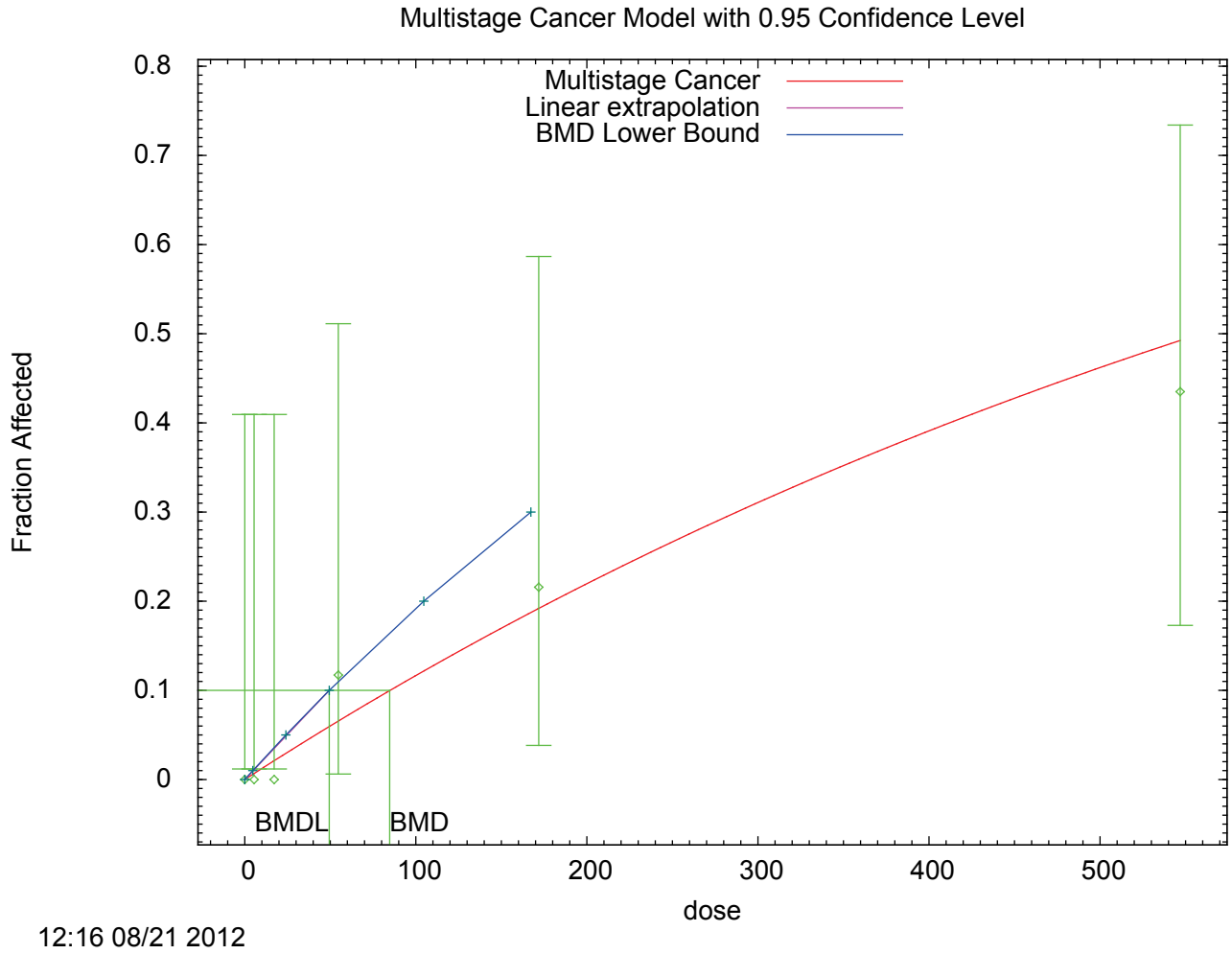
Run 73
Species: Mouse
Gender: Female
Organ: Pituitary gland
Response: Adenoma
Study: Placke et al. 1996 m = 3



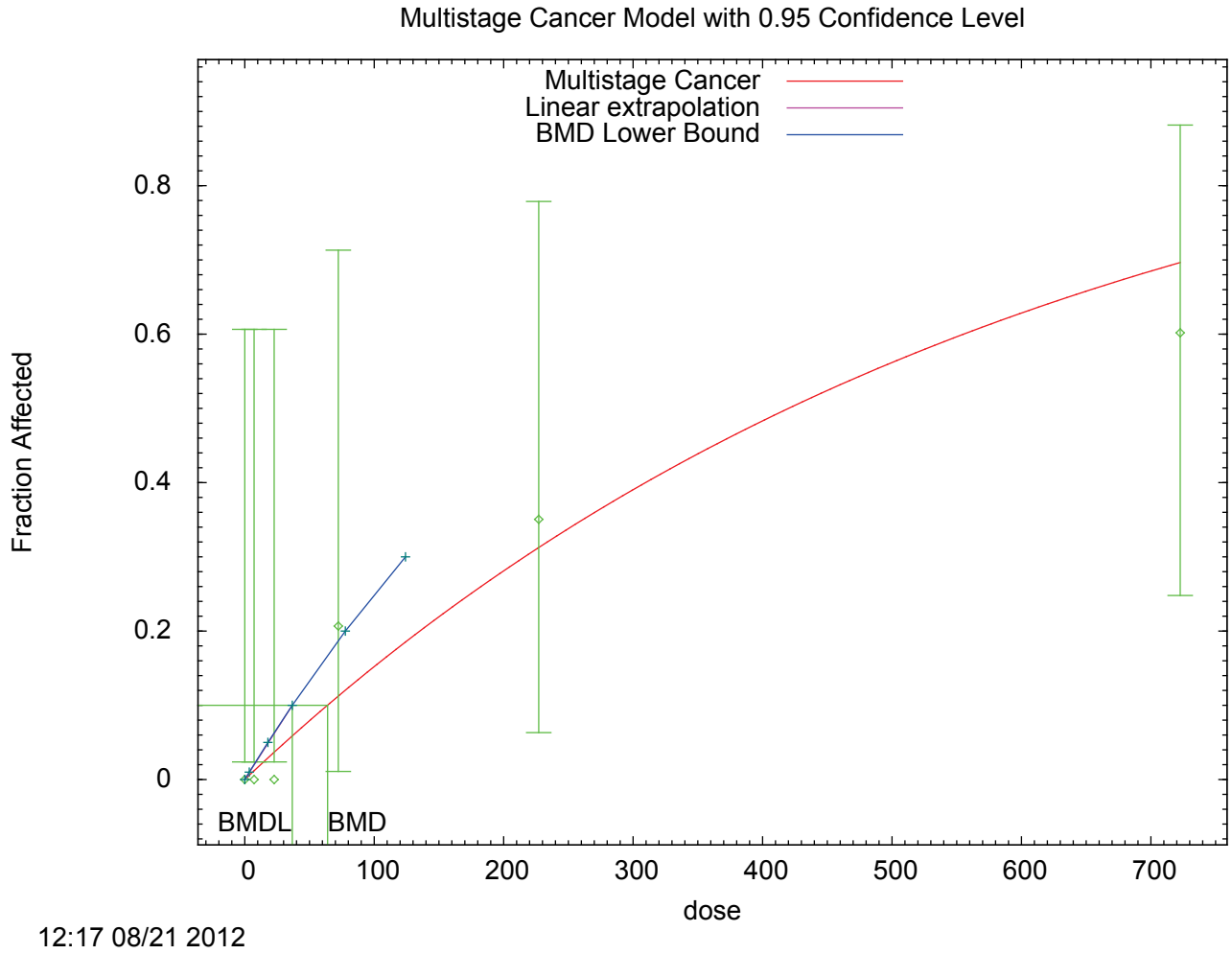
Run 74
Species: Mouse
Gender: Male
Organ: Forestomach
Response: Papilloma
Study: NTP 1994 m = 1



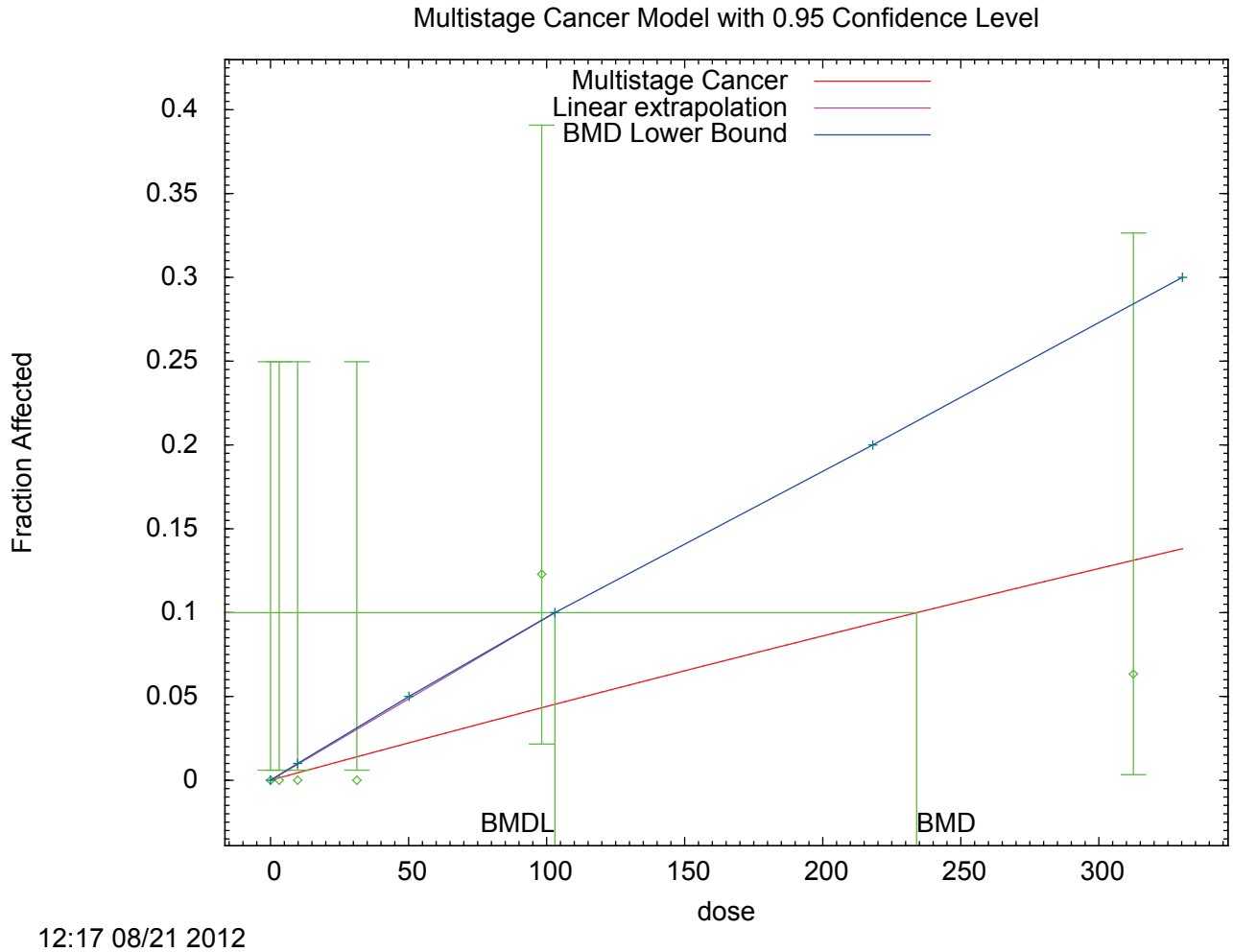
Run 75
Species: Mouse
Gender: Male
Organ: Forestomach
Response: Papilloma
Study: NTP 1994 m = 2



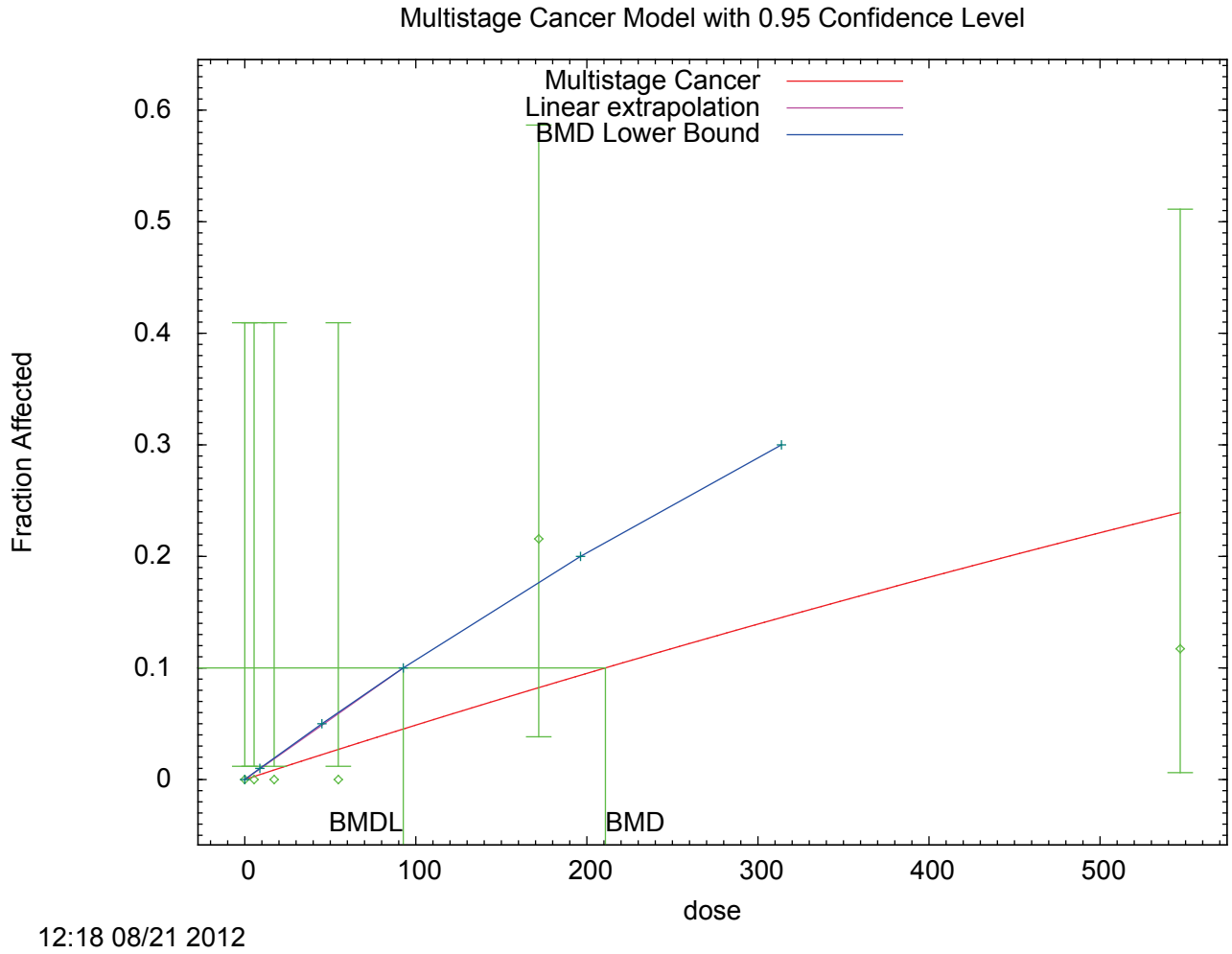
Run 76
Species: Mouse
Gender: Male
Organ: Forestomach
Response: Papilloma
Study: NTP 1994 m = 3



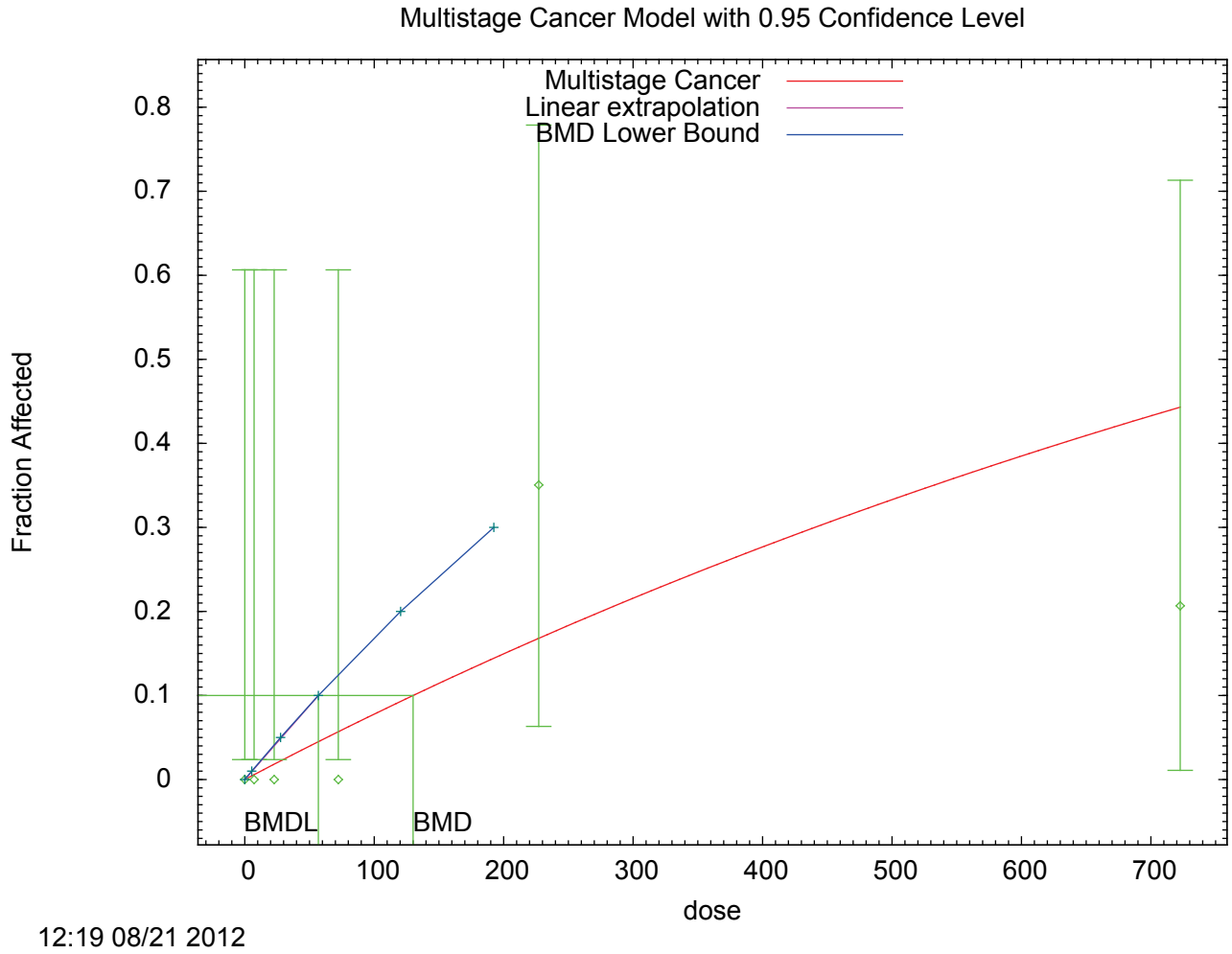
Run 77
Species: Mouse
Gender: Male
Organ: Forestomach
Response: Carcinoma
Study: NTP 1994 m = 1



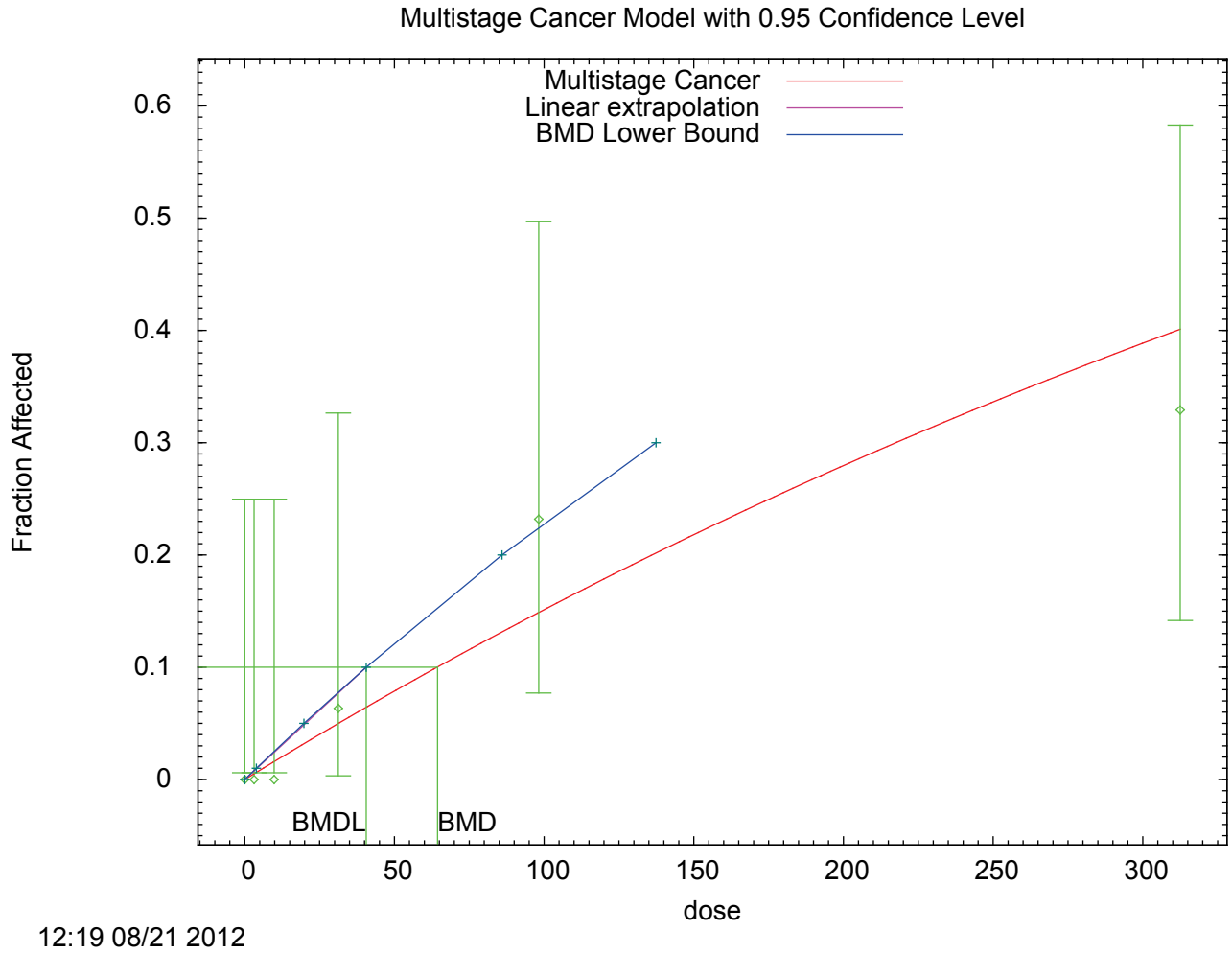
Run 78
Species: Mouse
Gender: Male
Organ: Forestomach
Response: Carcinoma
Study: NTP 1994 m = 2



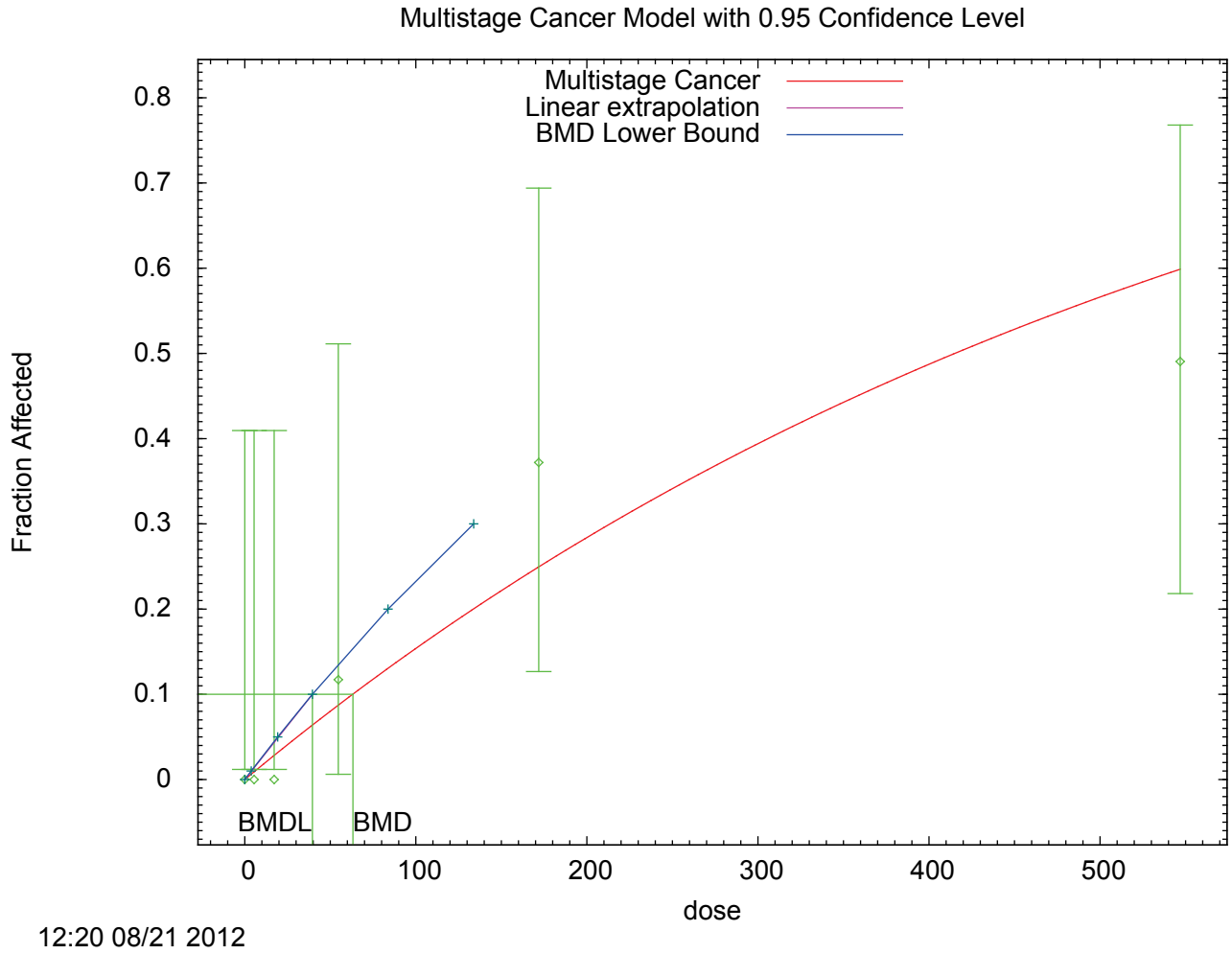
Run 79
Species: Mouse
Gender: Male
Organ: Forestomach
Response: Carcinoma
Study: NTP 1994 m = 3



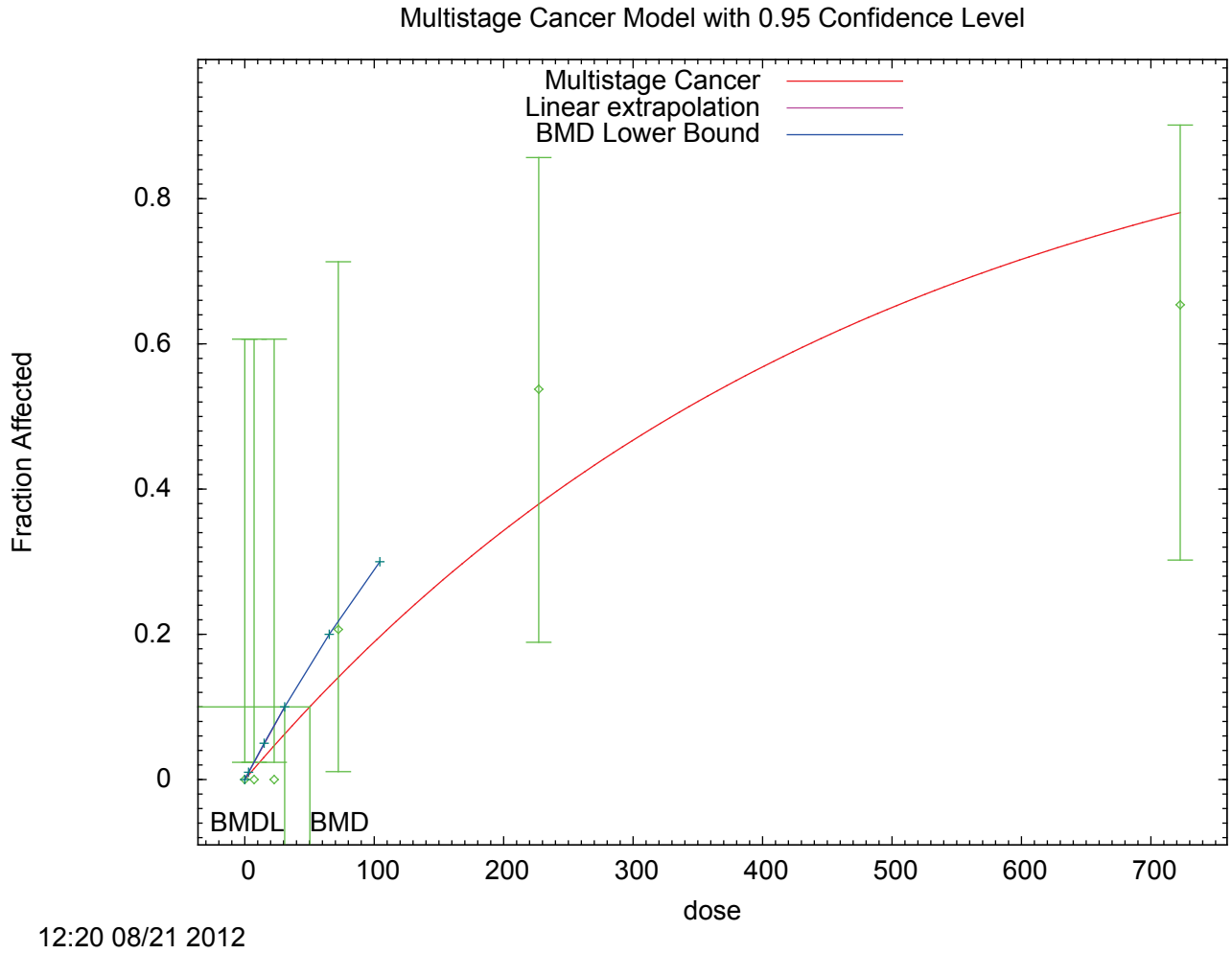
Run 80
Species: Mouse
Gender: Male
Organ: Forestomach
Response: Papilloma/Carcinoma
Study: NTP 1994 m = 1



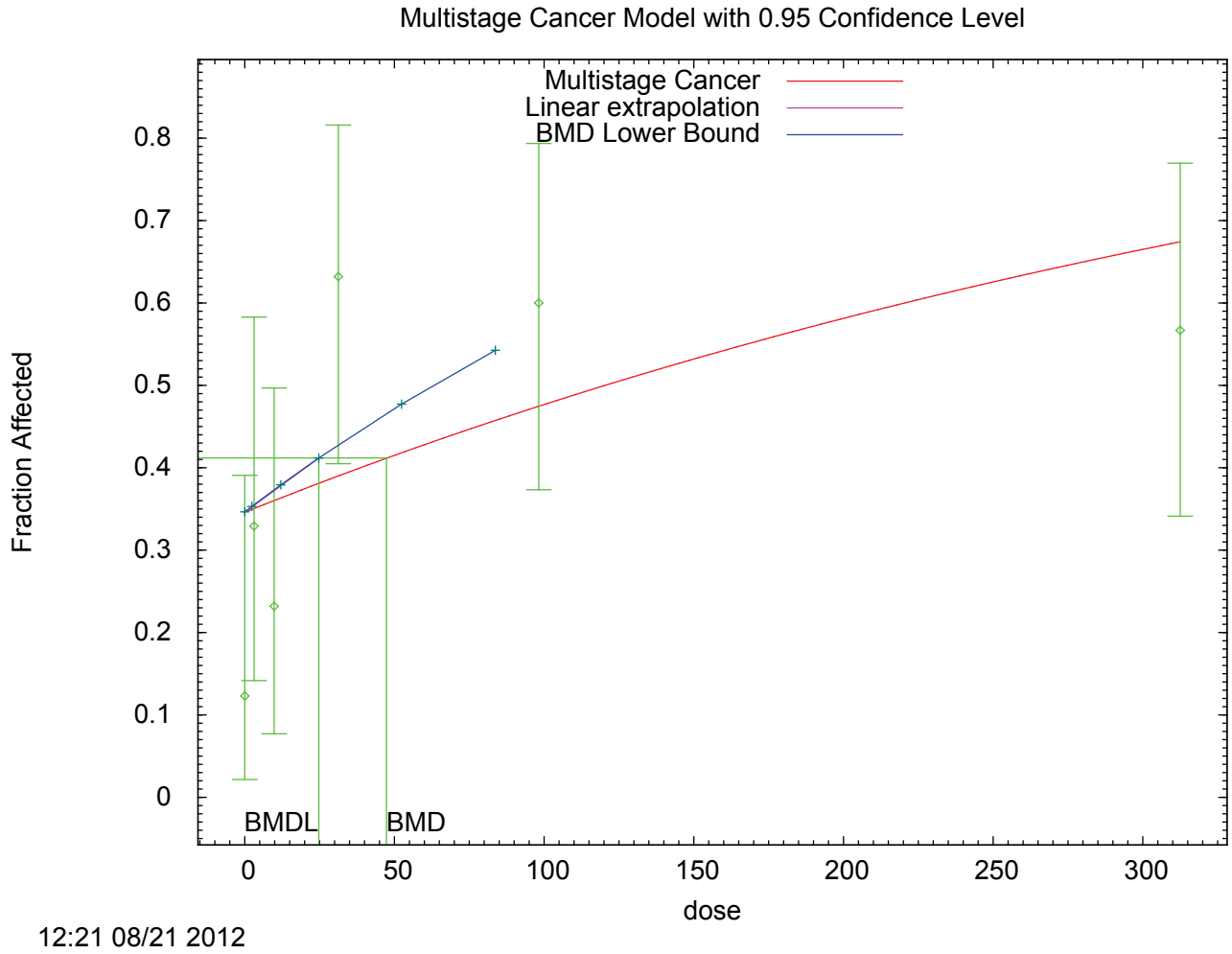
Run 81
Species: Mouse
Gender: Male
Organ: Forestomach
Response: Papilloma/Carcinoma
Study: NTP 1994 m = 2



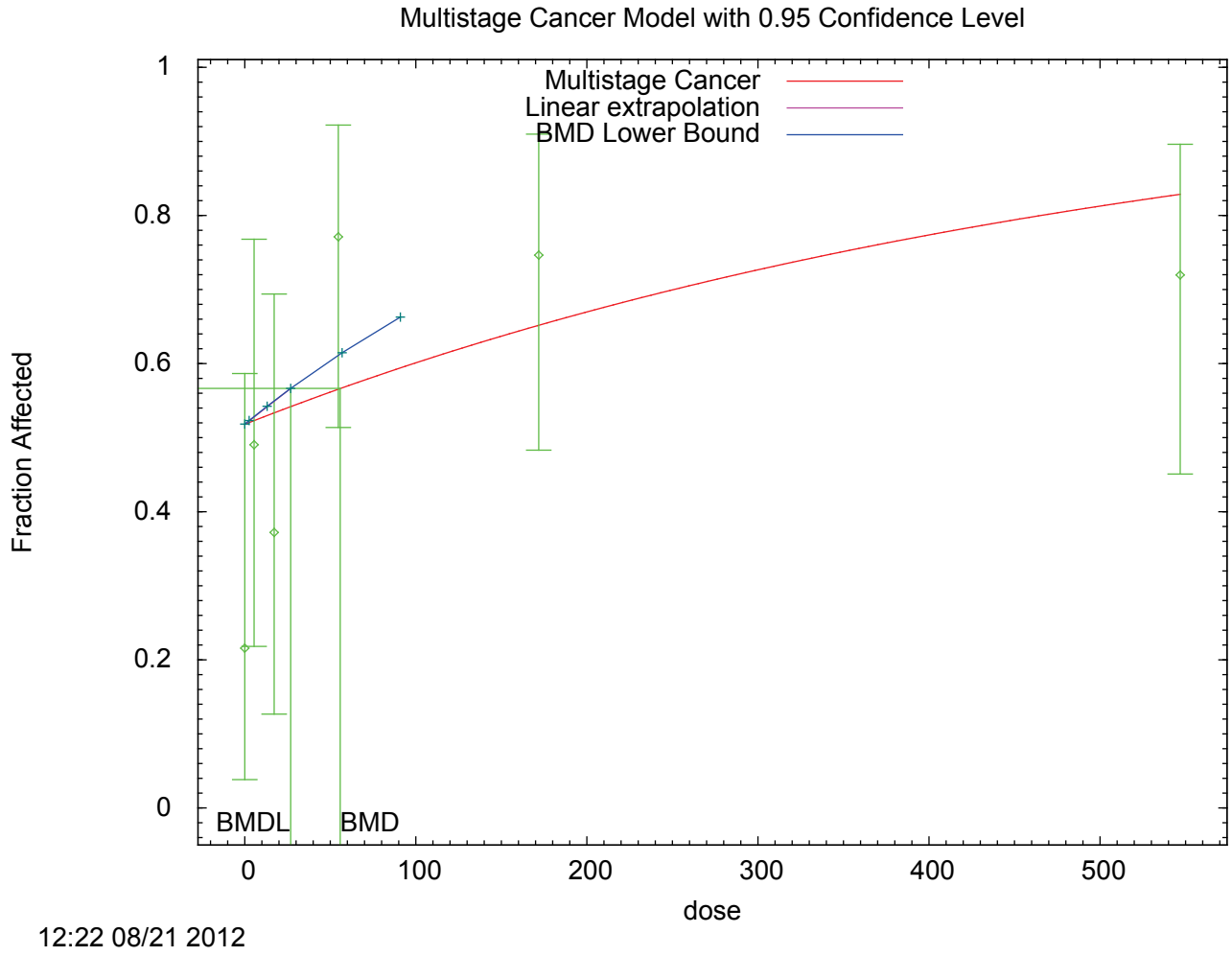
Run 82
Species: Mouse
Gender: Male
Organ: Forestomach
Response: Papilloma/Carcinoma
Study: NTP 1994 m = 3



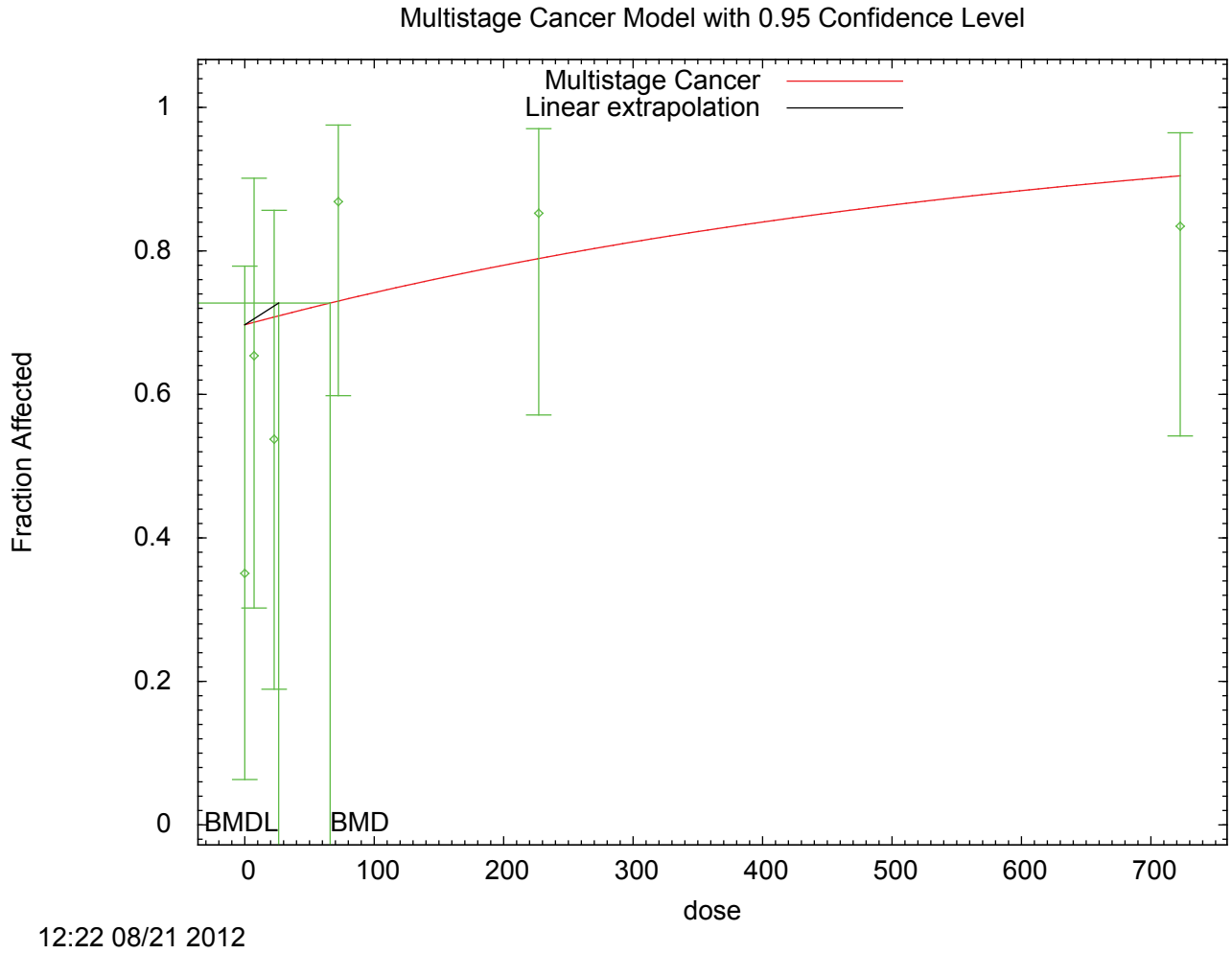
Run 83
Species: Mouse
Gender: Male
Organ: Harderian gland
Response: Adenoma
Study: NTP 1994 m = 1



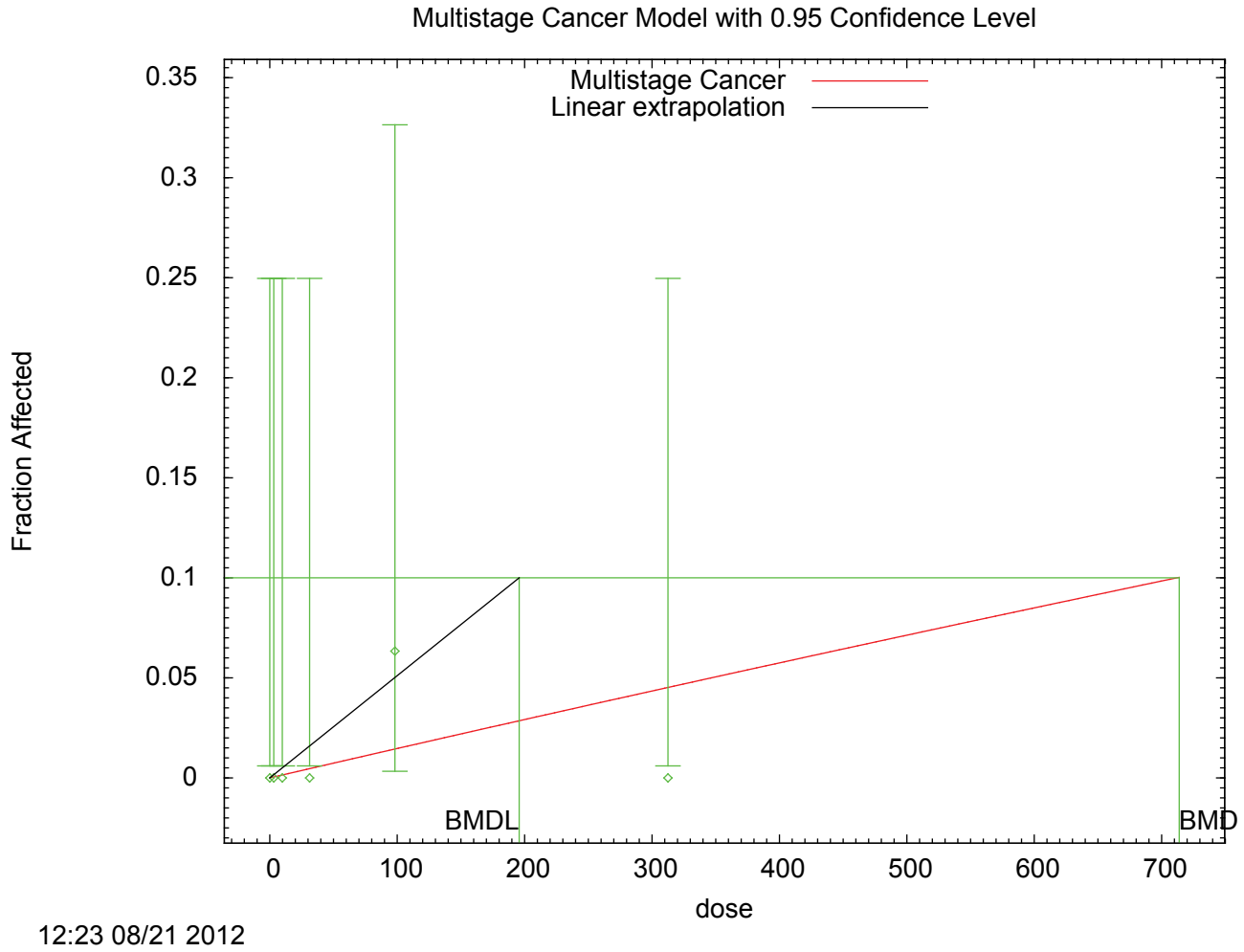
Run 84
Species: Mouse
Gender: Male
Organ: Harderian gland
Response: Adenoma
Study: NTP 1994 m = 2



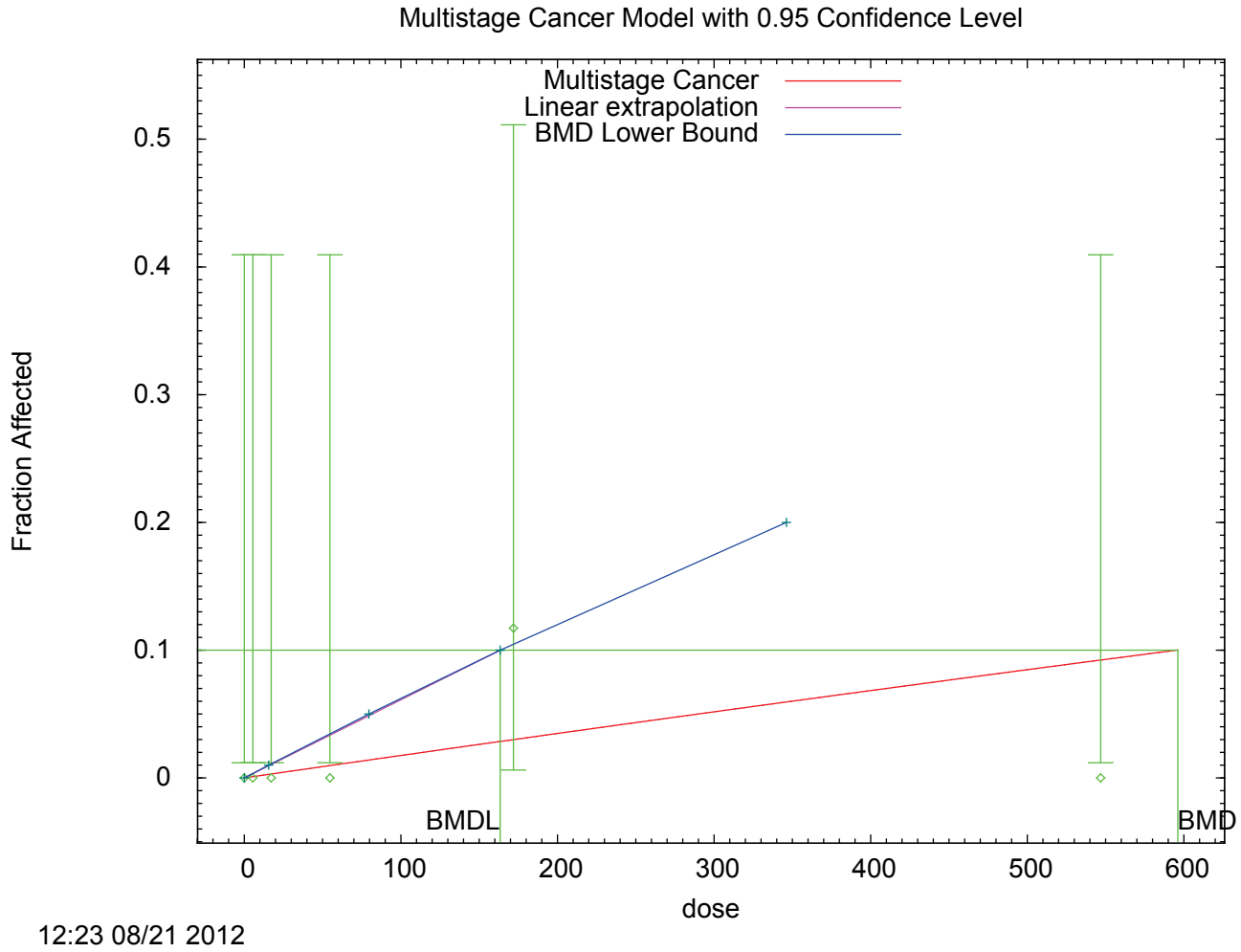
Run 85
Species: Mouse
Gender: Male
Organ: Harderian gland
Response: Adenoma
Study: NTP 1994 m = 3



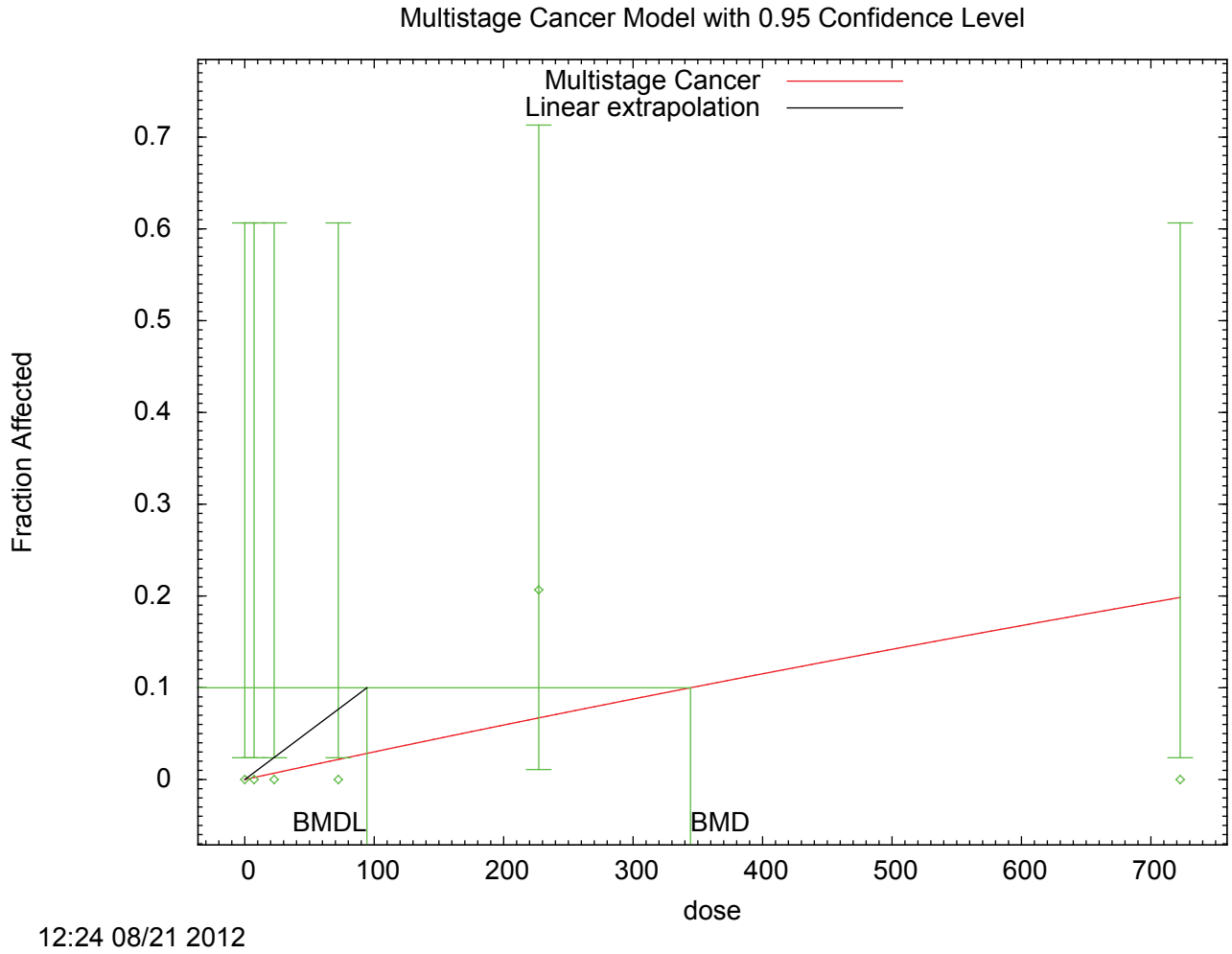
Run 86
Species: Mouse
Gender: Male
Organ: Harderian gland
Response: Carcinoma
Study: NTP 1994 m = 1



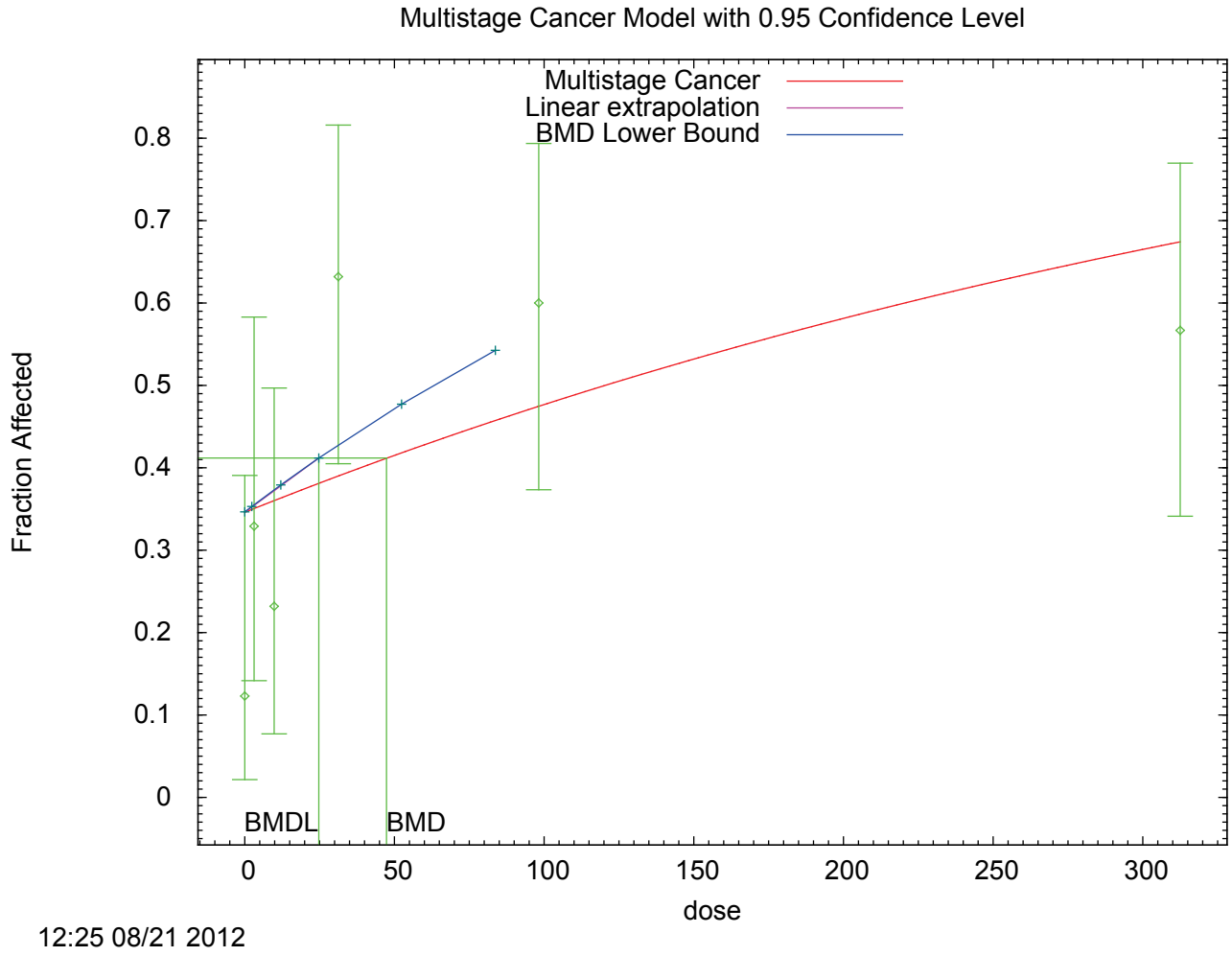
Run 87
Species: Mouse
Gender: Male
Organ: Harderian gland
Response: Carcinoma
Study: NTP 1994 m = 2



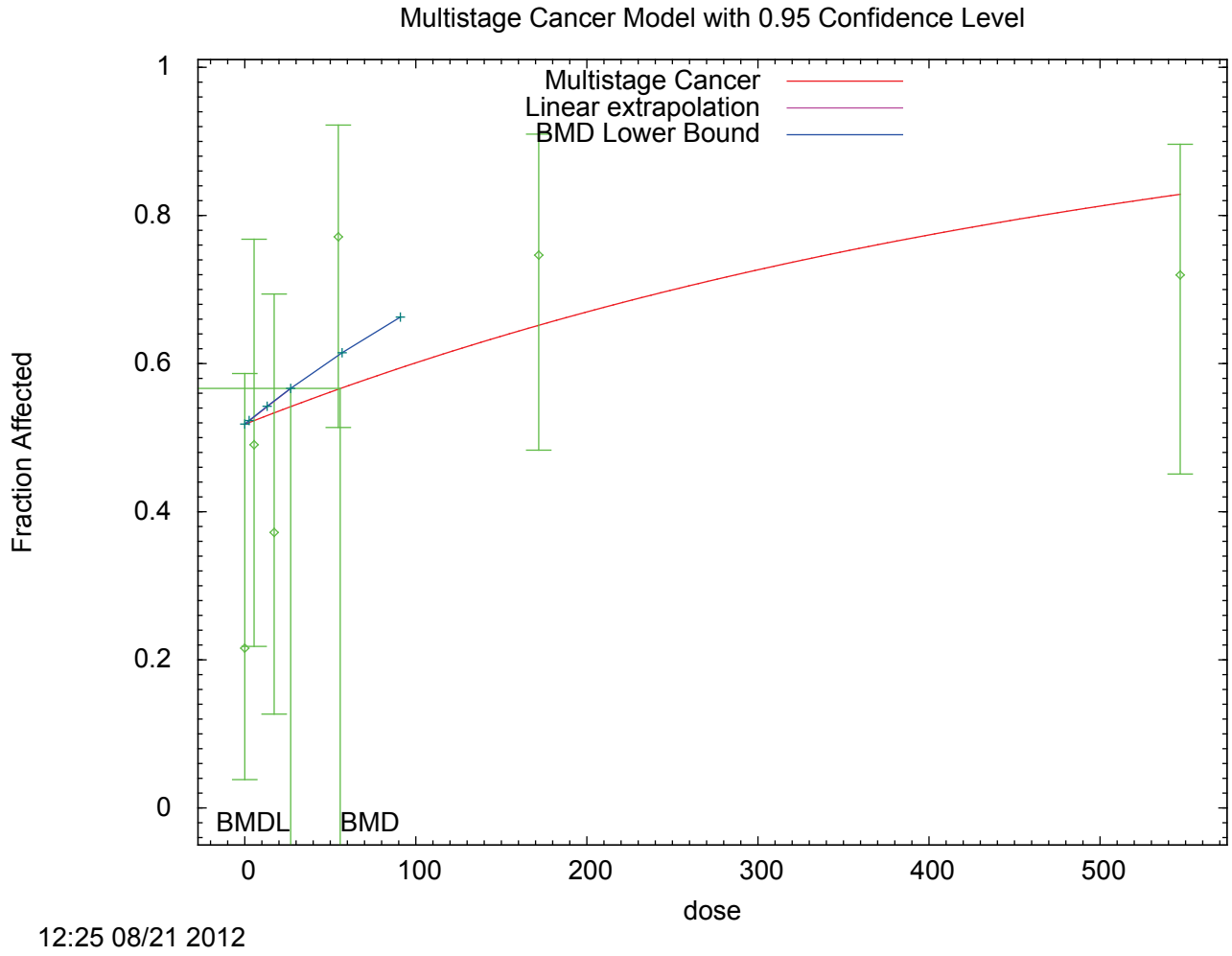
Run 88
Species: Mouse
Gender: Male
Organ: Harderian gland
Response: Carcinoma
Study: NTP 1994 m = 3



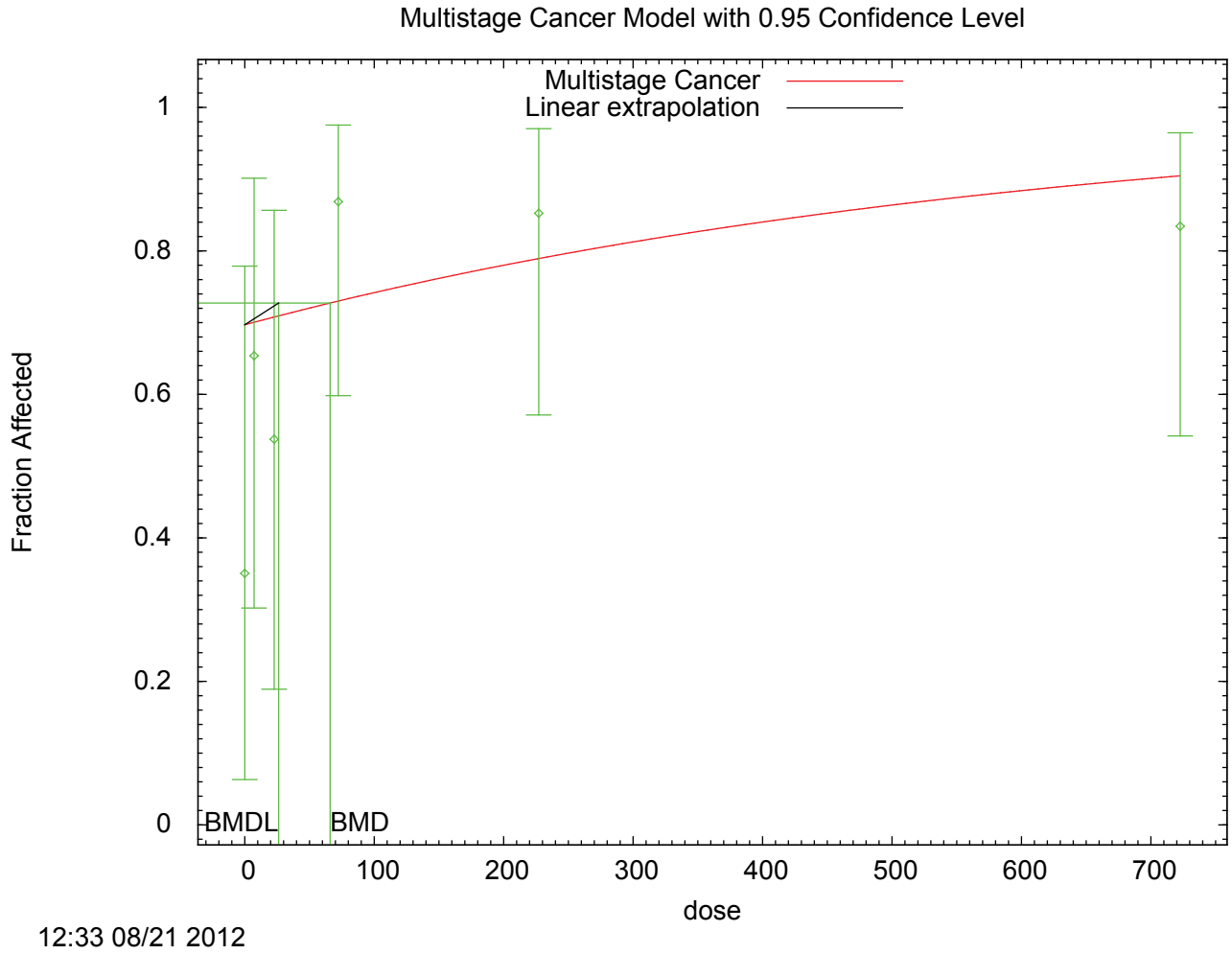
Run 89
Species: Mouse
Gender: Male
Organ: Harderian gland
Response: Adenoma/Carcinoma
Study: NTP 1994 m = 1



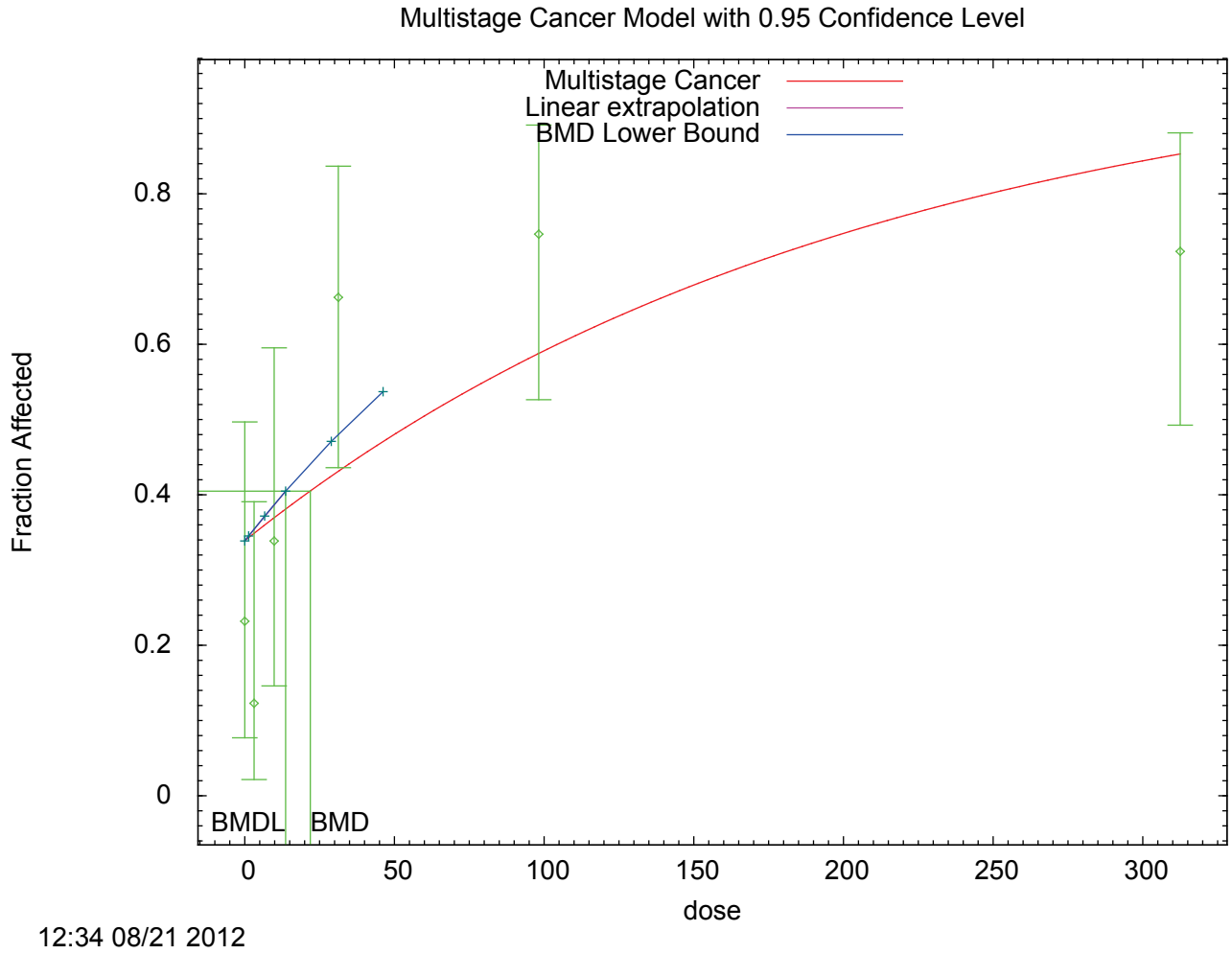
Run 90
Species: Mouse
Gender: Male
Organ: Harderian gland
Response: Adenoma/Carcinoma
Study: NTP 1994 m = 2



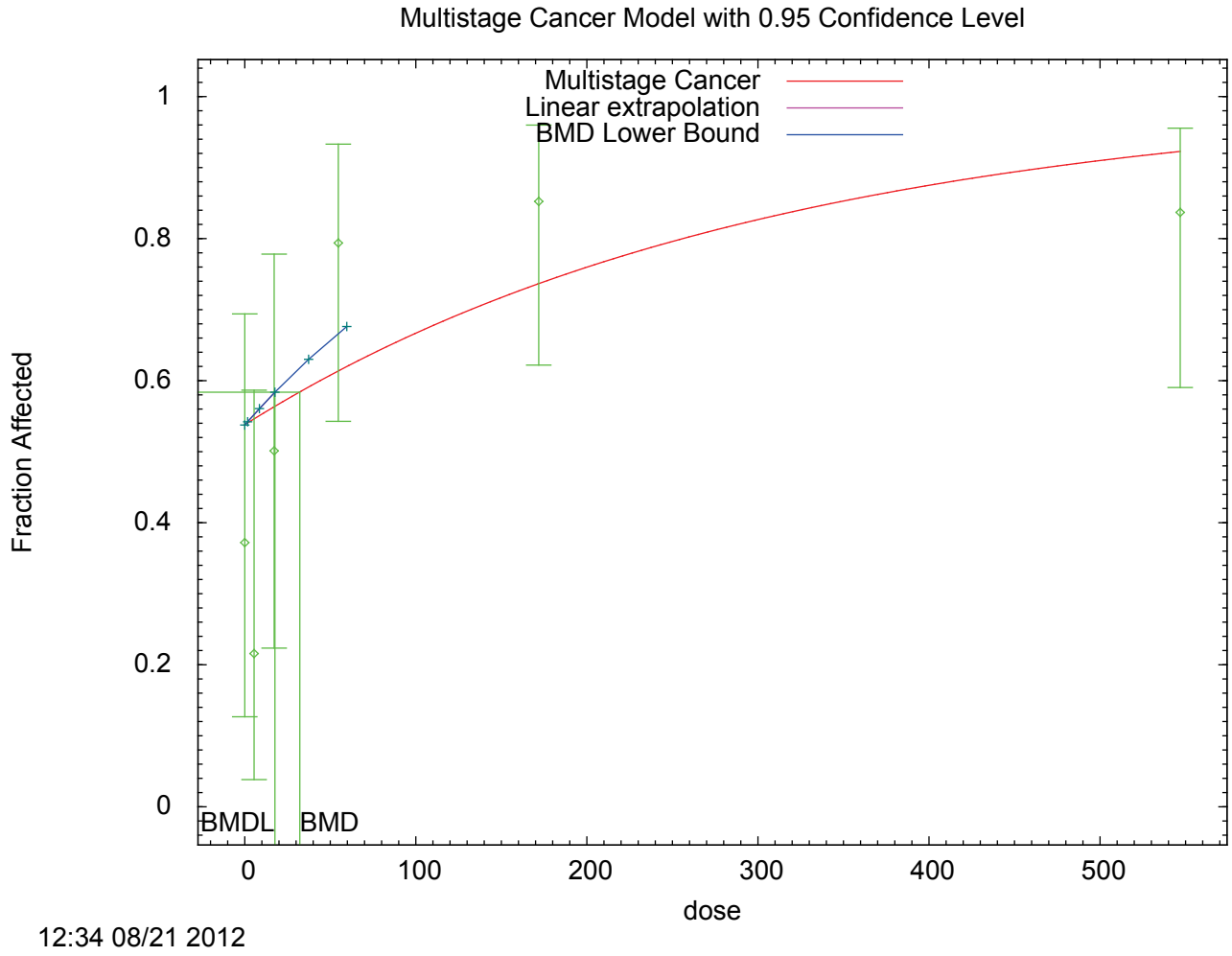
Run 91
Species: Mouse
Gender: Male
Organ: Harderian gland
Response: Adenoma/Carcinoma
Study: NTP 1994 m = 3



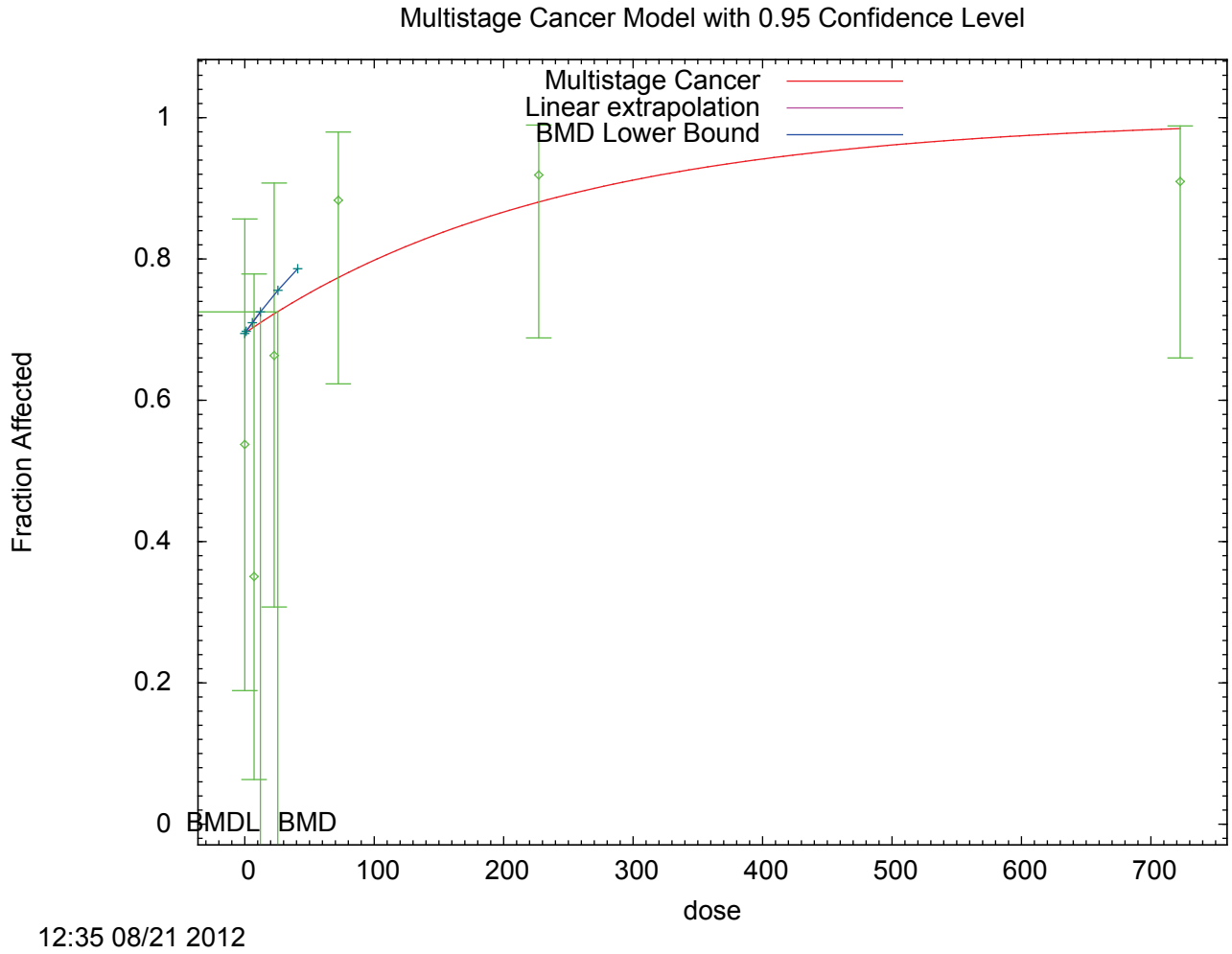
Run 92
Species: Mouse
Gender: Male
Organ: Liver
Response: Adenoma
Study: NTP 1994 m = 1



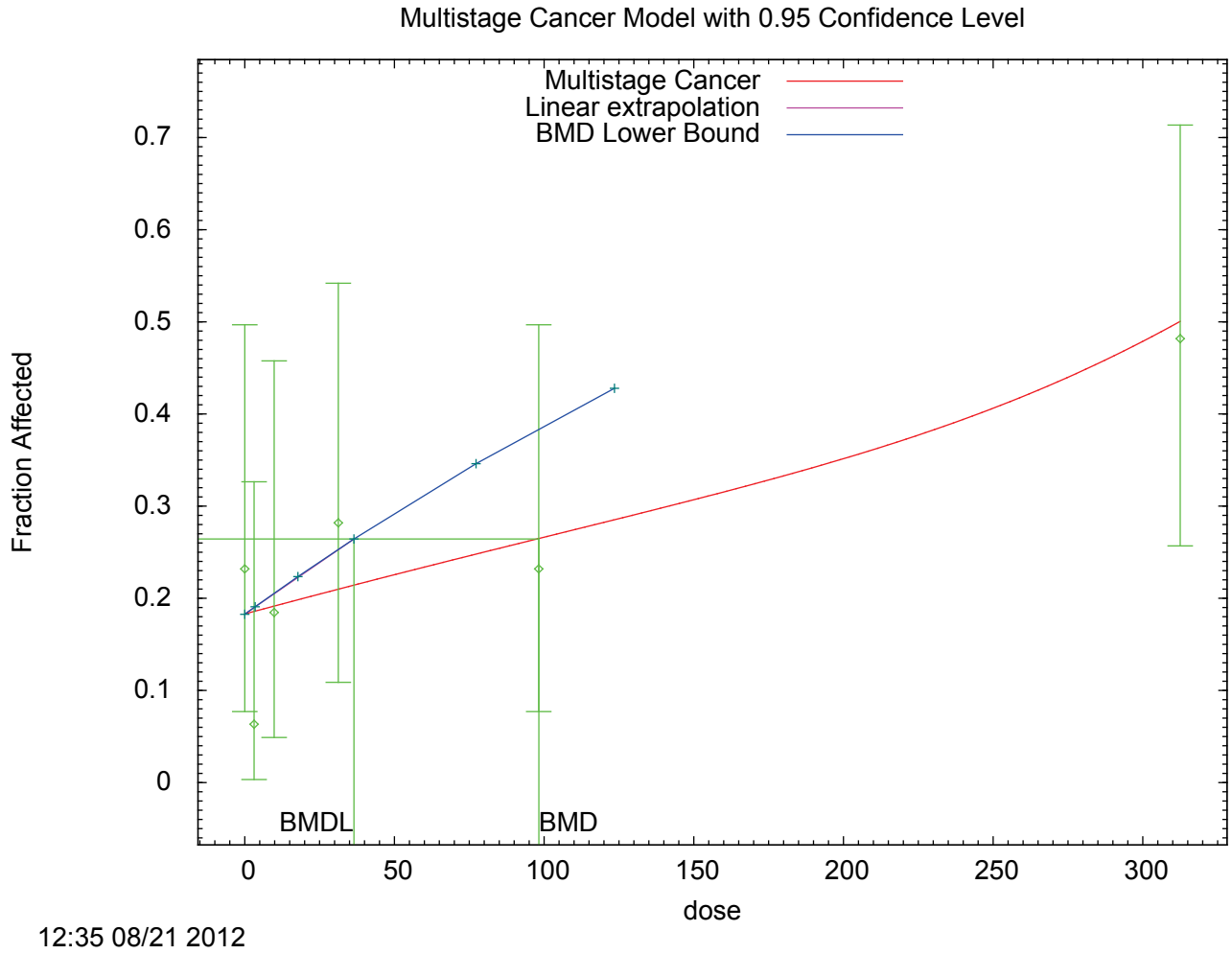
Run 93
Species: Mouse
Gender: Male
Organ: Liver
Response: Adenoma
Study: NTP 1994 m = 2



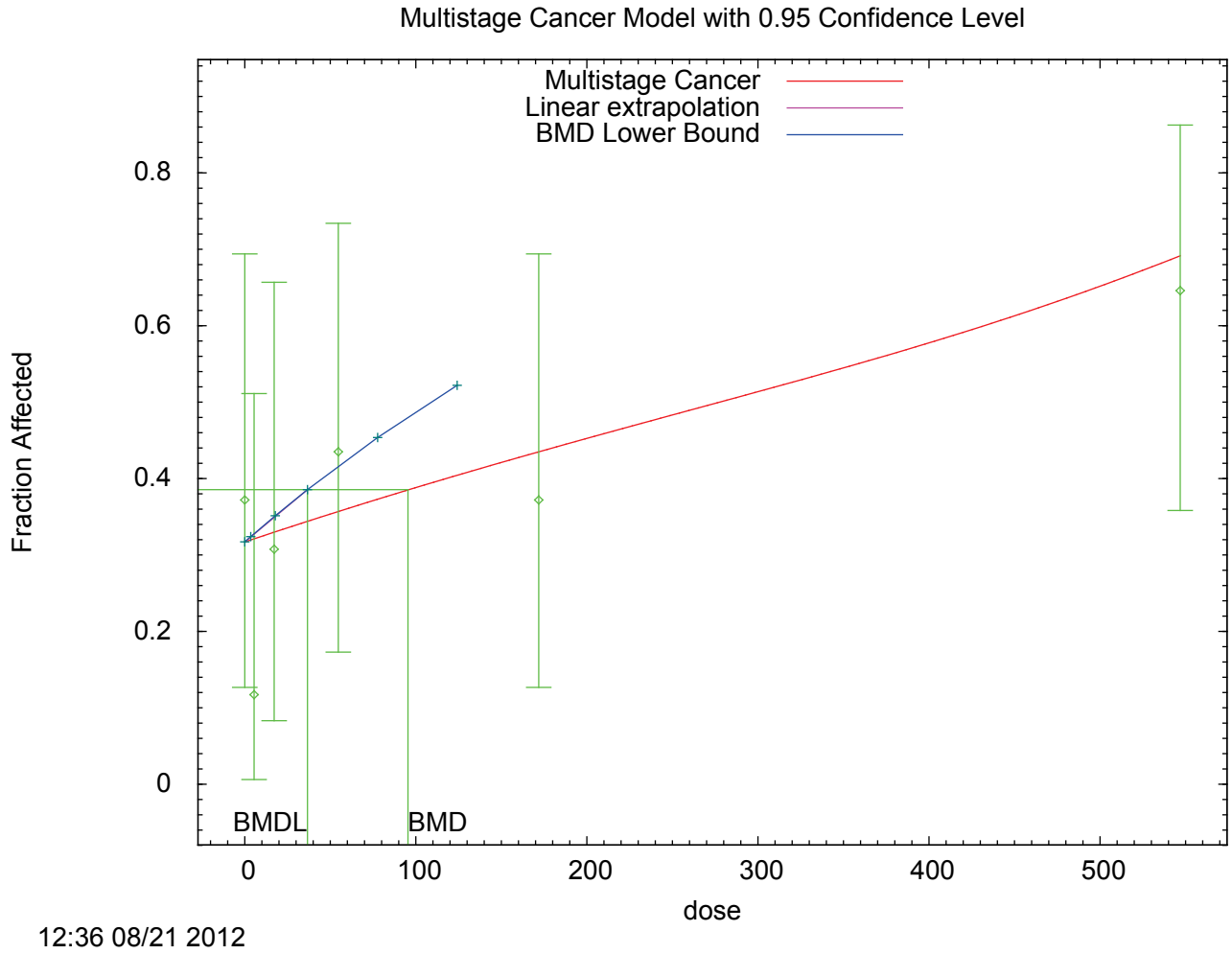
Run 94
Species: Mouse
Gender: Male
Organ: Liver
Response: Adenoma
Study: NTP 1994 m = 3



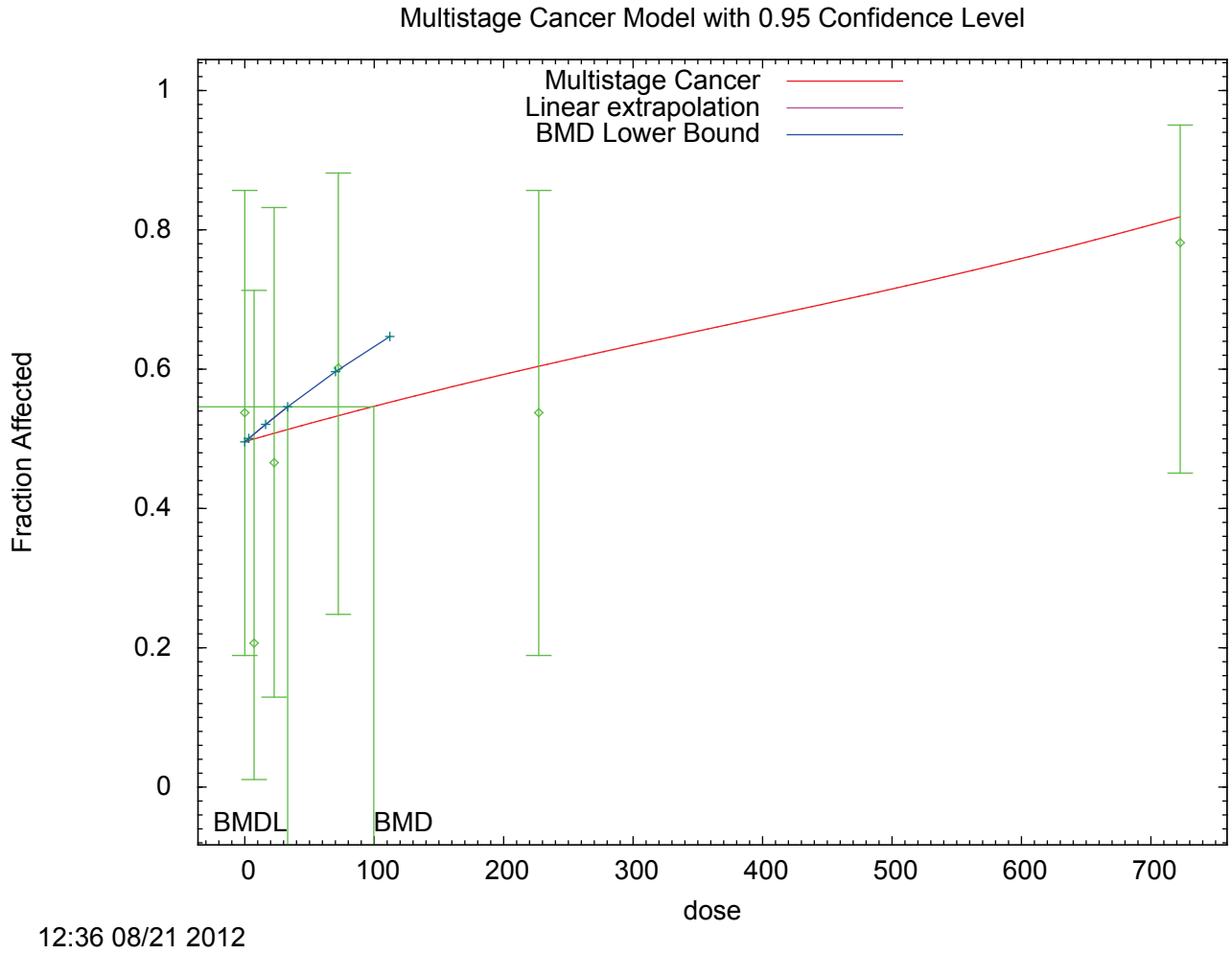
Run 95
Species: Mouse
Gender: Male
Organ: Liver
Response: Carcinoma Adenoma
Study: NTP 1994 m = 1



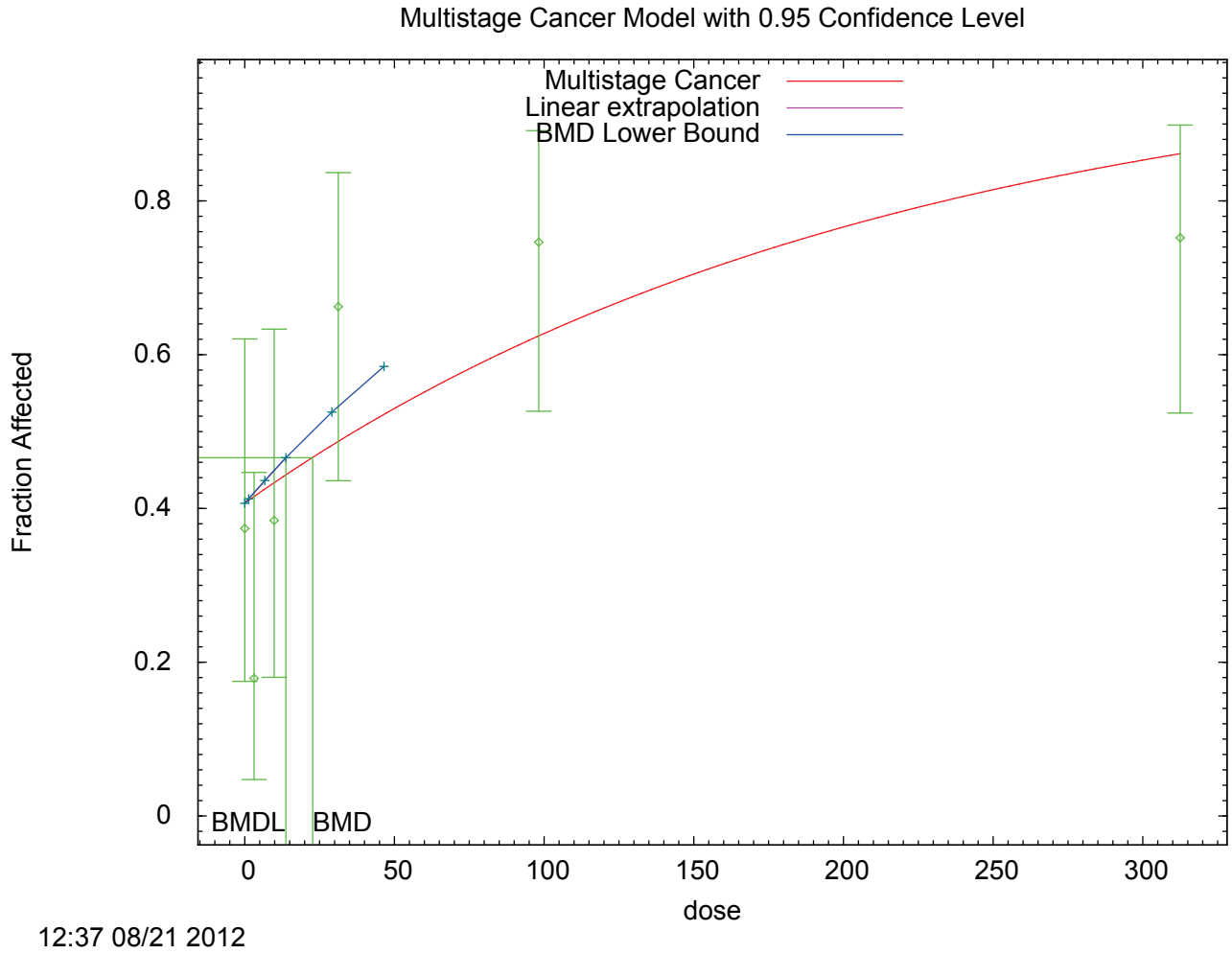
Run 96
Species: Mouse
Gender: Male
Organ: Liver
Response: Carcinoma
Study: NTP 1994 m = 2



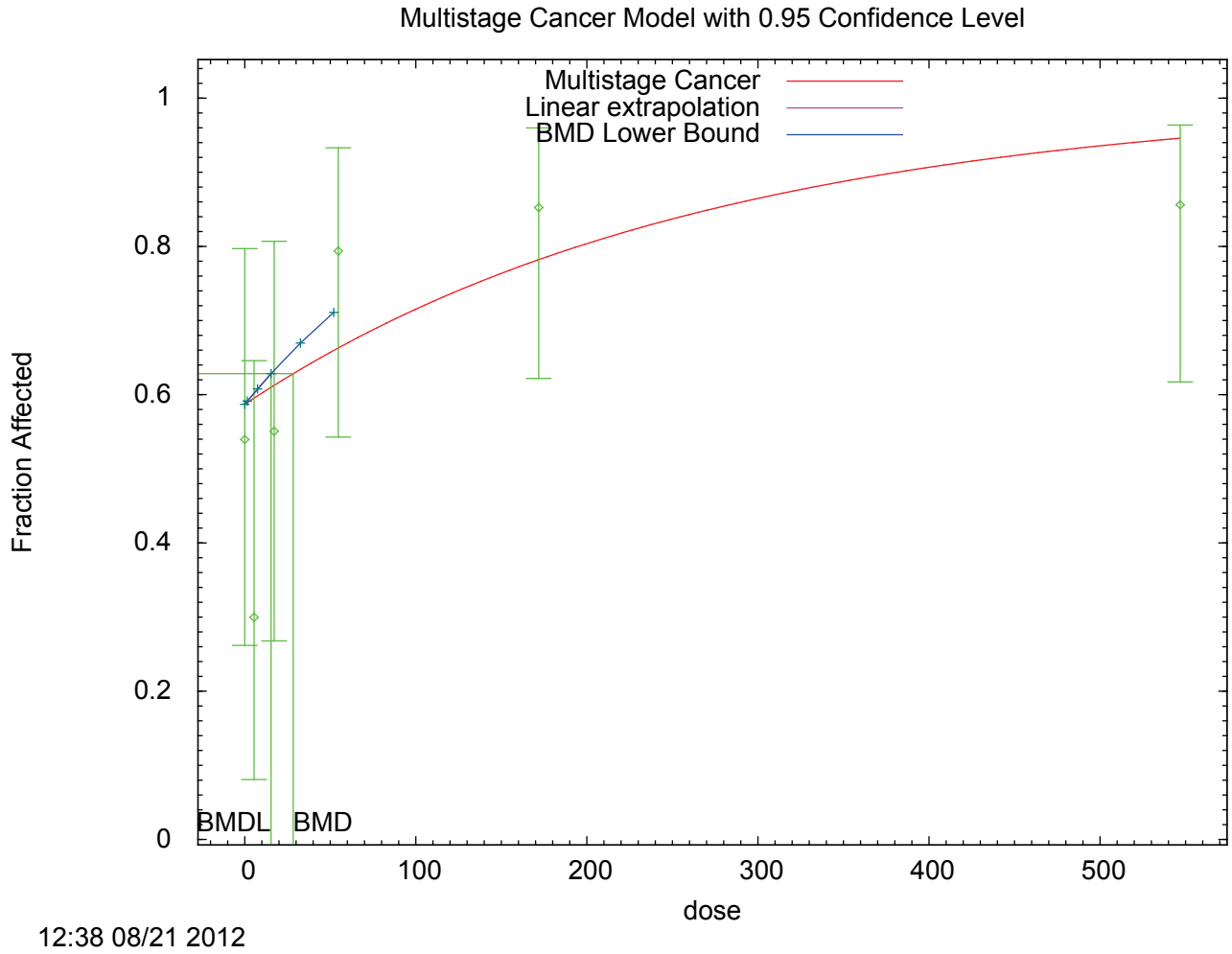
Run 97
Species: Mouse
Gender: Male
Organ: Liver
Response: Carcinoma
Study: NTP 1994 m = 3



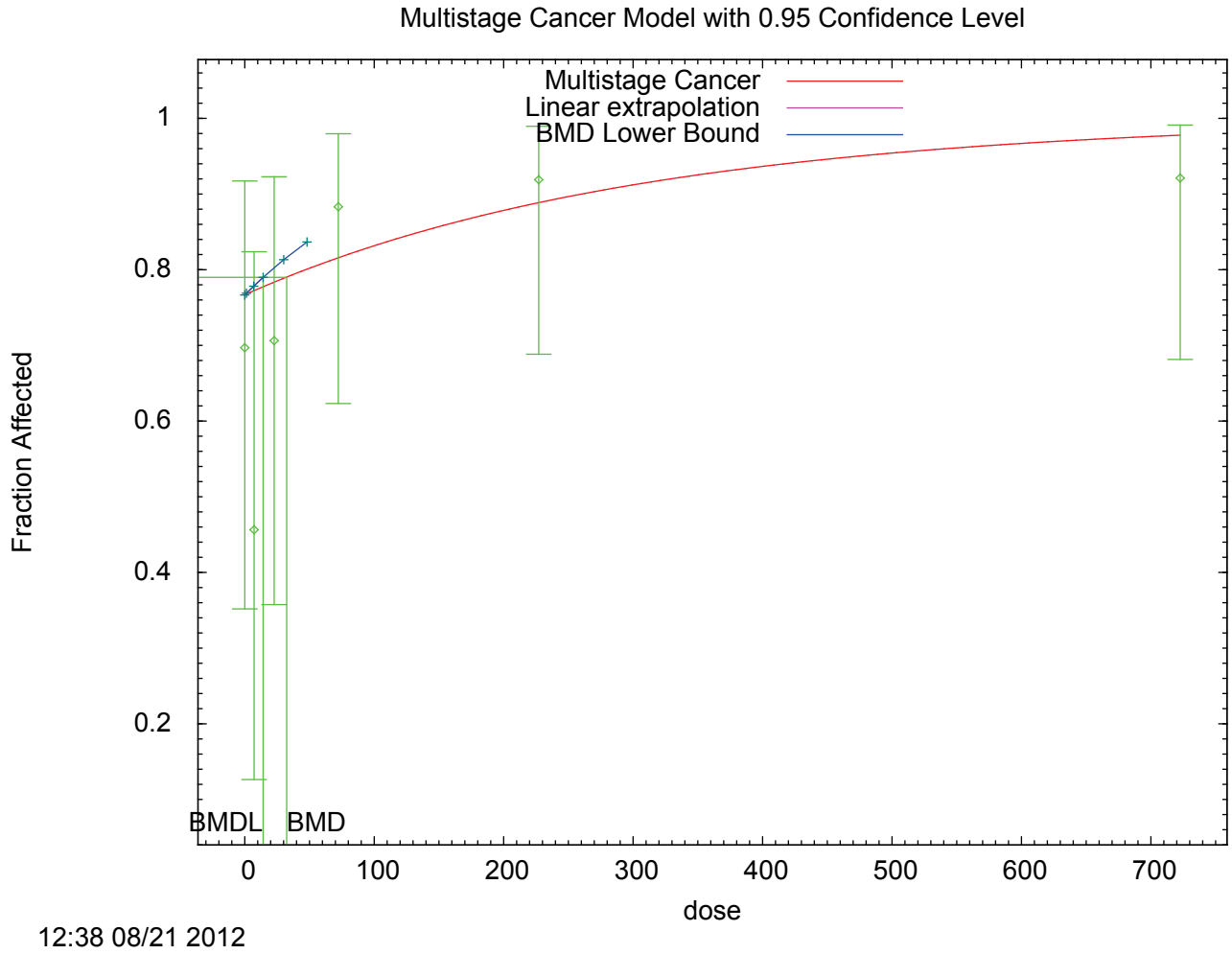
Run 98
Species: Mouse
Gender: Male
Organ: Liver
Response: Adenoma/Carcinoma
Study: NTP 1994 m = 1



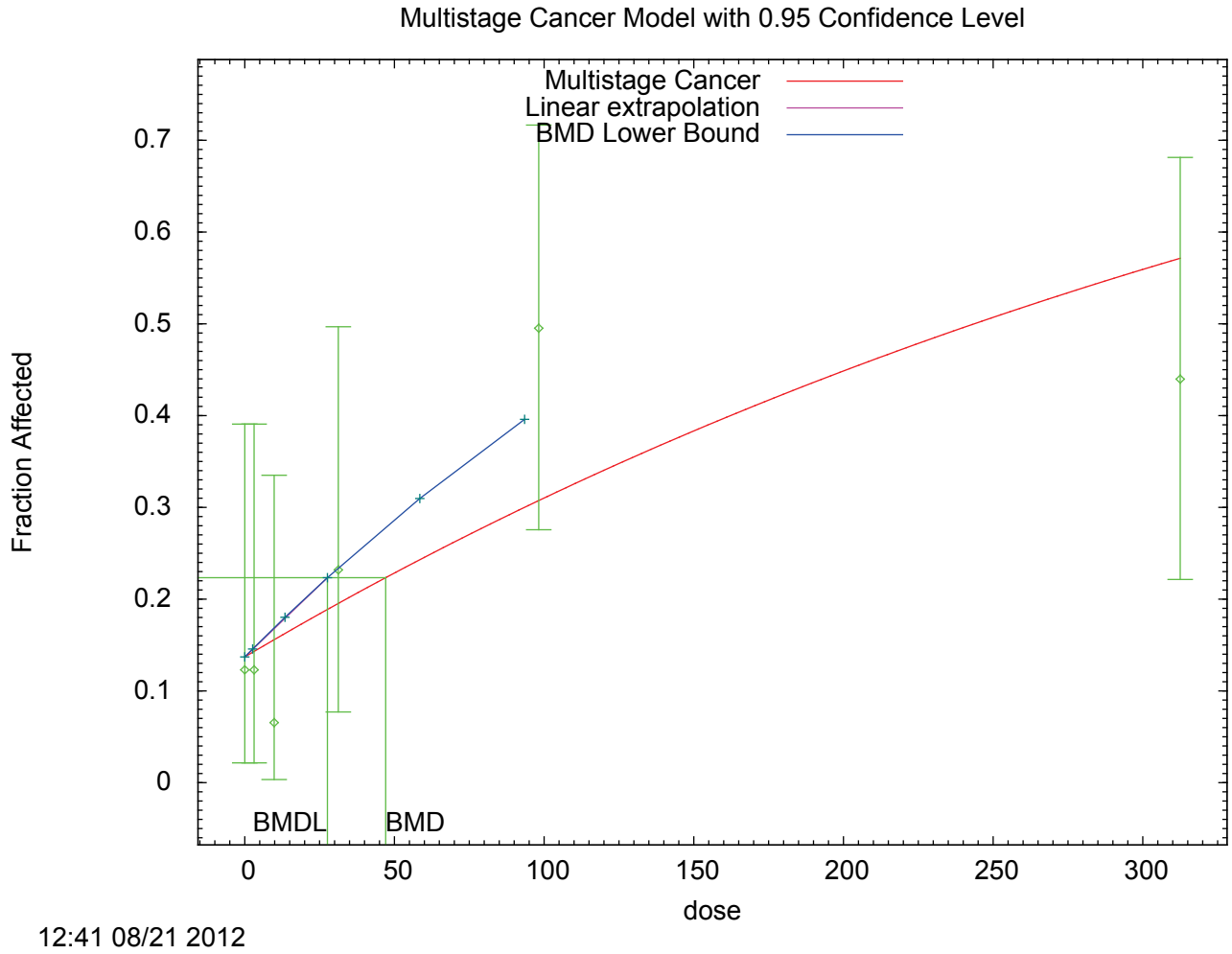
Run 99
Species: Mouse
Gender: Male
Organ: Liver
Response: Adenoma/Carcinoma
Study: NTP 1994 m = 2



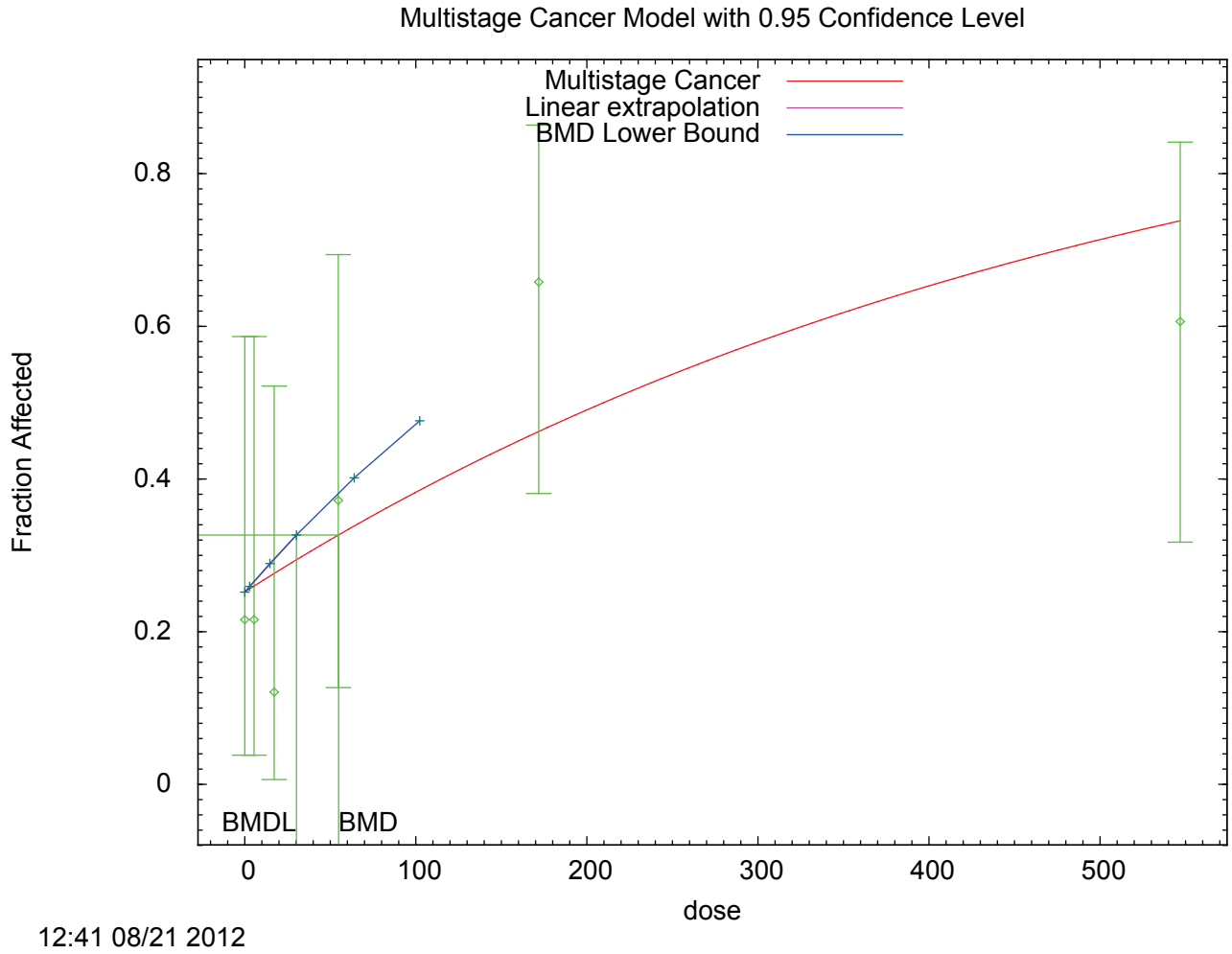
Run 100
Species: Mouse
Gender: Male
Organ: Liver
Response: Adenoma/Carcinoma
Study: NTP 1994 m = 3



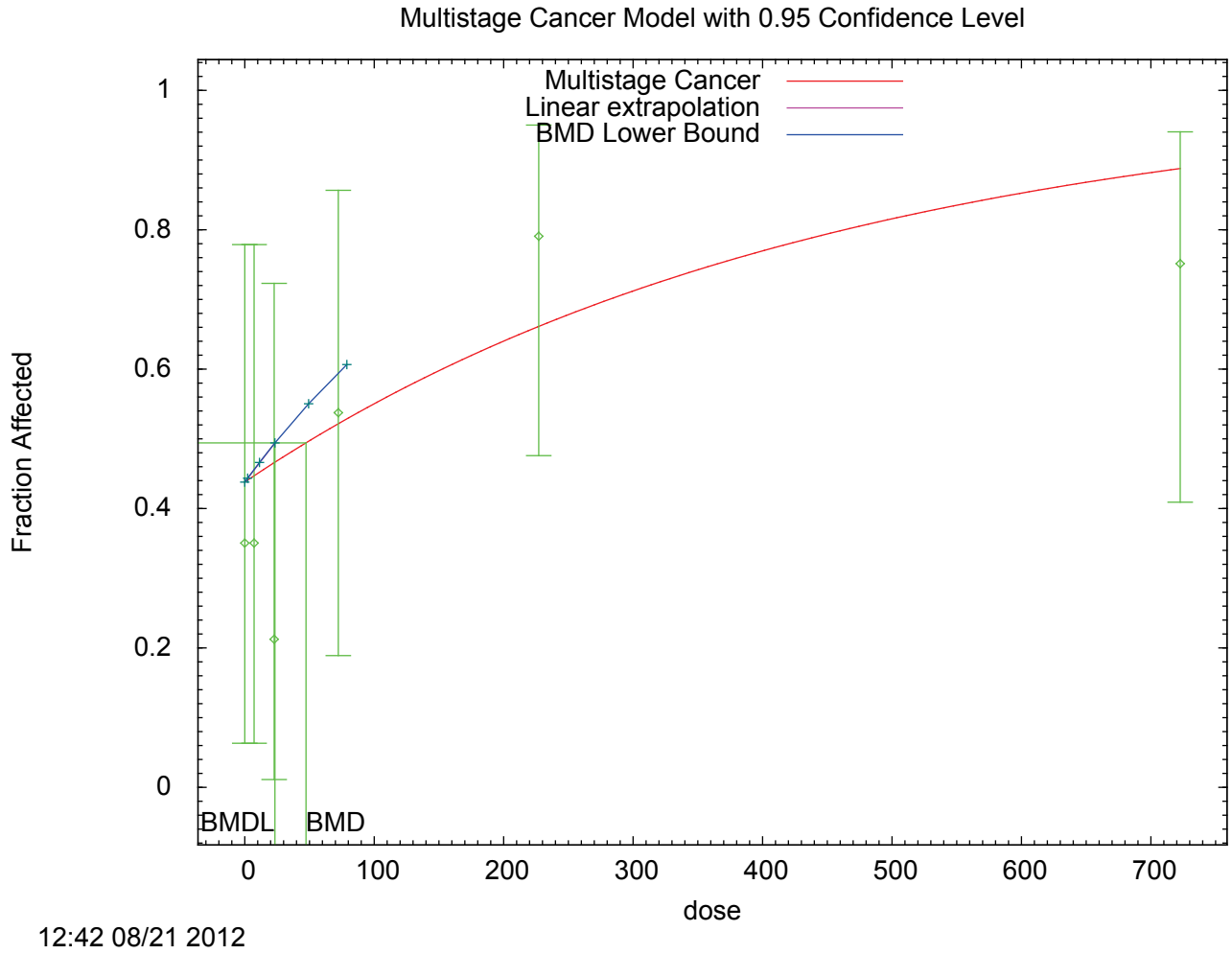
Run 101
Species: Mouse
Gender: Male
Organ: Lung
Response: Adenoma
Study: NTP 1994 m = 1



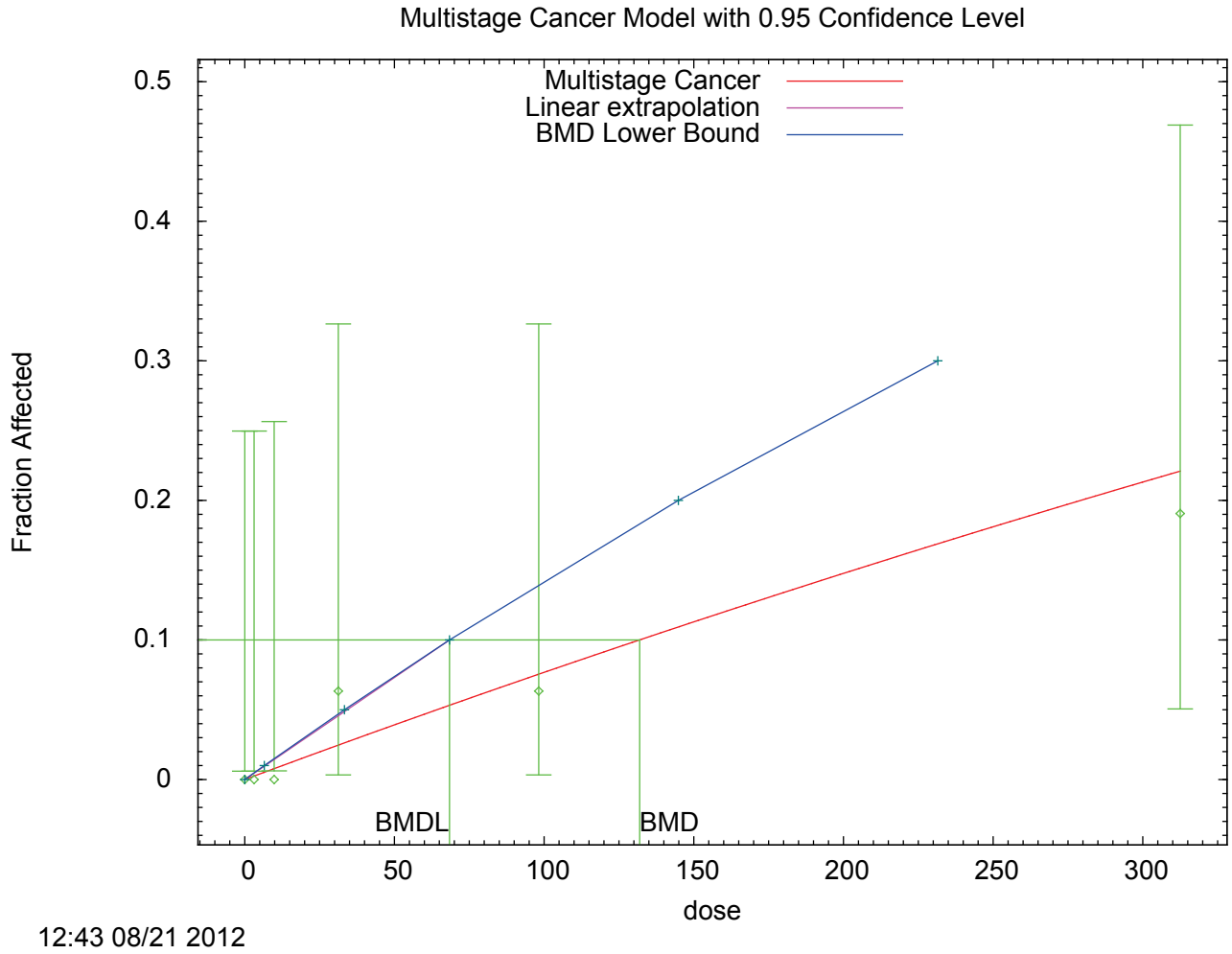
Run 102
Species: Mouse
Gender: Male
Organ: Lung
Response: Adenoma
Study: NTP 1994 m = 2



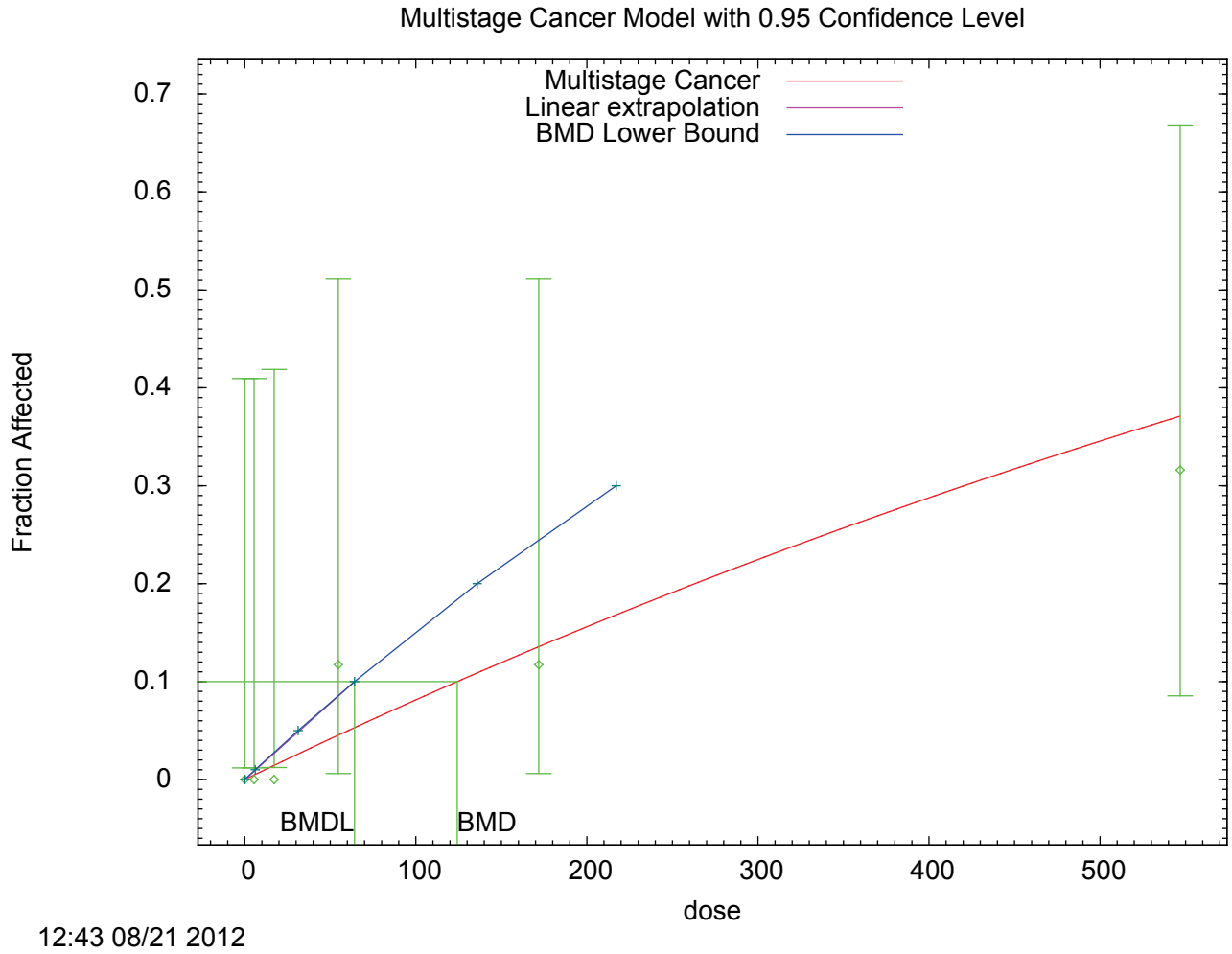
Run 103
Species: Mouse
Gender: Male
Organ: Lung
Response: Adenoma
Study: NTP 1994 m = 3



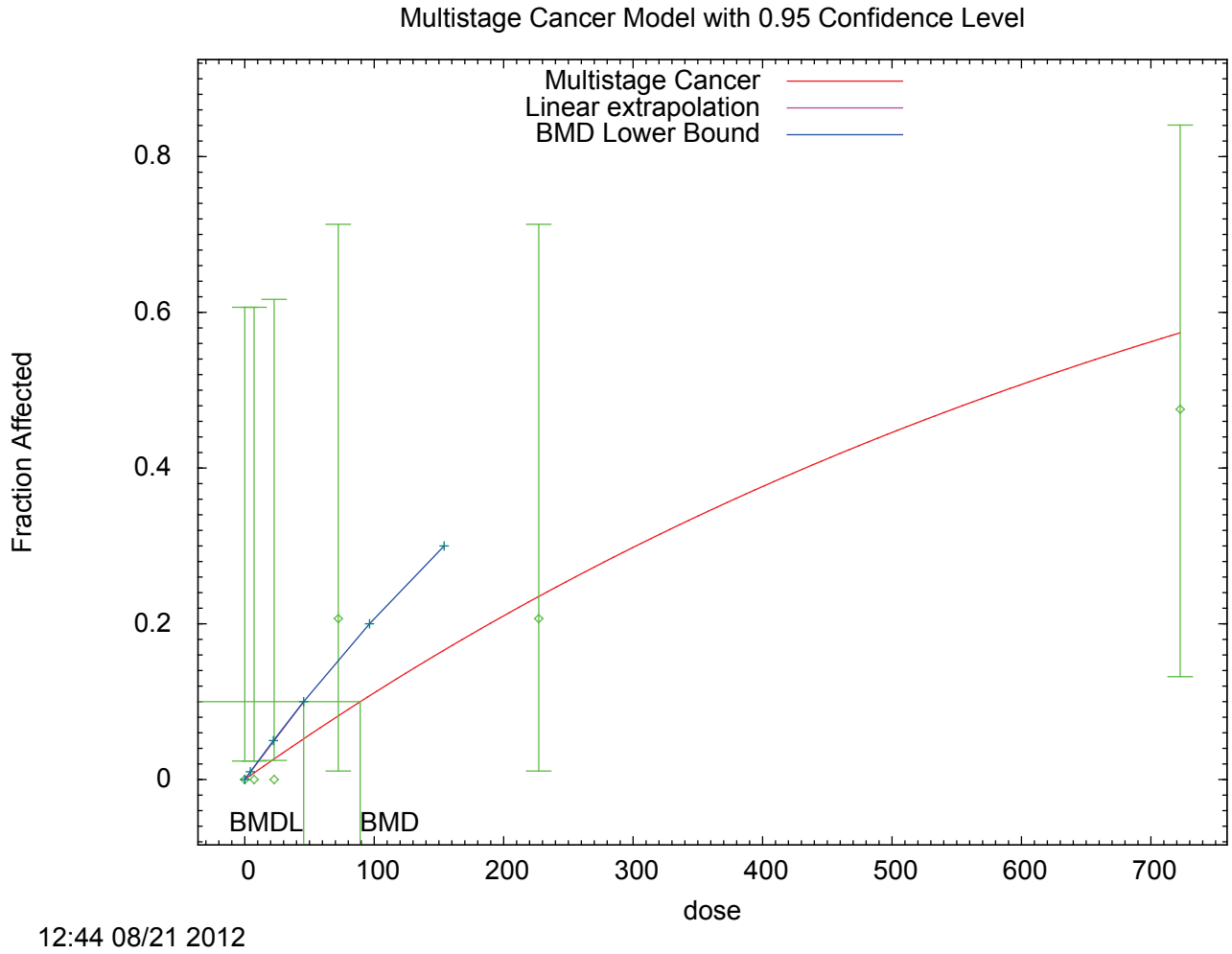
Run 104
Species: Mouse
Gender: Male
Organ: Lung
Response: Carcinoma
Study: NTP 1994 m = 1



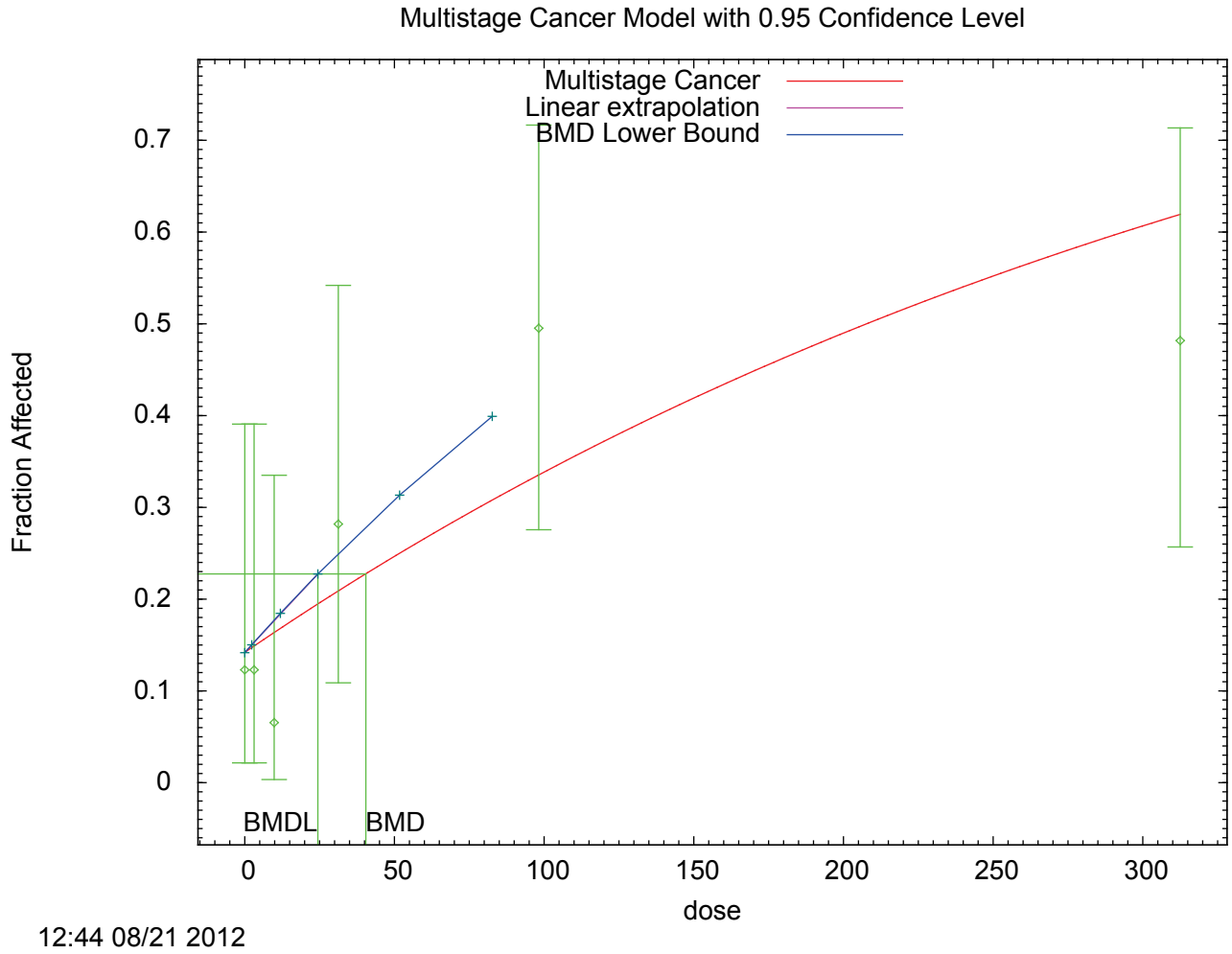
Run 105
Species: Mouse
Gender: Male
Organ: Lung
Response: Carcinoma
Study: NTP 1994 m = 2



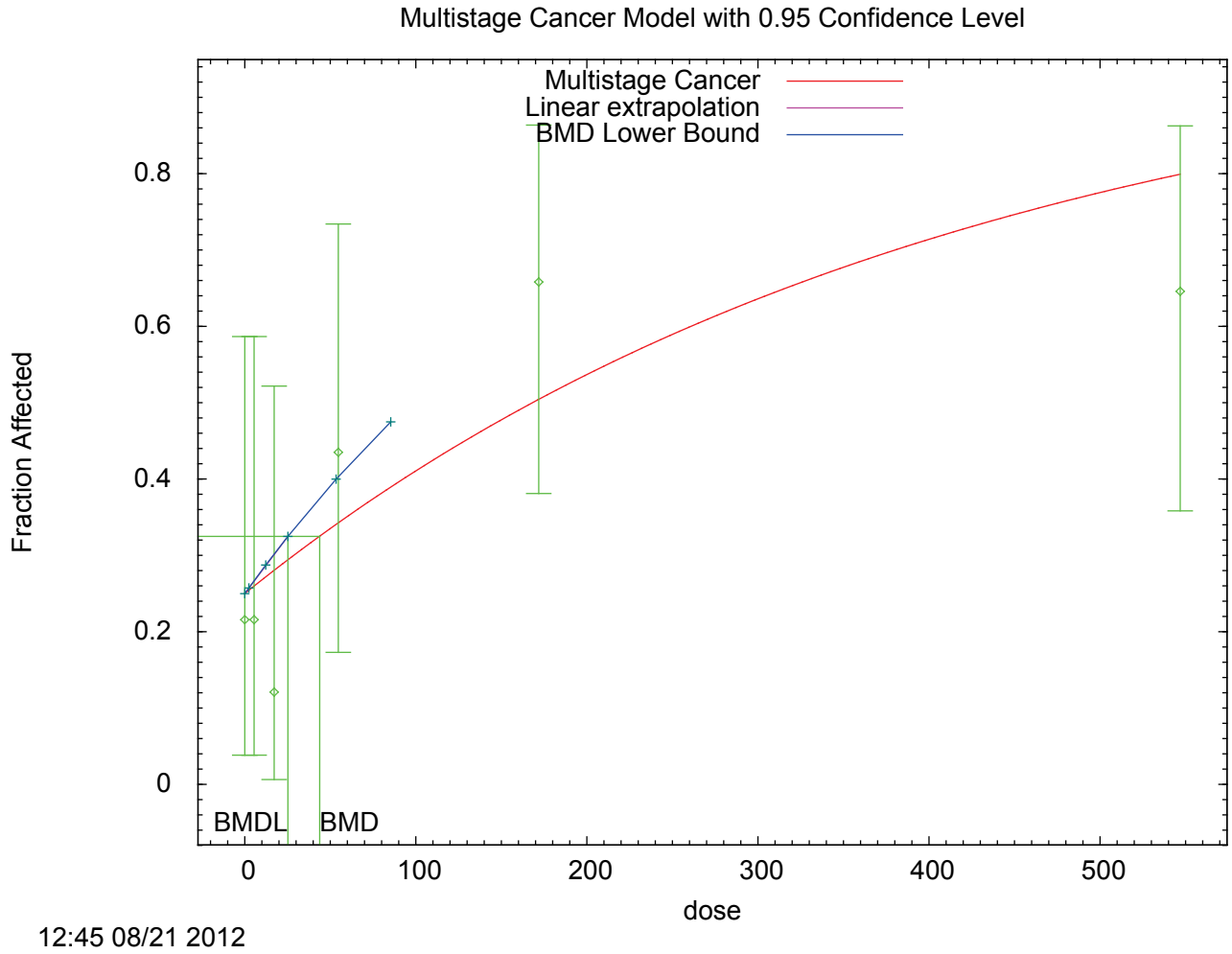
Run 106
Species: Mouse
Gender: Male
Organ: Lung
Response: Carcinoma
Study: NTP 1994 m = 3



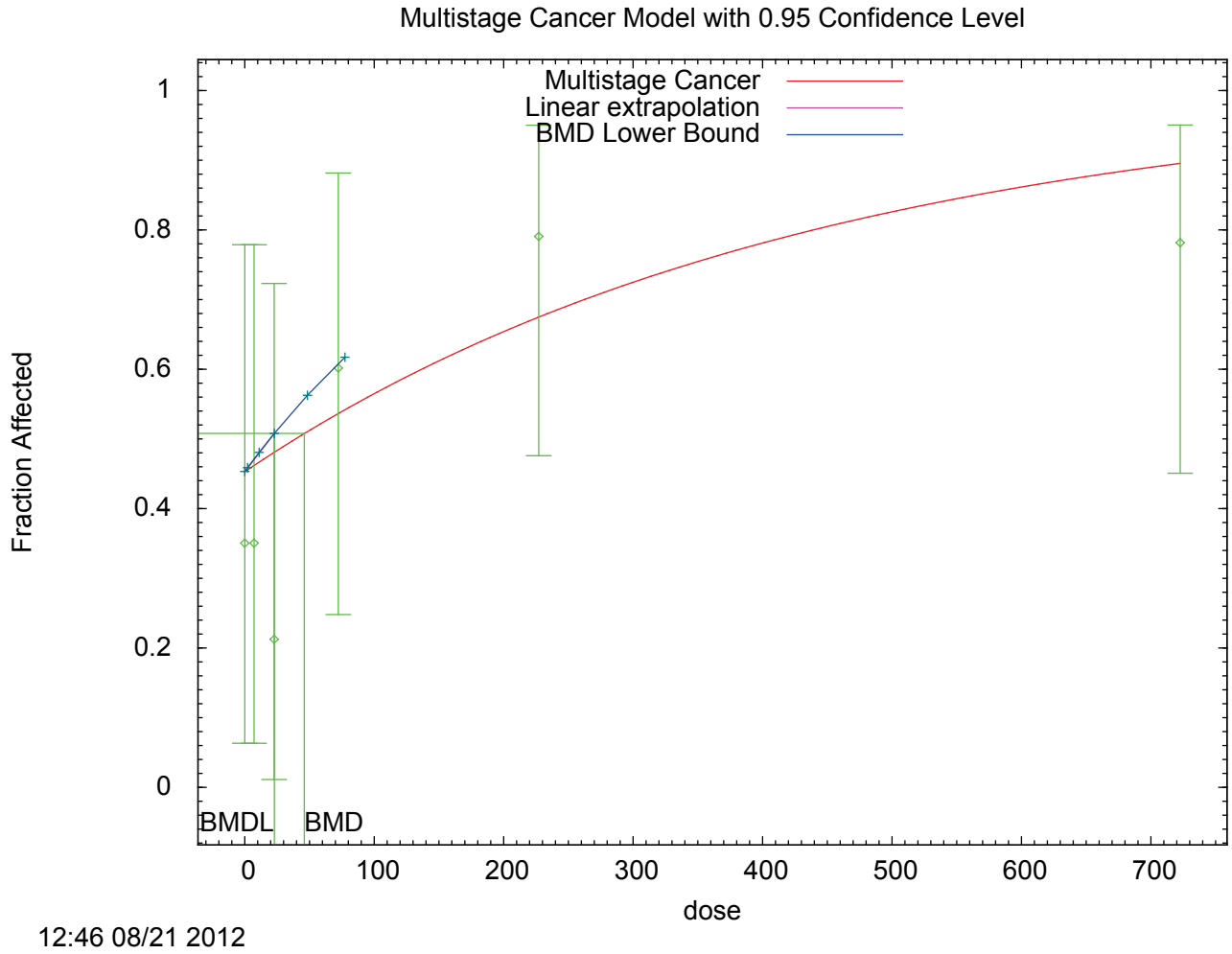
Run 107
Species: Mouse
Gender: Male
Organ: Lung
Response: Adenoma/Carcinoma
Study: NTP 1994 m = 1



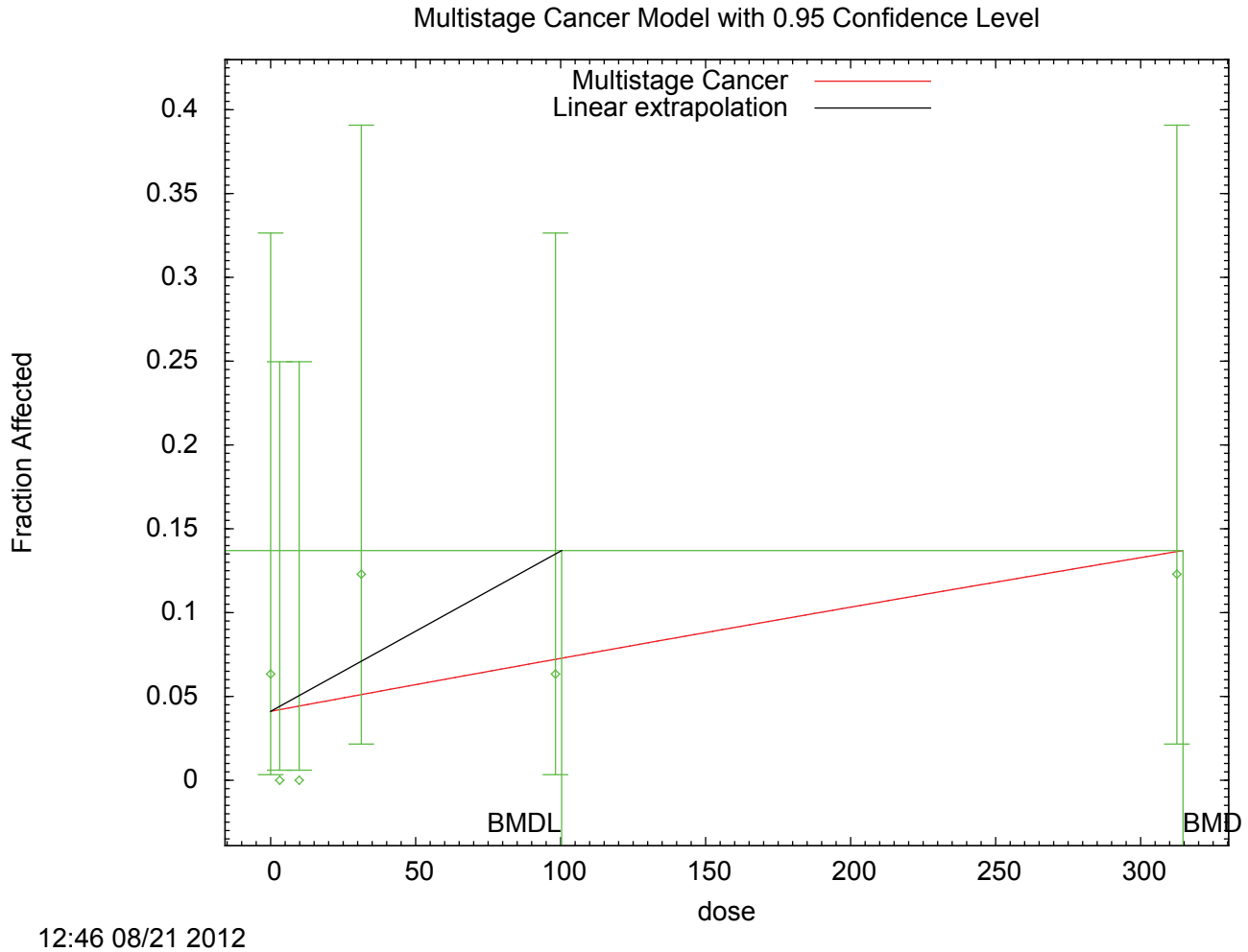
Run 108
Species: Mouse
Gender: Male
Organ: Lung
Response: Adenoma/Carcinoma
Study: NTP 1994 m = 2



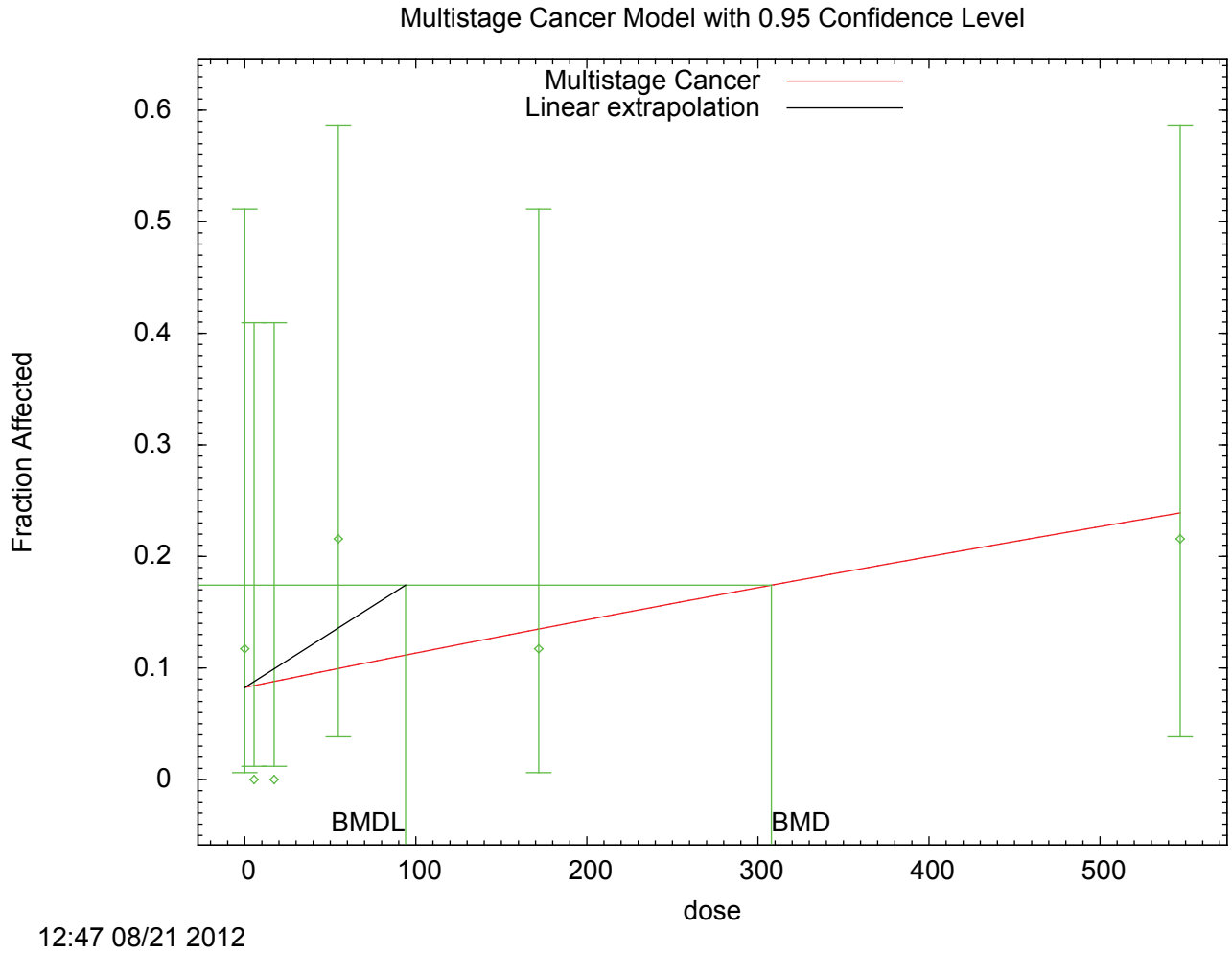
Run 109
Species: Mouse
Gender: Male
Organ: Lung
Response: Adenoma/Carcinoma
Study: NTP 1994 m = 3



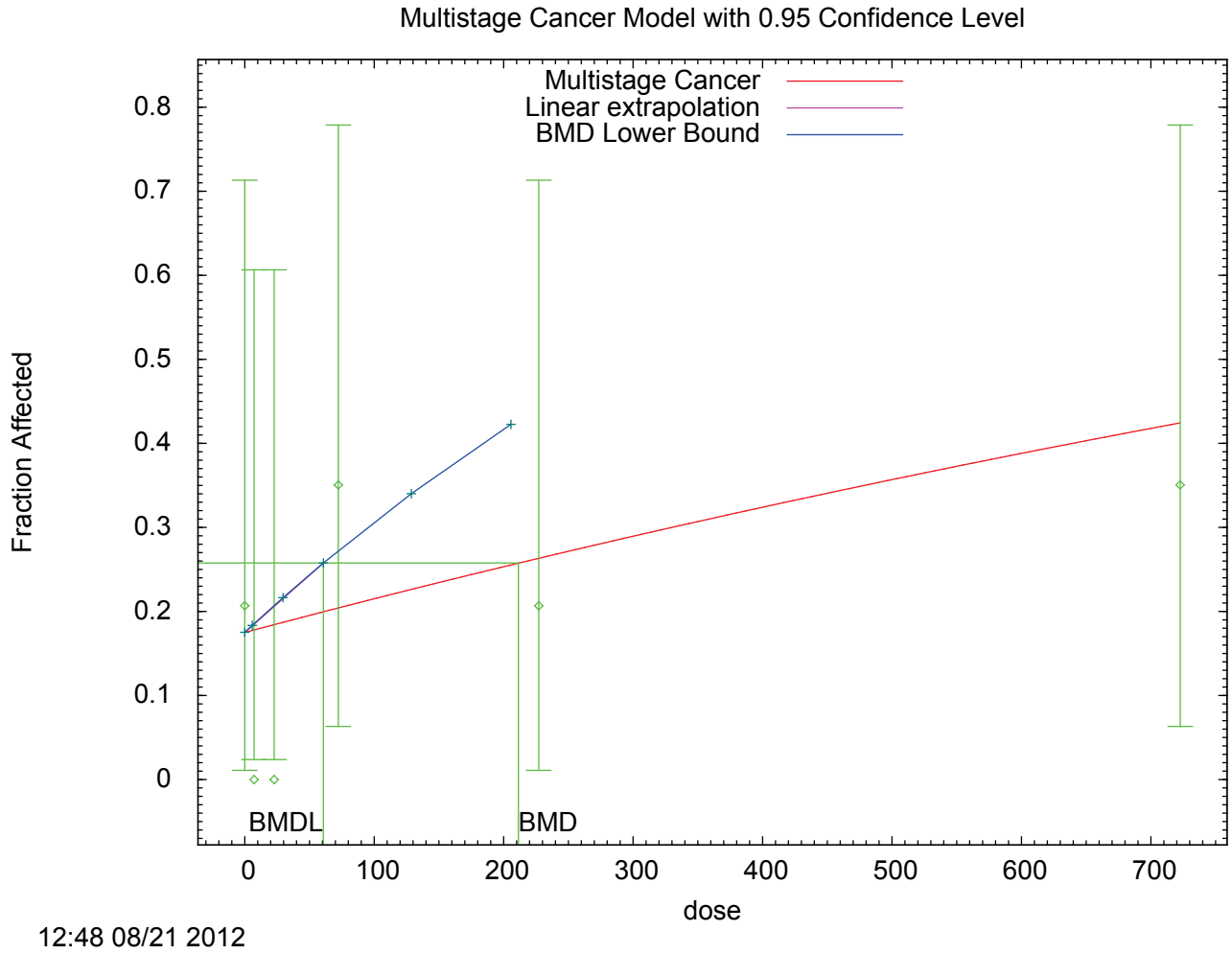
Run 110
Species: Mouse
Gender: Male
Organ: Hematopoietic
Response: any lymphoma
Study: NTP 1994 m = 1



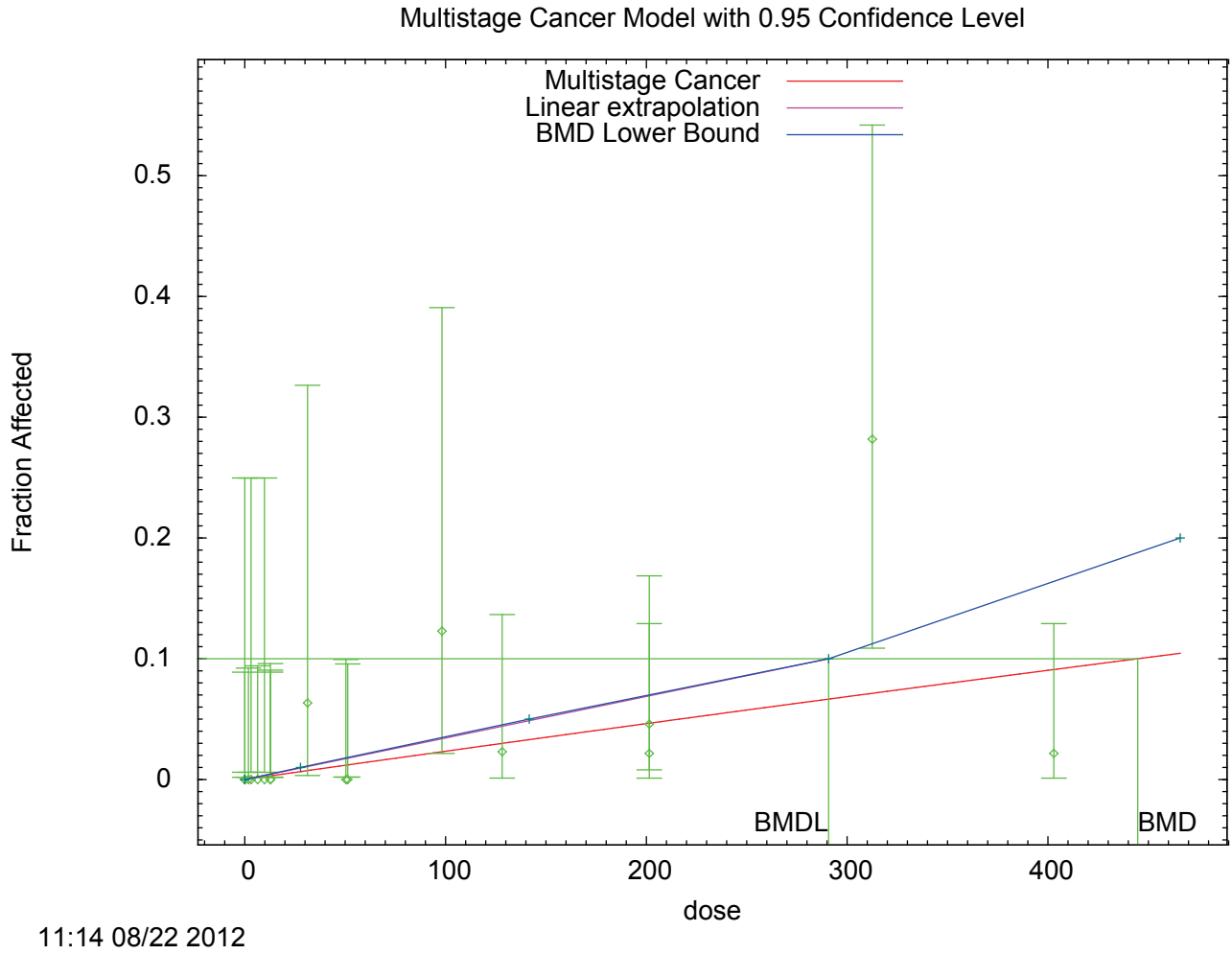
Run 111
Species: Mouse
Gender: Male
Organ: Hematopoietic
Response: any lymphoma
Study: NTP 1994 m = 2



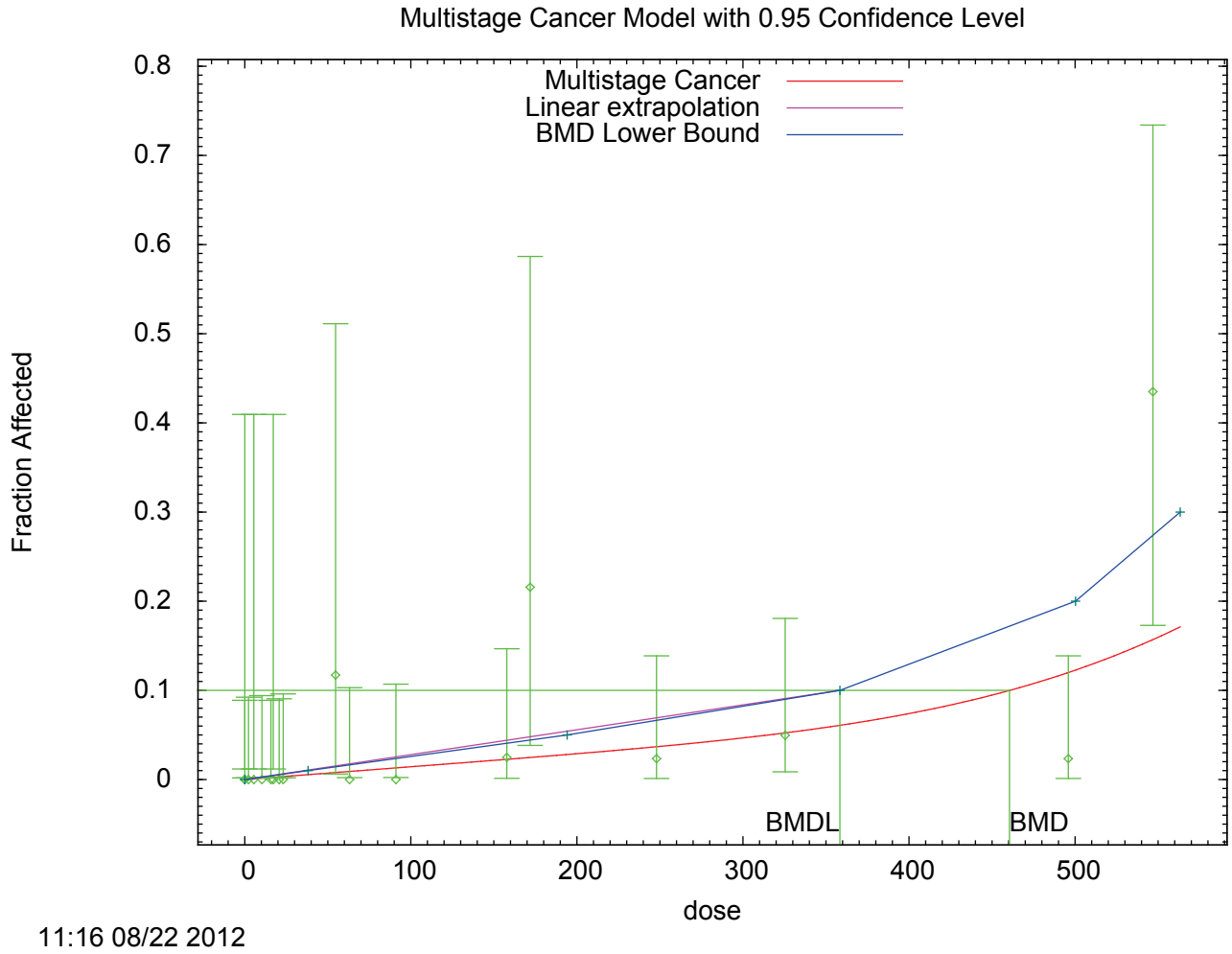
Run 112
Species: Mouse
Gender: Male
Organ: Hematopoietic
Response: any lymphoma
Study: NTP 1994 m = 3



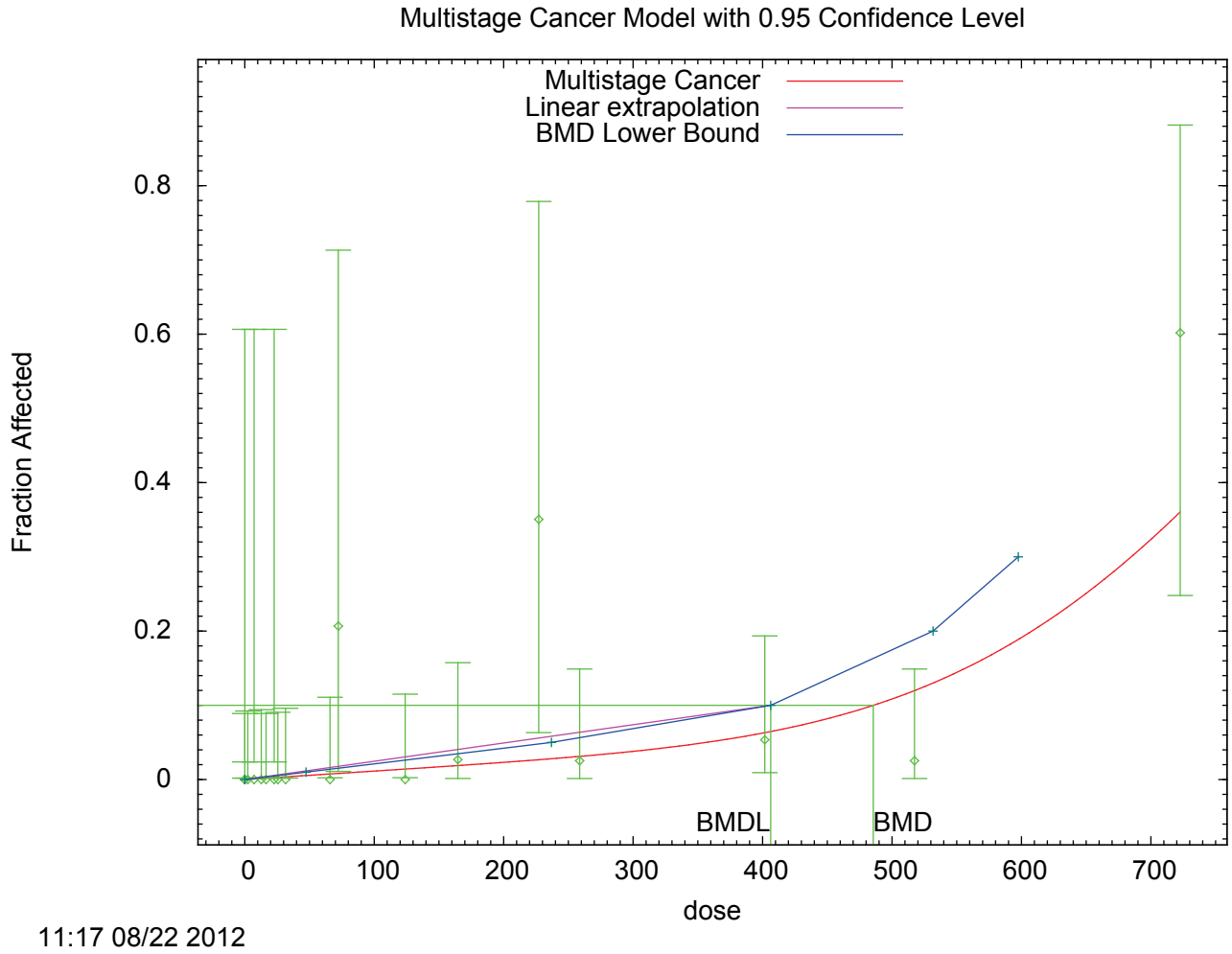
Run 113
Species: Mouse
Gender: Male
Organ: Forestomach
Response: Papilloma
Study: NTP 1994 and Placke et al. 1996 Combined m = 1



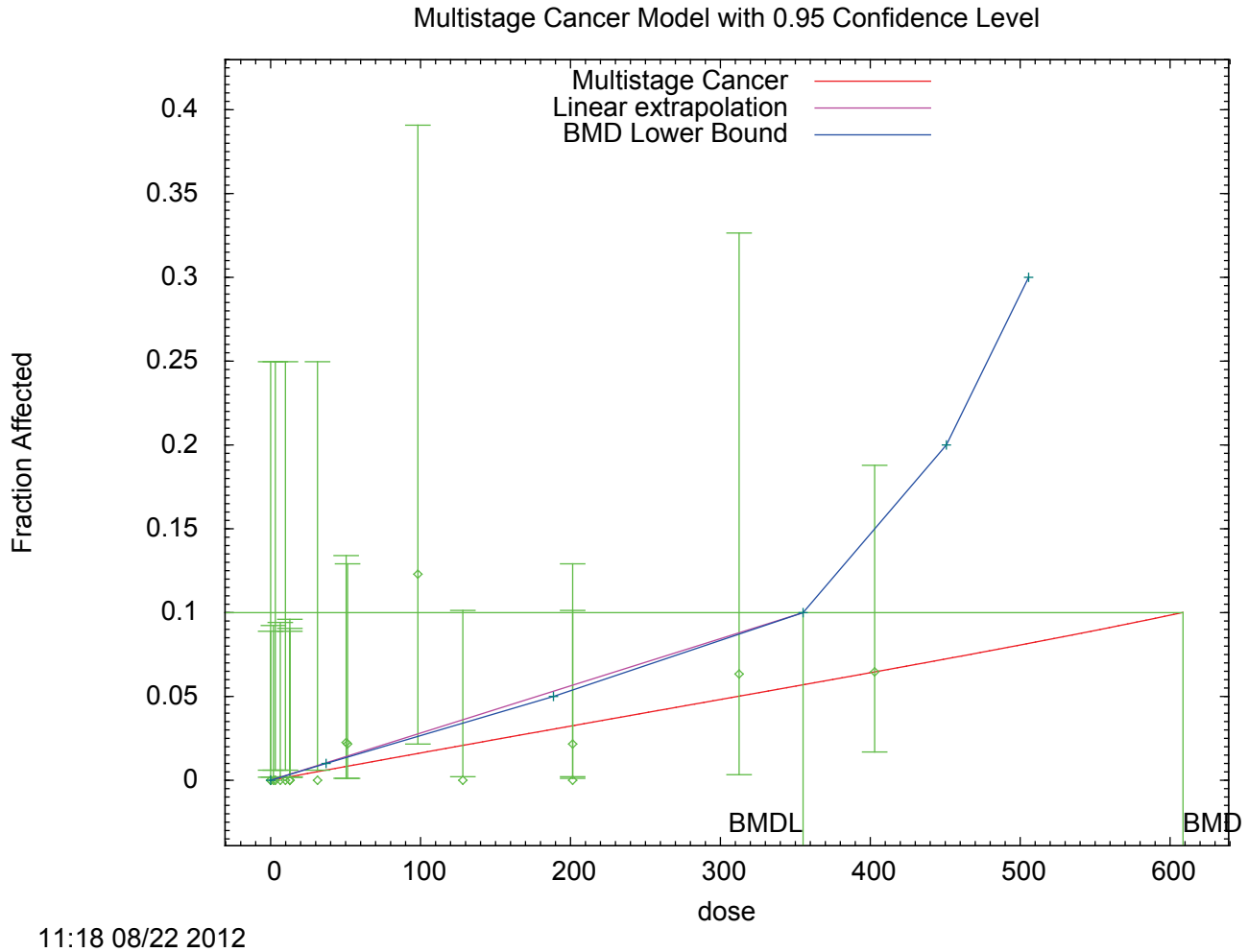
Run 114
Species: Mouse
Gender: Male
Organ: Forestomach
Response: Papilloma
Study: NTP 1994 and Placke et al. 1996 Combined m = 2



Run 115
Species: Mouse
Gender: Male
Organ: Forestomach
Response: Papilloma
Study: NTP 1994 and Placke et al. 1996 Combined m = 3

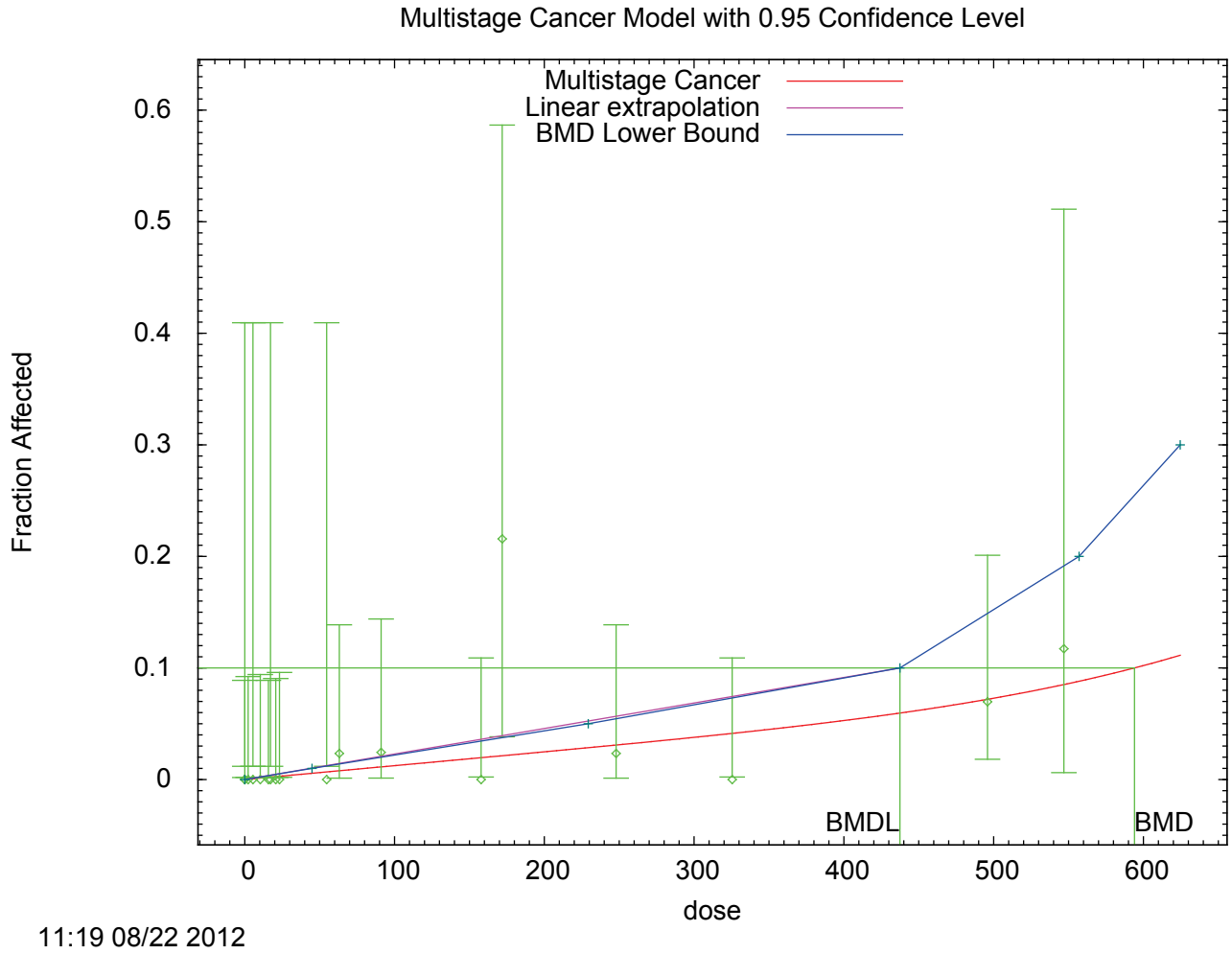


Run 116
Species: Mouse
Gender: Male
Organ: Forestomach
Response: Carcinoma
Study: NTP 1994 and Placke et al. 1996 Combined m = 1

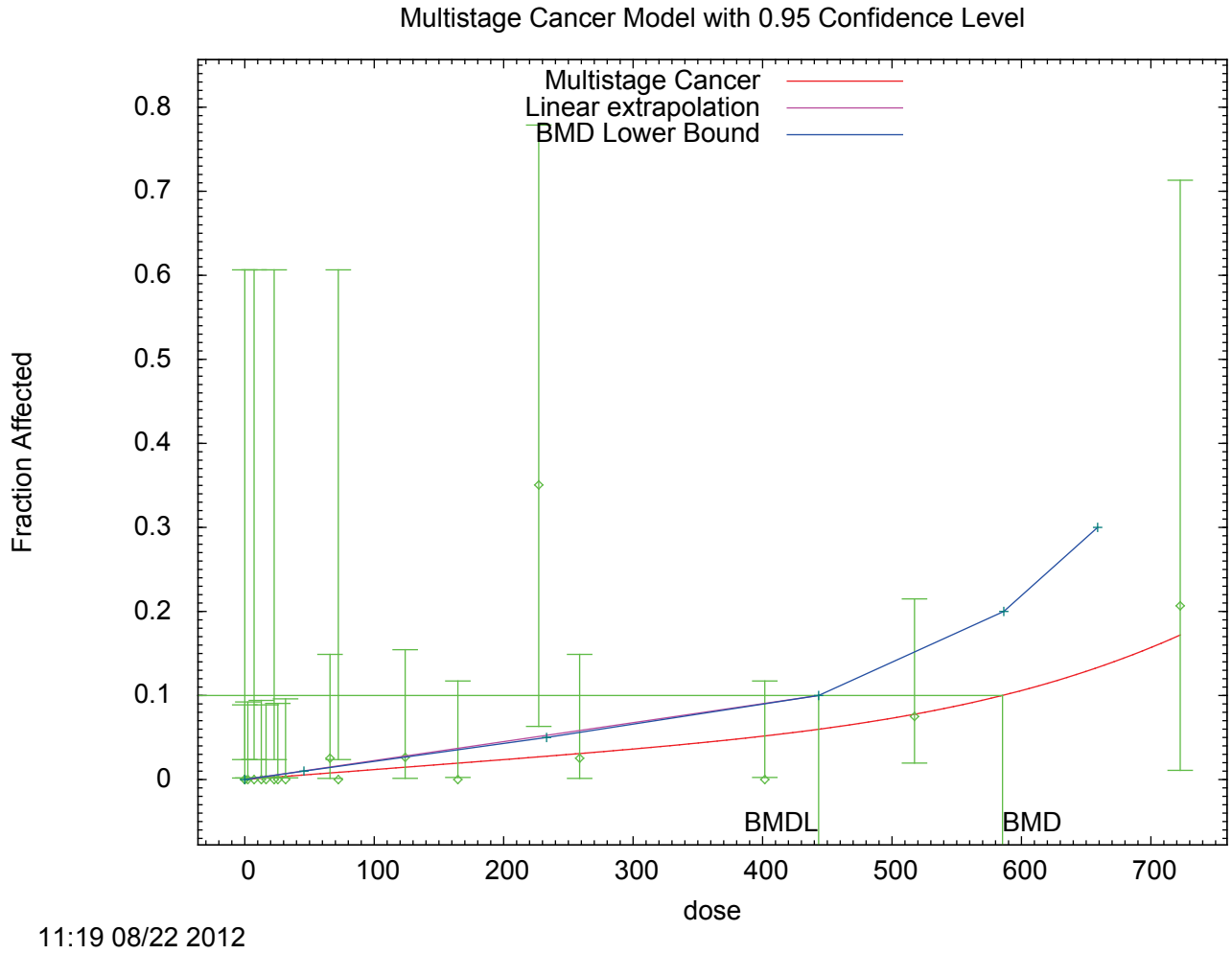


11:18 08/22 2012

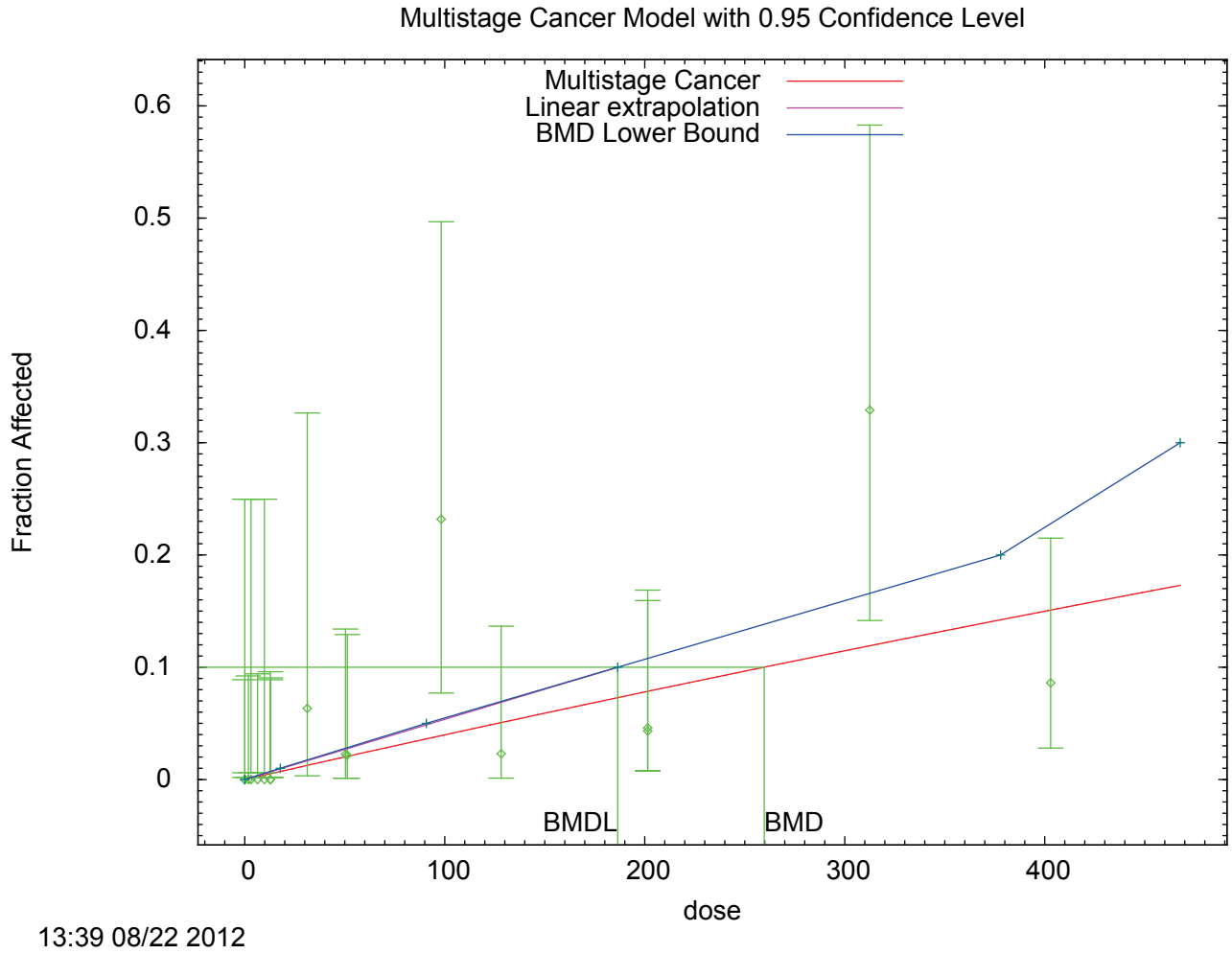
Run 117
Species: Mouse
Gender: Male
Organ: Forestomach
Response: Carcinoma
Study: NTP 1994 and Placke et al. 1996 Combined m = 2



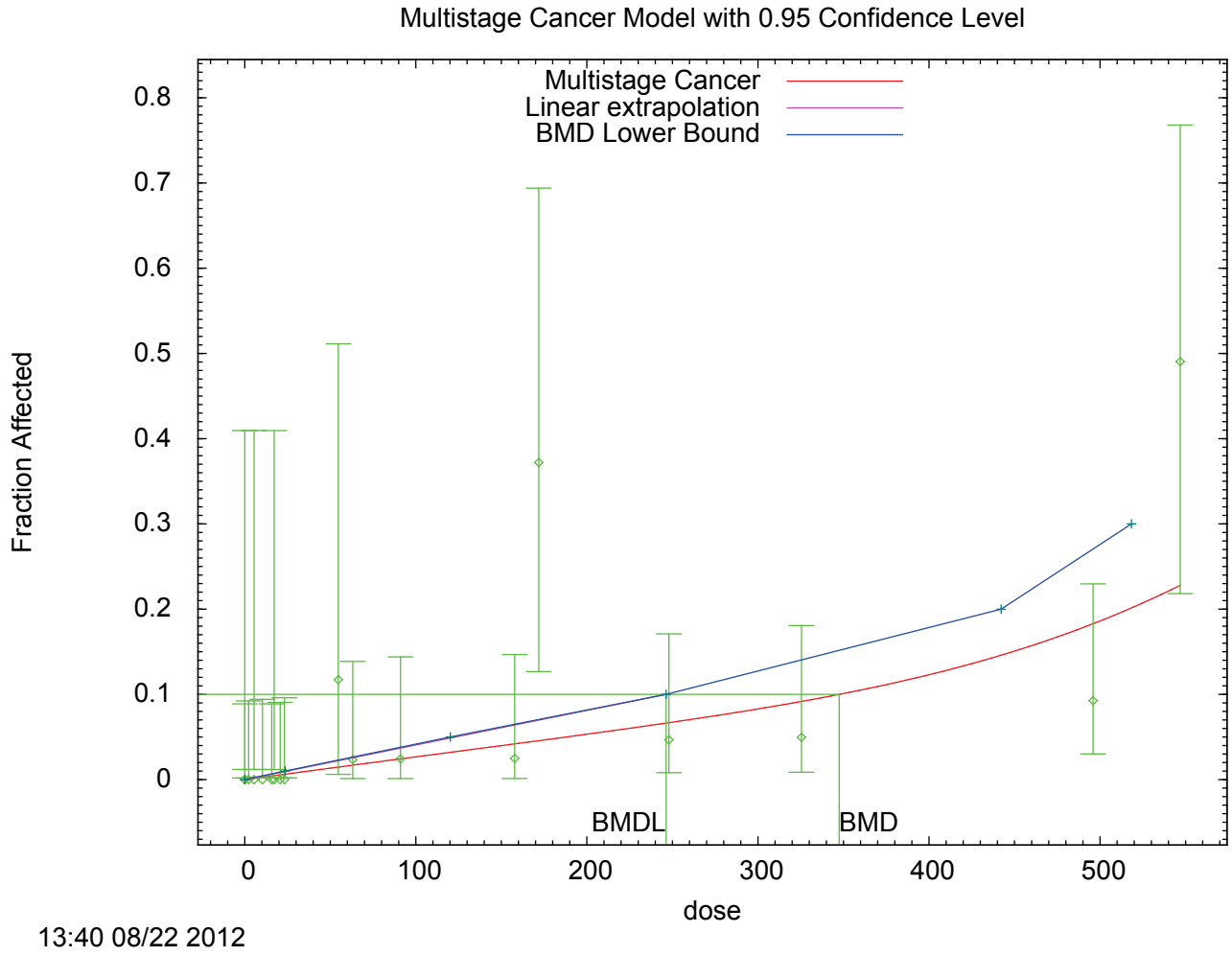
Run 118
Species: Mouse
Gender: Male
Organ: Forestomach
Response: Carcinoma
Study: NTP 1994 and Placke et al. 1996 Combined m = 3



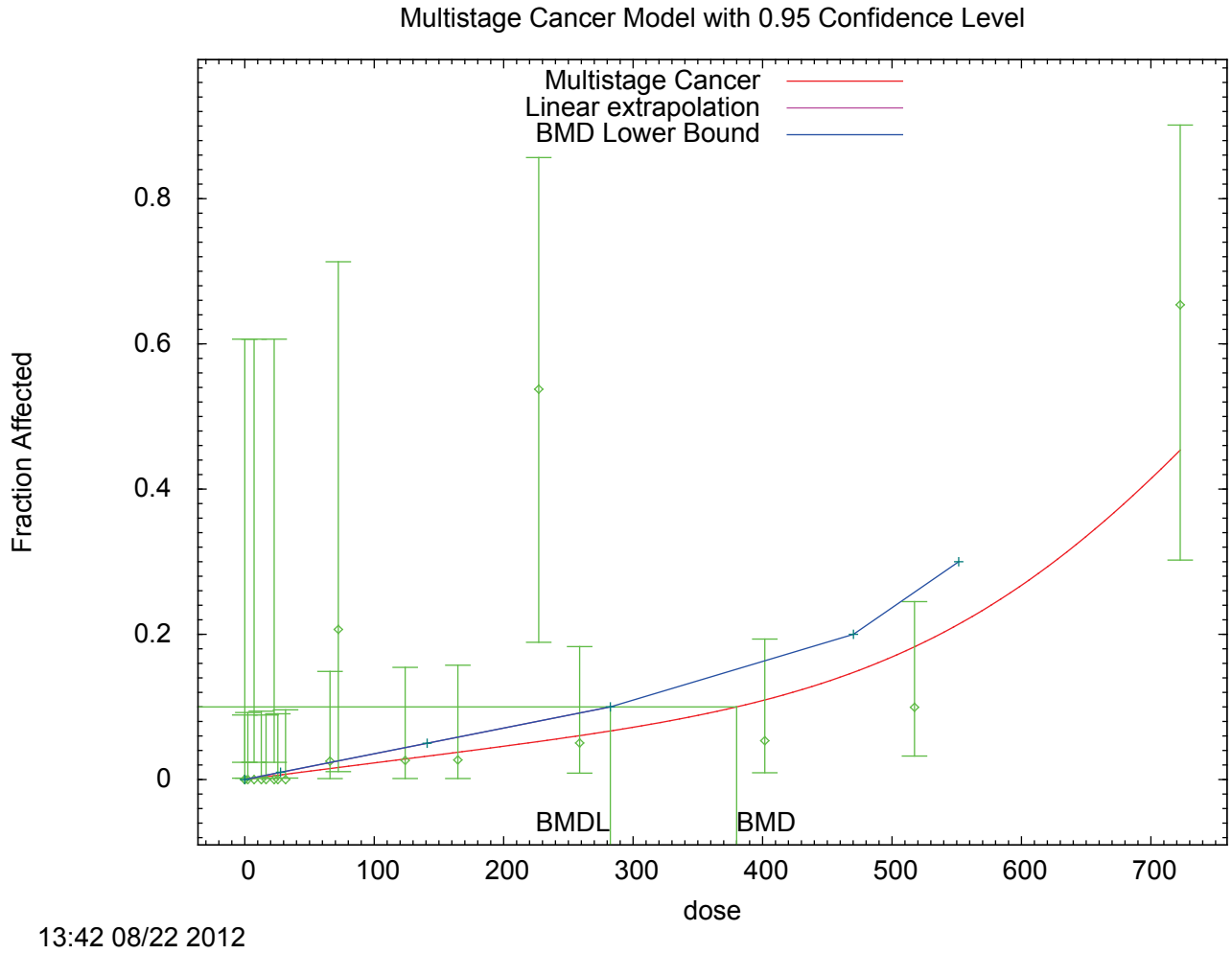
Run 119
Species: Mouse
Gender: Male
Organ: Forestomach
Response: Papilloma/Carcinoma
Study: NTP 1994 and Placke et al. 1996 Combined m = 1



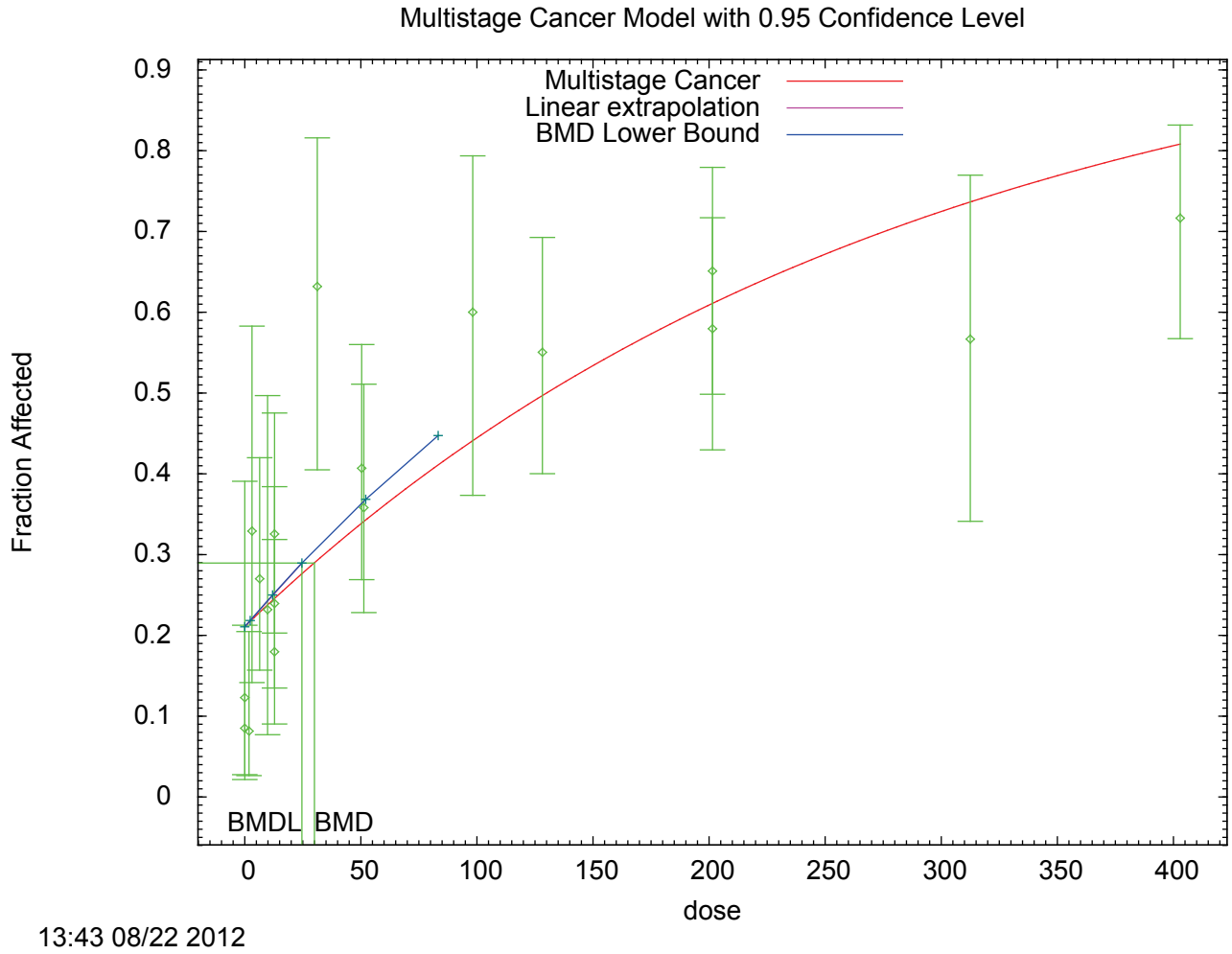
Run 120
Species: Mouse
Gender: Male
Organ: Forestomach
Response: Papilloma/Carcinoma
Study: NTP 1994 and Placke et al. 1996 Combined m = 2



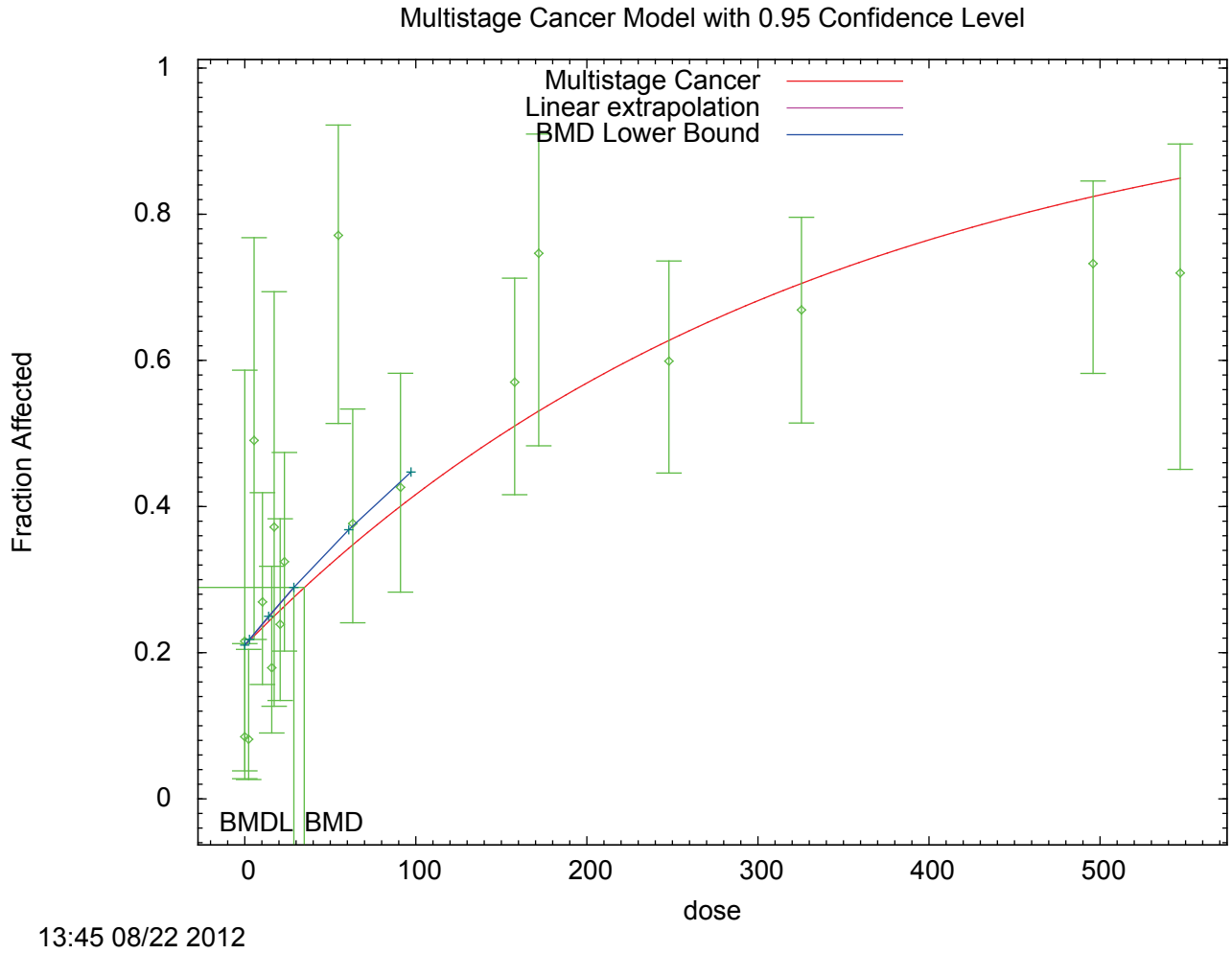
Run 121
Species: Mouse
Gender: Male
Organ: Forestomach
Response: Papilloma/Carcinoma
Study: NTP 1994 and Placke et al. 1996 Combined m = 3



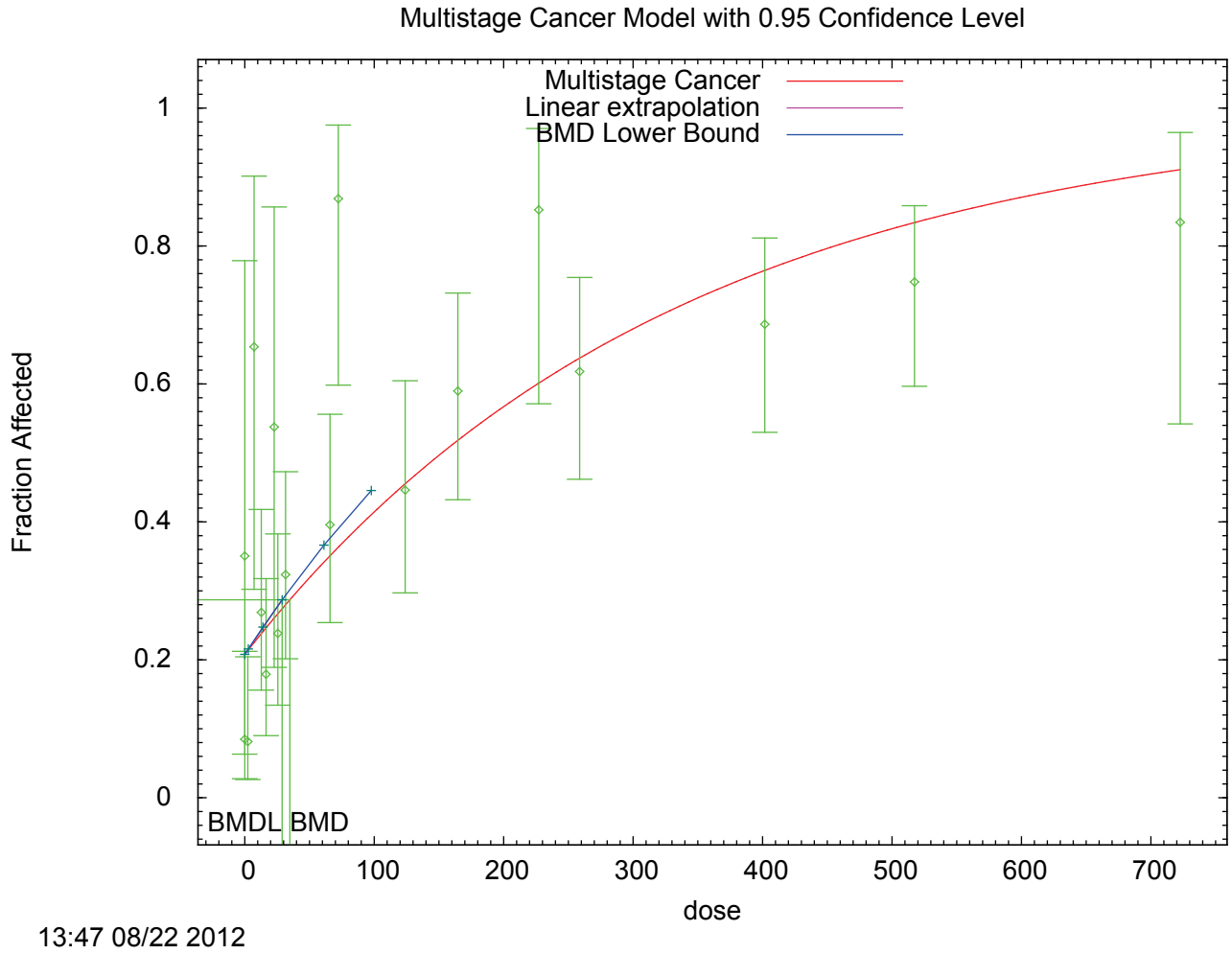
Run 122
Species: Mouse
Gender: Male
Organ: Harderian gland
Response: Adenoma
Study: NTP 1994 and Placke et al. 1996 Combined m = 1



Run 123
Species: Mouse
Gender: Male
Organ: Harderian gland
Response: Adenoma
Study: NTP 1994 and Placke et al. 1996 Combined m = 2

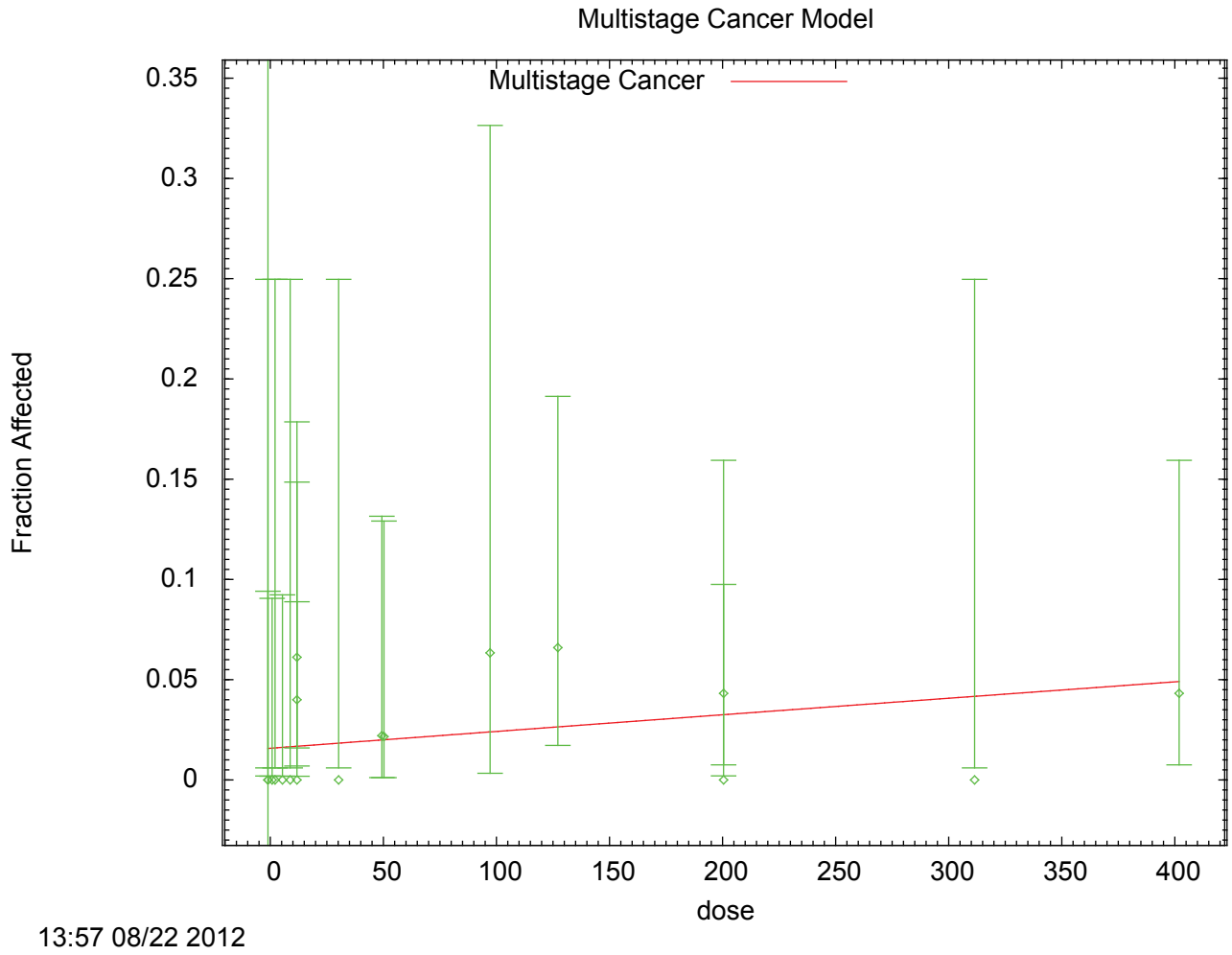


Run 124
Species: Mouse
Gender: Male
Organ: Harderian gland
Response: Adenoma
Study: NTP 1994 and Placke et al. 1996 Combined m = 3

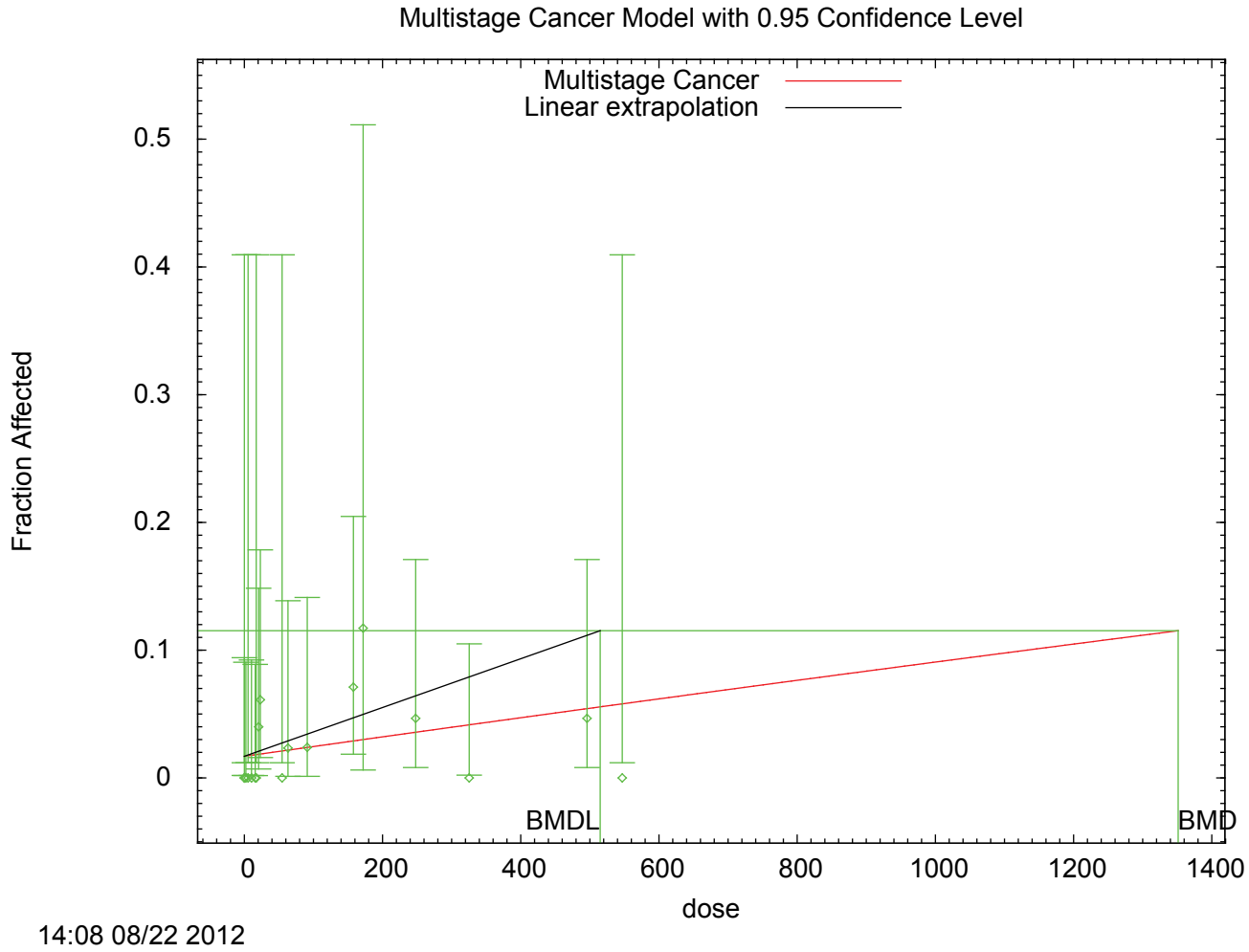


Run 125
Species: Mouse
Gender: Male
Organ: Harderian gland
Response: Carcinoma
Study: NTP 1994 and Placke et al. 1996 Combined m = 1

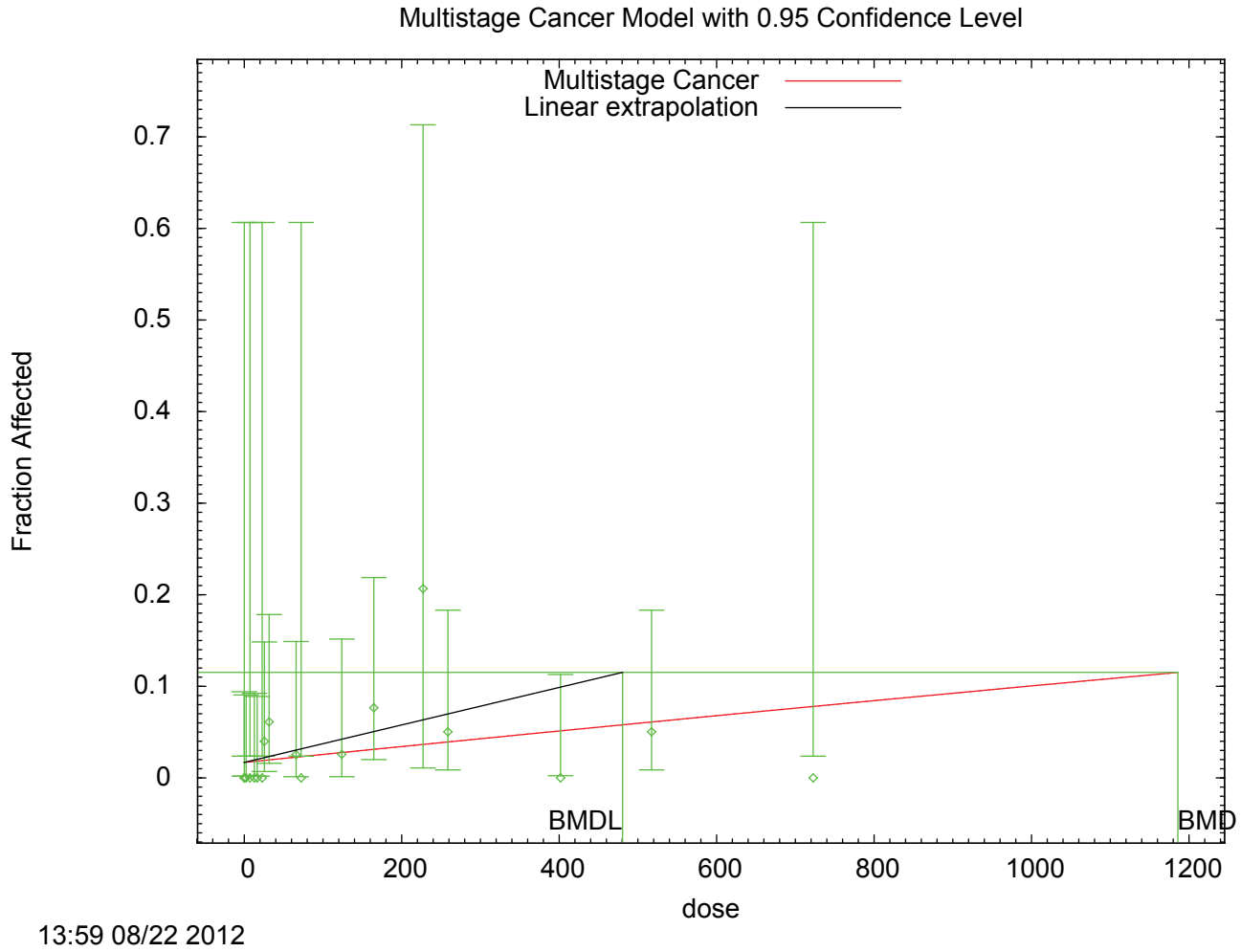
```
BMD computation failed. BMD is larger than three times maximum input doses.
```



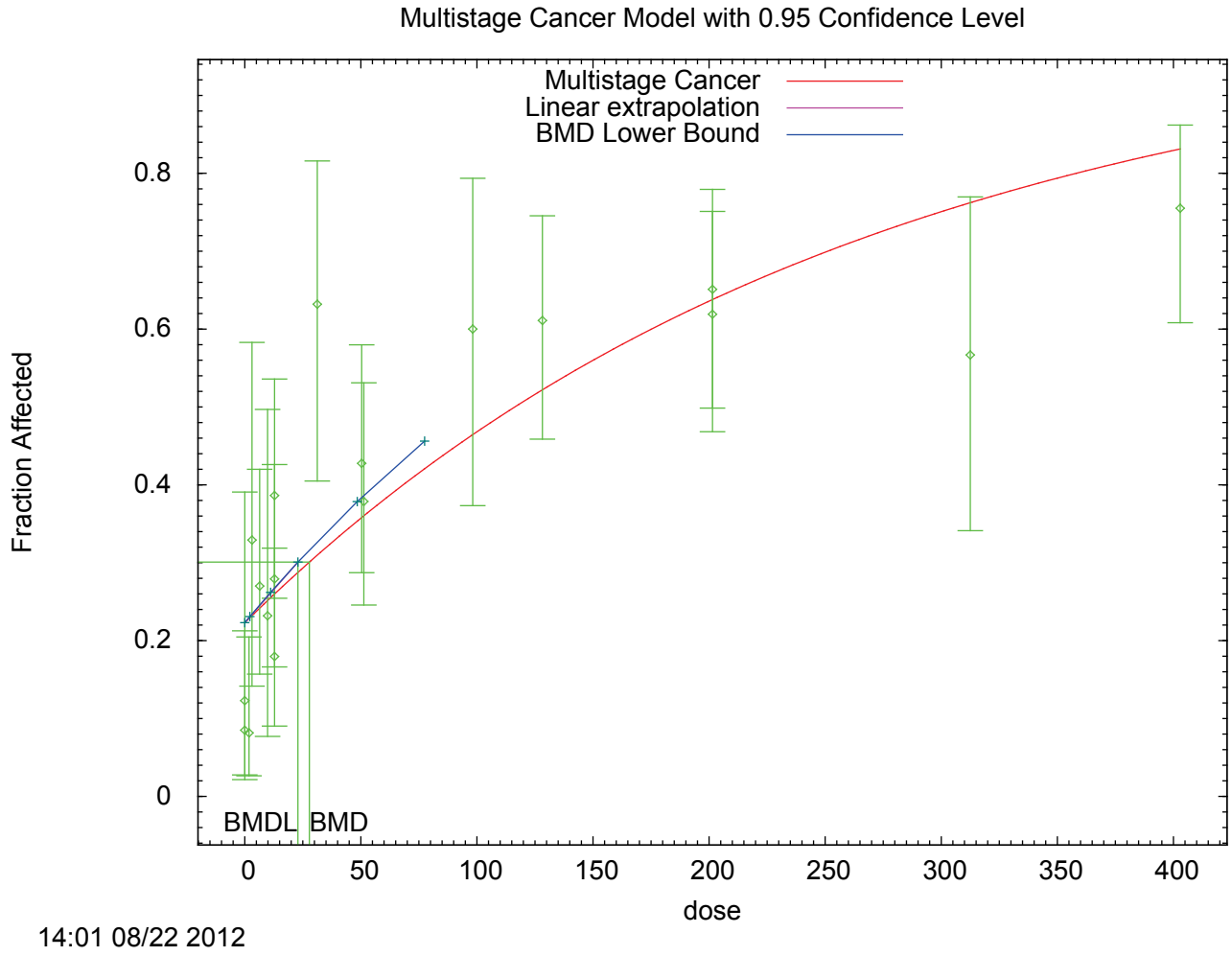
Run 126
Species: Mouse
Gender: Male
Organ: Harderian gland
Response: Carcinoma
Study: NTP 1994 and Placke et al. 1996 Combined m = 2



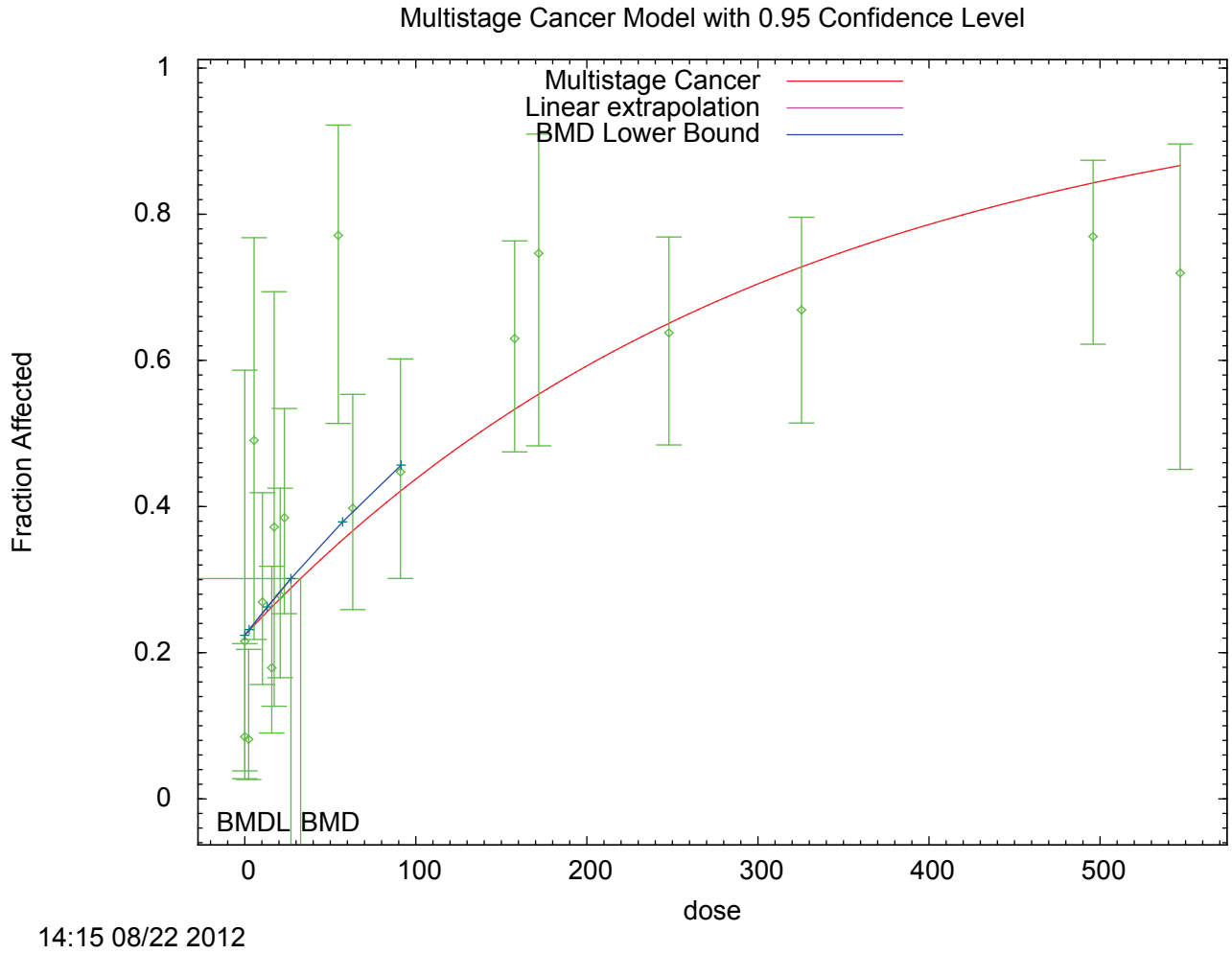
Run 127
Species: Mouse
Gender: Male
Organ: Harderian gland
Response: Carcinoma
Study: NTP 1994 and Placke et al. 1996 Combined m = 3



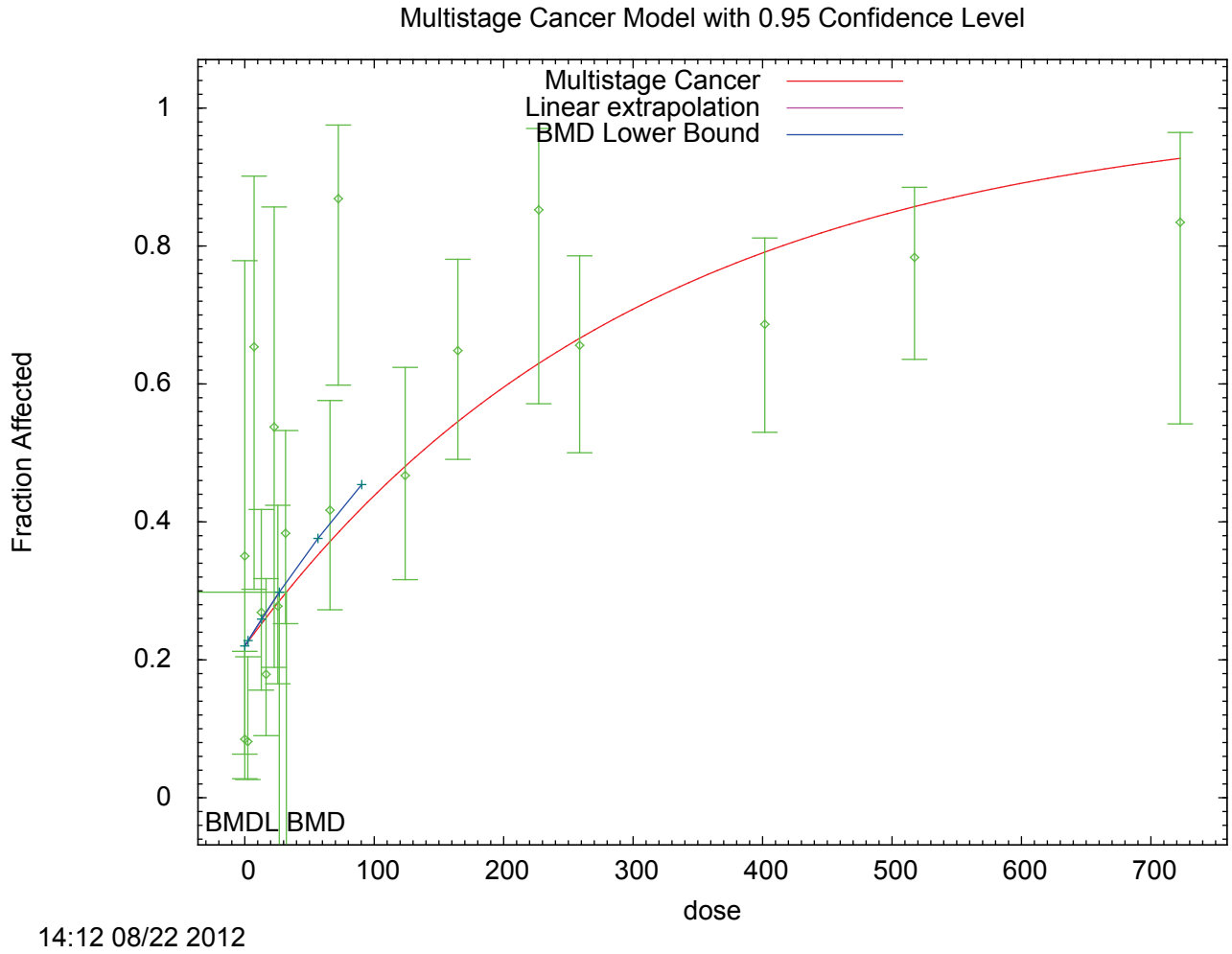
Run 128
Species: Mouse
Gender: Male
Organ: Harderian gland
Response: Aden./Carc.
Study: NTP 1994 and Placke et al. 1996 Combined m = 1



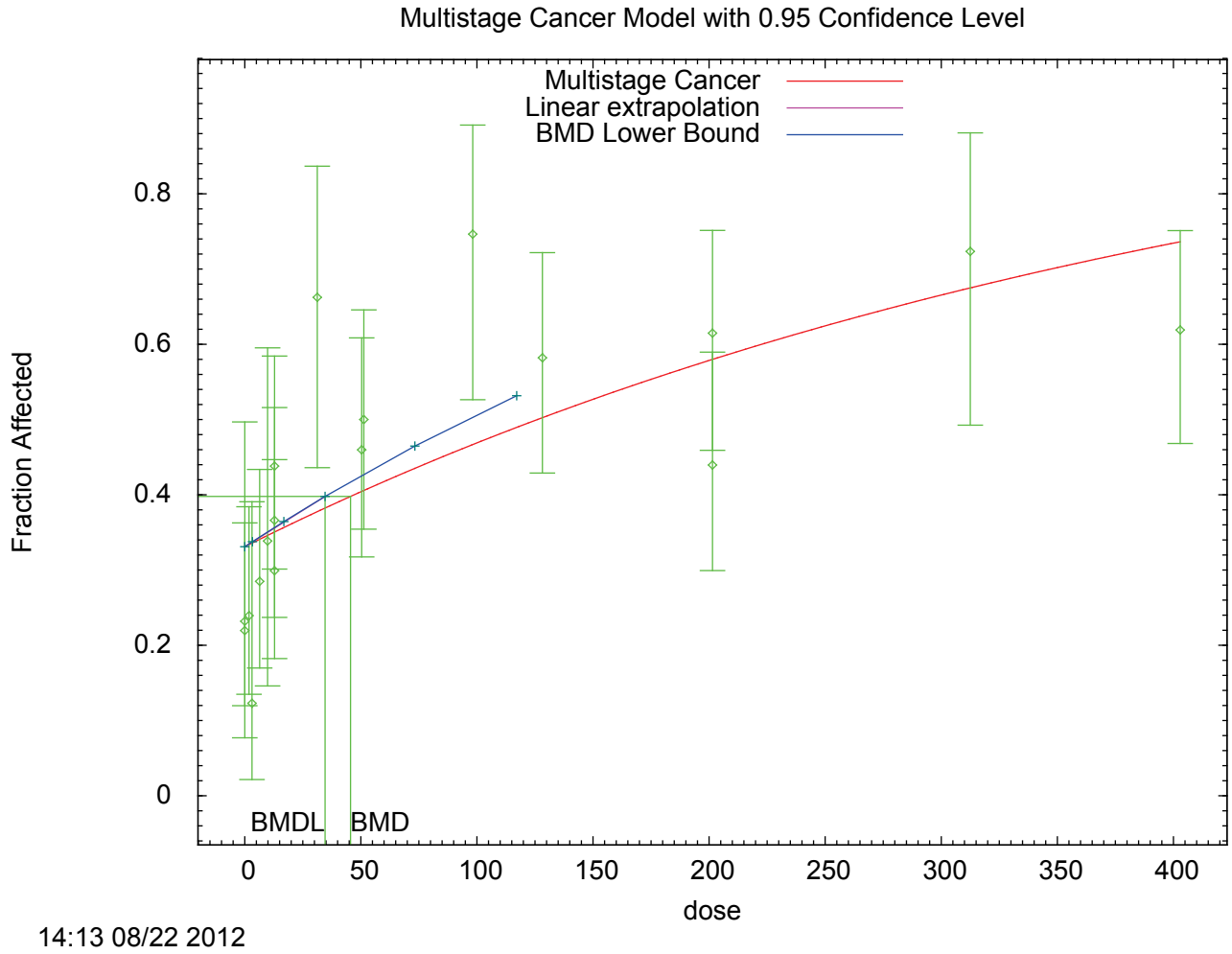
Run 129
Species: Mouse
Gender: Male
Organ: Harderian gland
Response: Aden./Carc.
Study: NTP 1994 and Placke et al. 1996 Combined m = 2



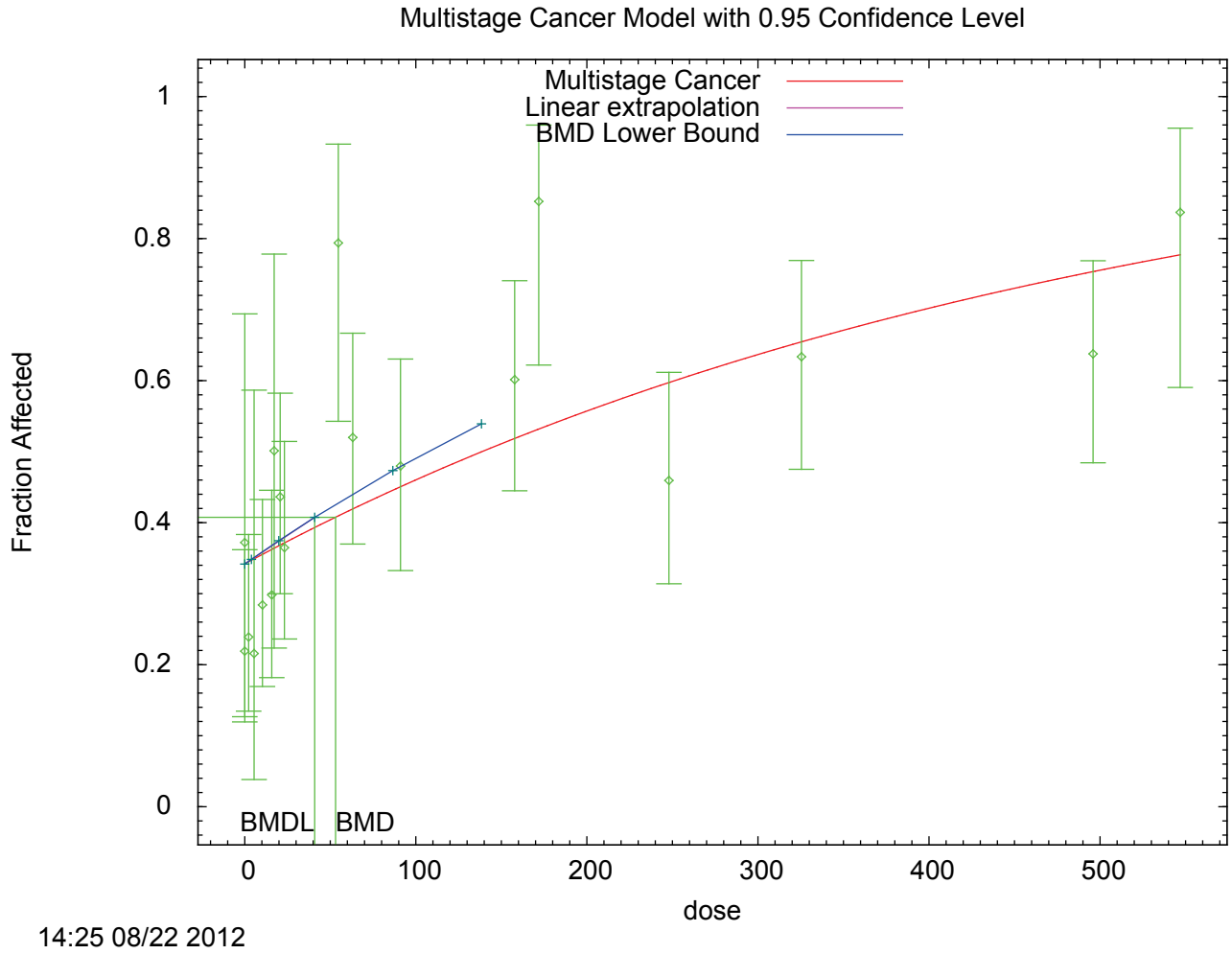
Run 130
Species: Mouse
Gender: Male
Organ: Harderian gland
Response: Aden./Carc.
Study: NTP 1994 and Placke et al. 1996 Combined m = 3



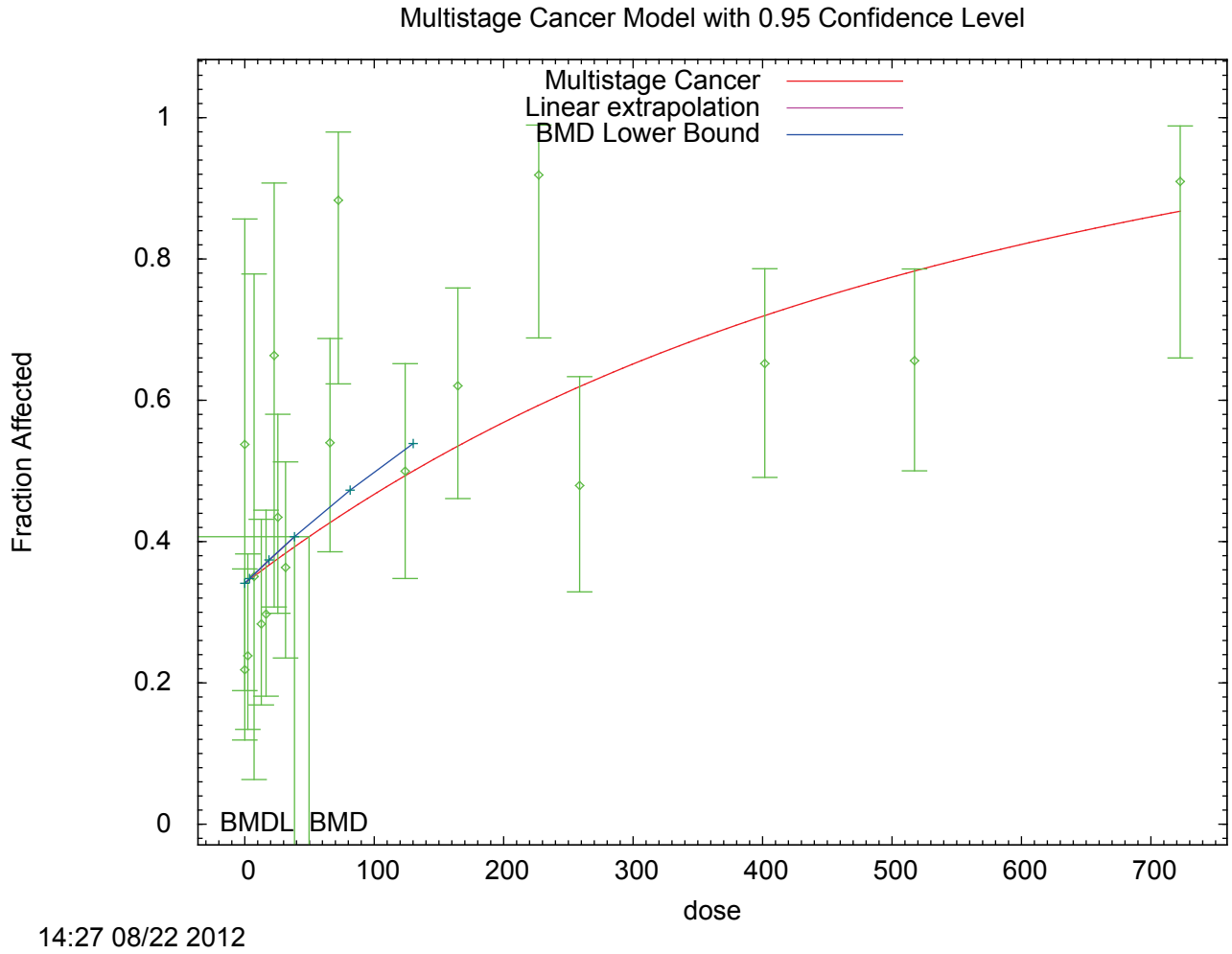
Run 131
Species: Mouse
Gender: Male
Organ: Liver
Response: Adenoma
Study: NTP 1994 and Placke et al. 1996 Combined m = 1



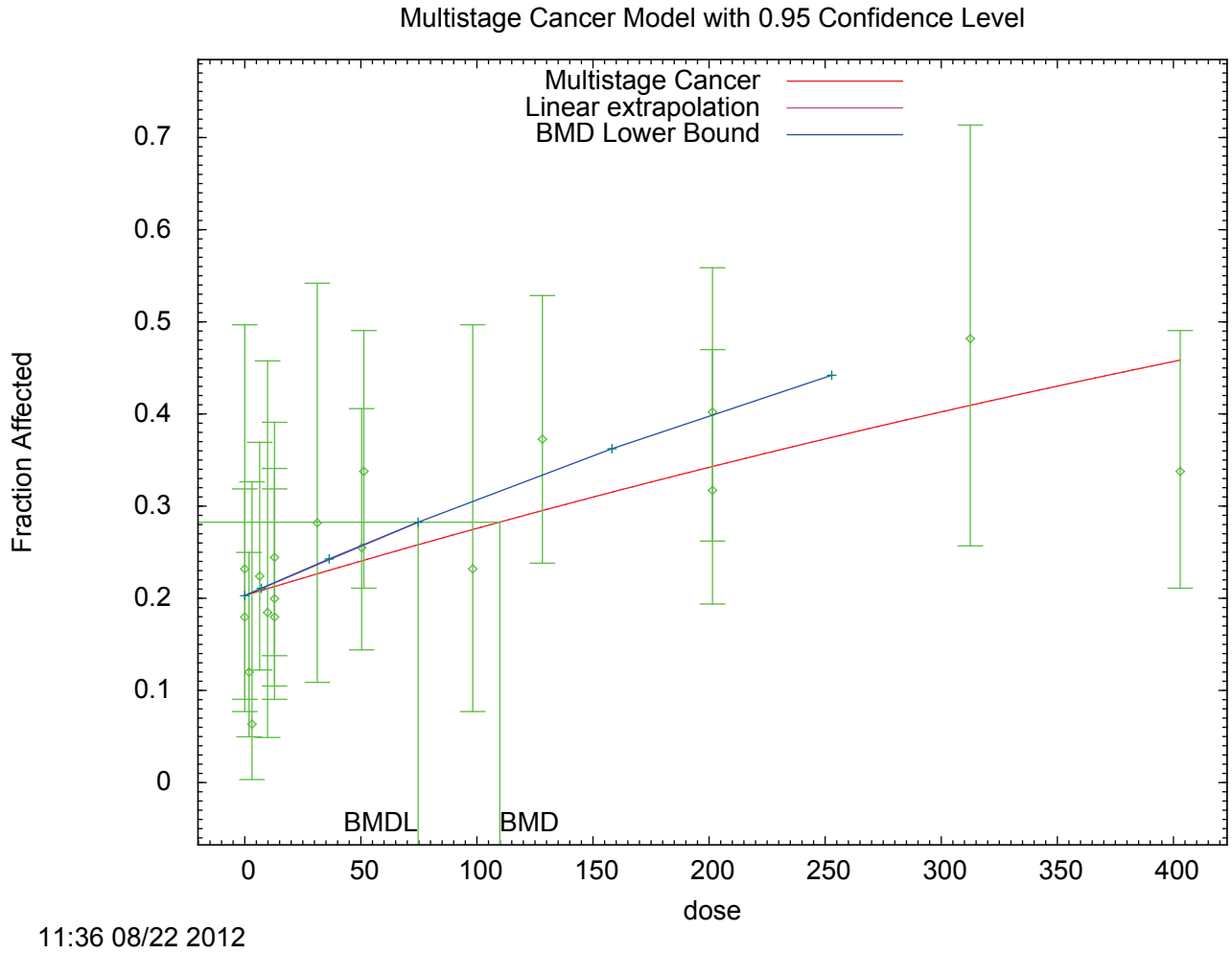
Run 132
Species: Mouse
Gender: Male
Organ: Liver
Response: Adenoma
Study: NTP 1994 and Placke et al. 1996 Combined m = 2



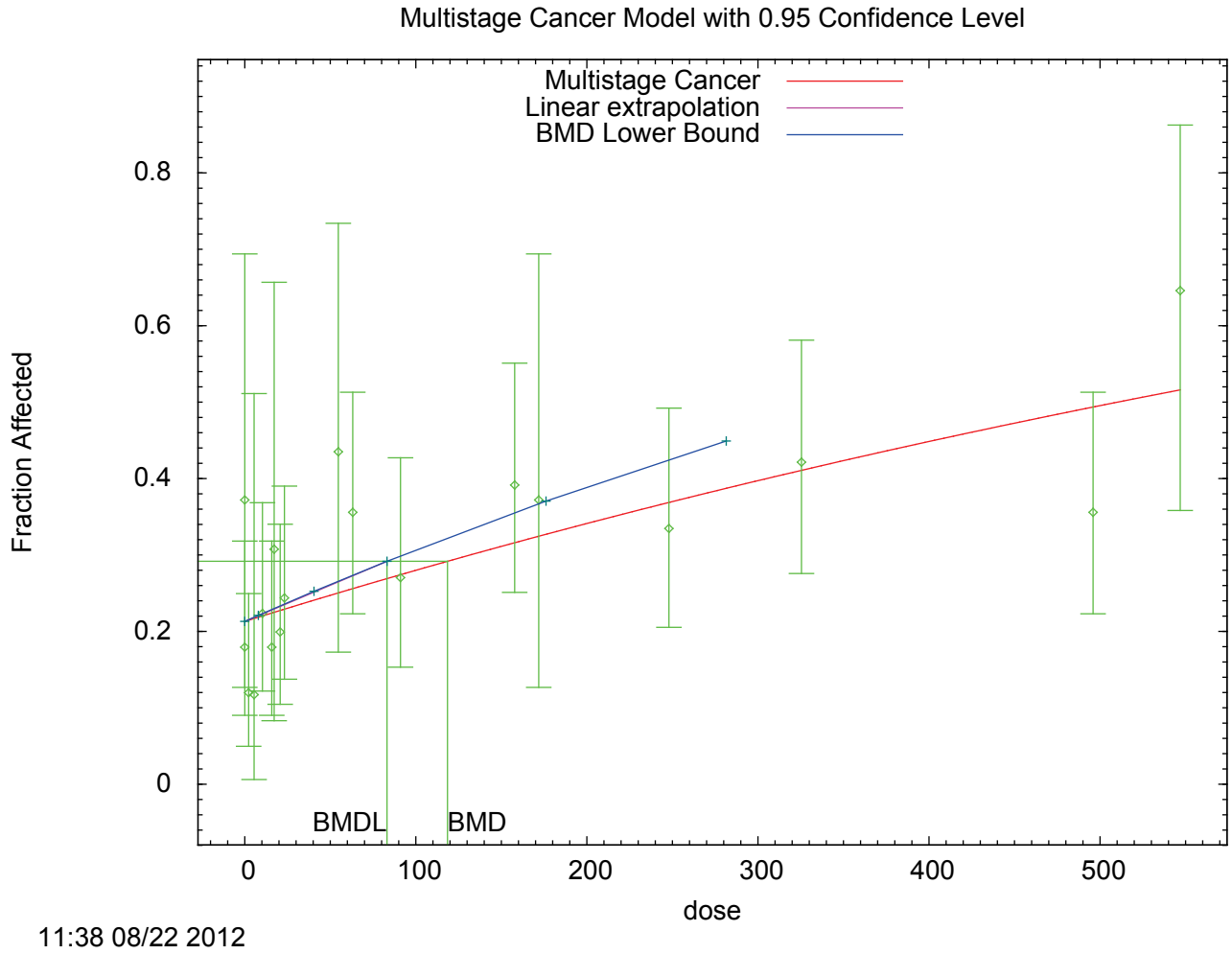
Run 133
Species: Mouse
Gender: Male
Organ: Liver
Response: Adenoma
Study: NTP 1994 and Placke et al. 1996 Combined m = 3



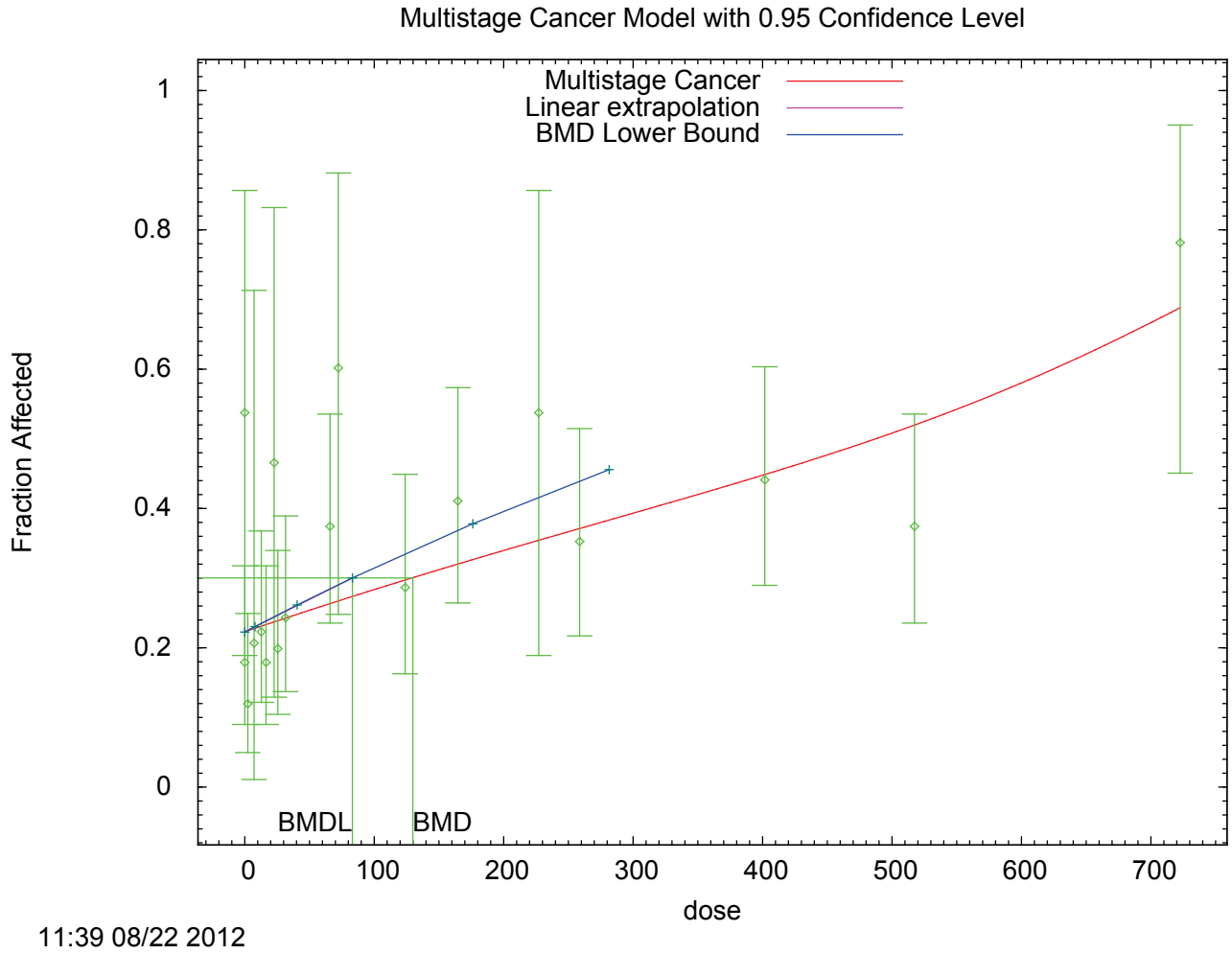
Run 134
Species: Mouse
Gender: Male
Organ: Liver
Response: Carcinoma
Study: NTP 1994 and Placke et al. 1996 Combined m = 1



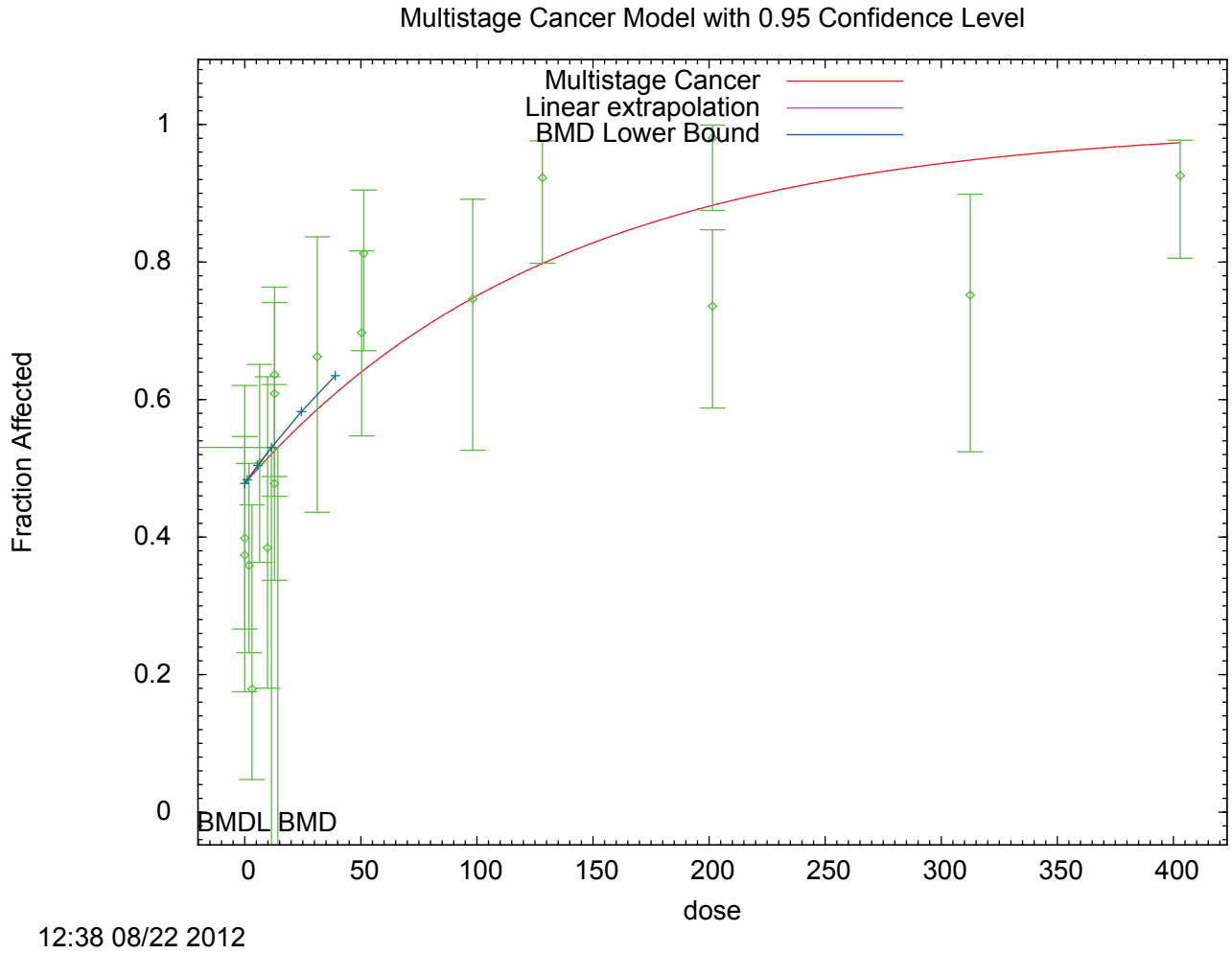
Run 135
Species: Mouse
Gender: Male
Organ: Liver
Response: Carcinoma
Study: NTP 1994 and Placke et al. 1996 Combined m = 2



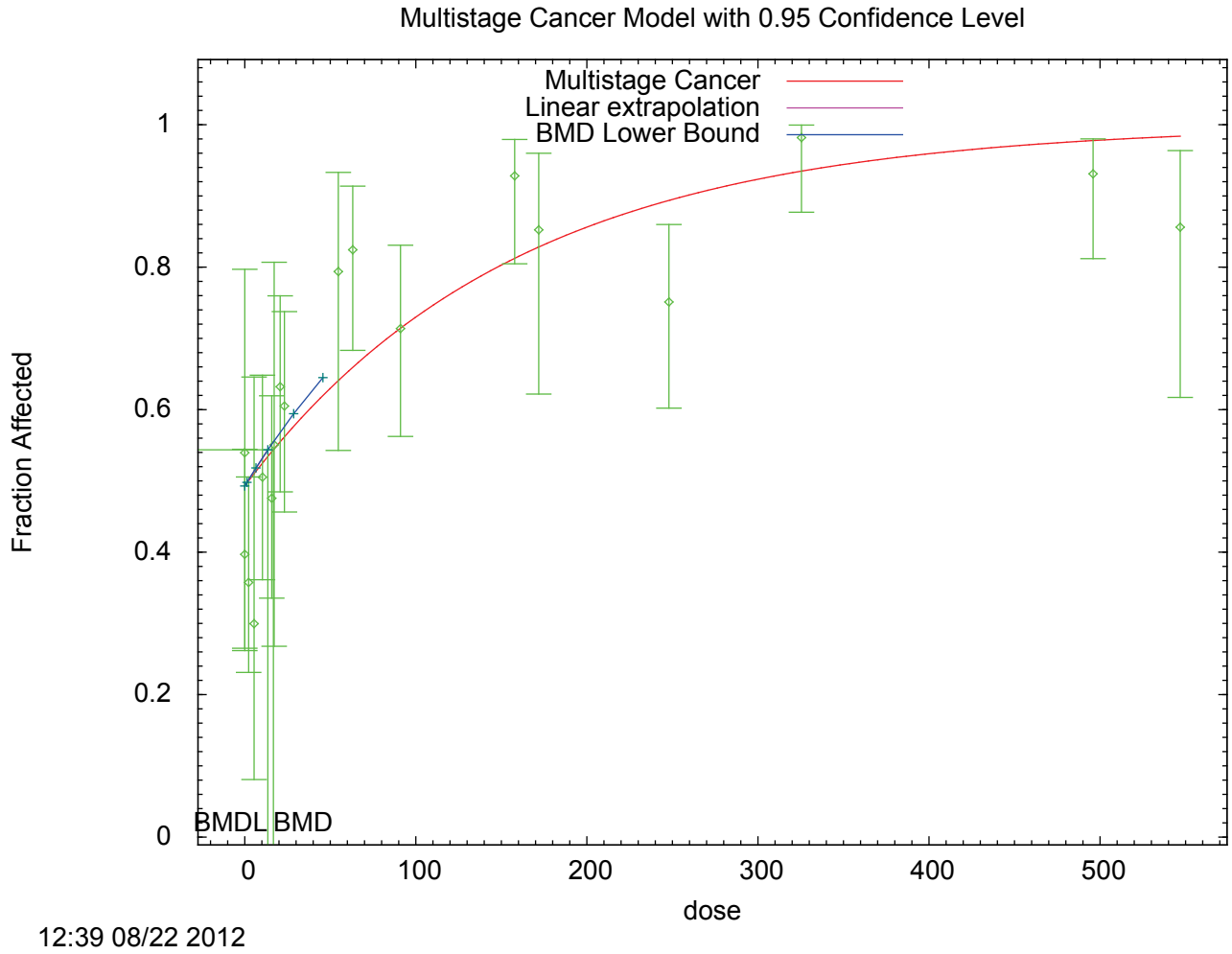
Run 136
Species: Mouse
Gender: Male
Organ: Liver
Response: Carcinoma
Study: NTP 1994 and Placke et al. 1996 Combined m = 3



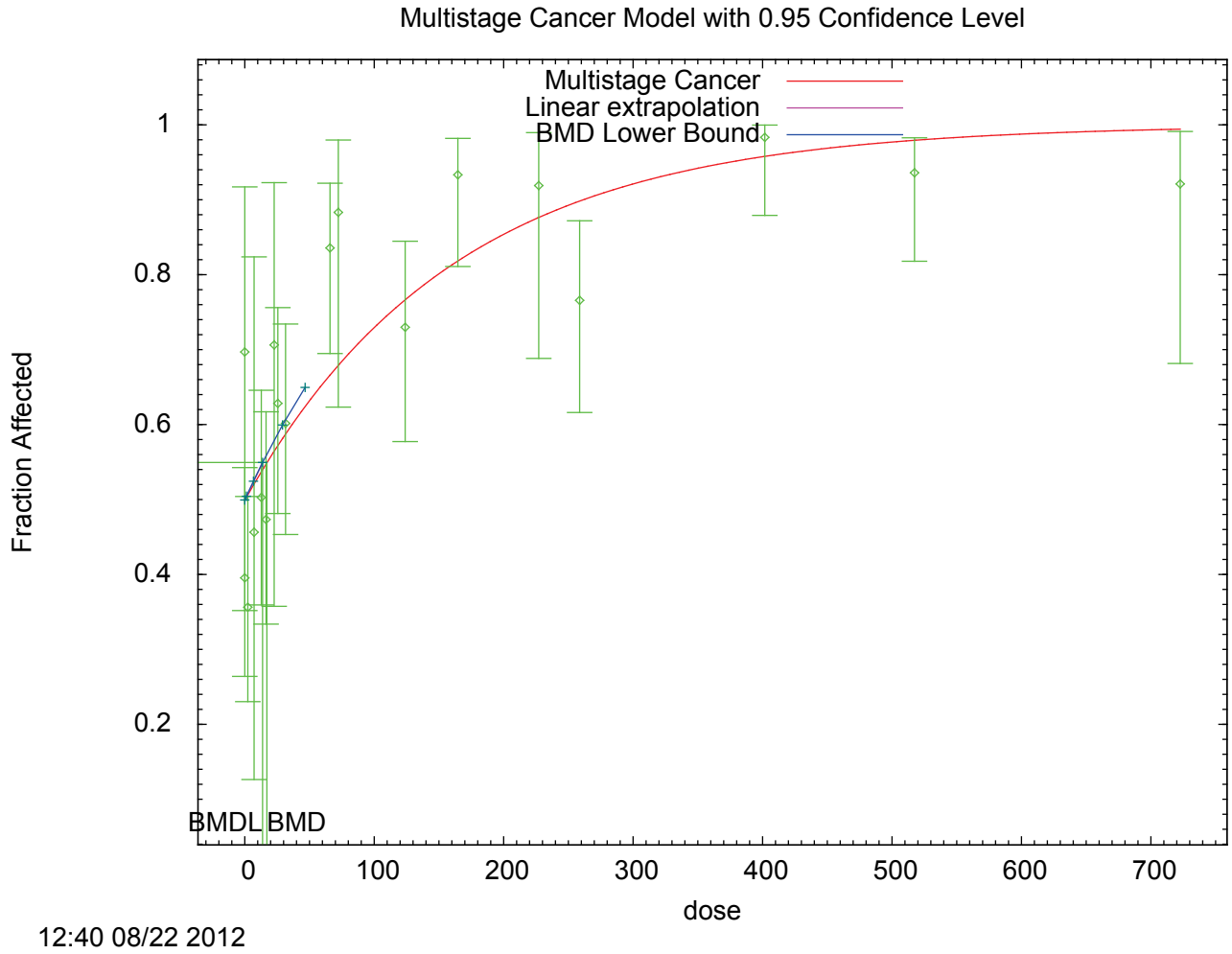
Run 137
Species: Mouse
Gender: Male
Organ: Liver
Response: Aden./Carc.
Study: NTP 1994 and Placke et al. 1996 Combined m = 1



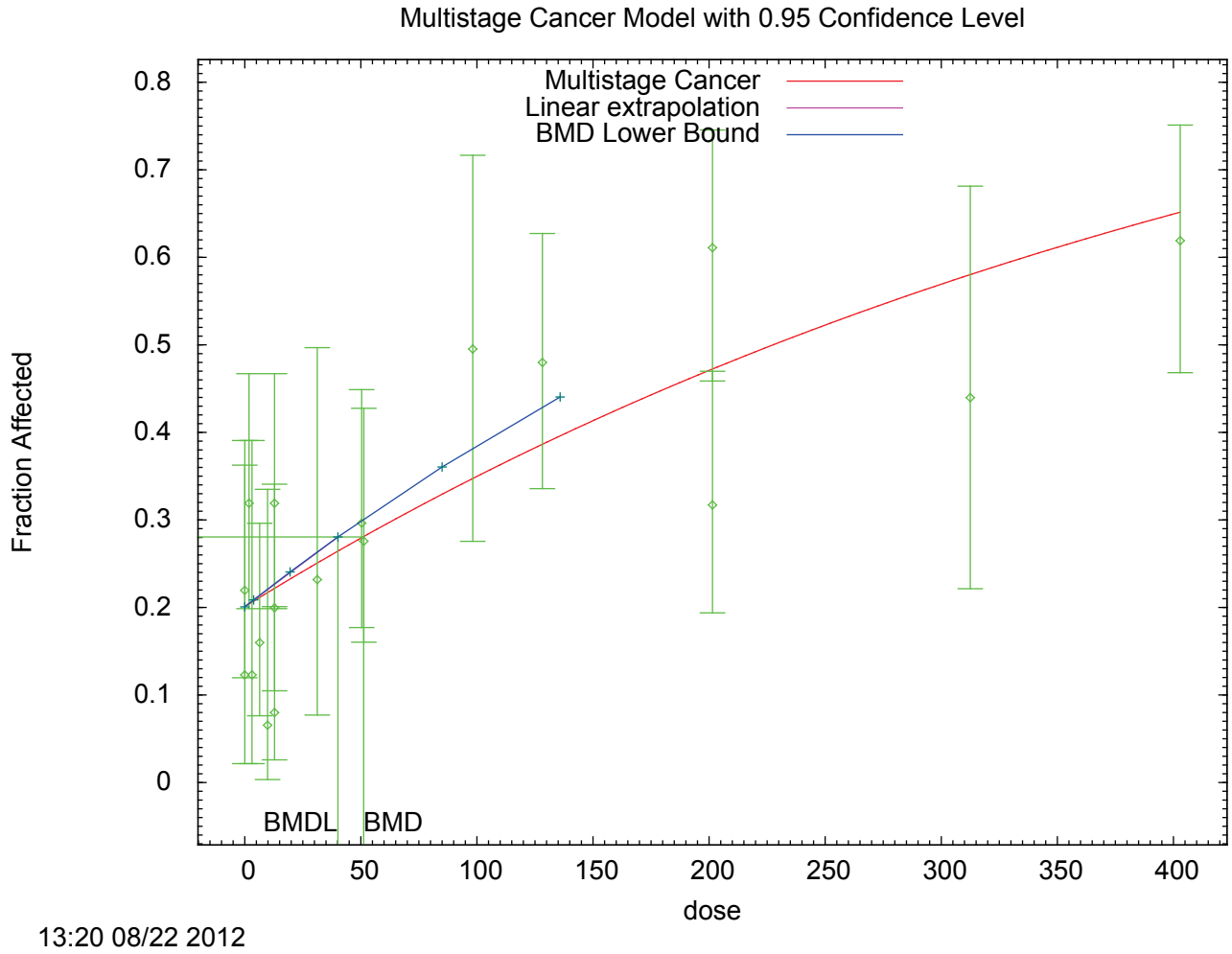
Run 138
Species: Mouse
Gender: Male
Organ: Liver
Response: Aden./Carc.
Study: NTP 1994 and Placke et al. 1996 Combined m = 2



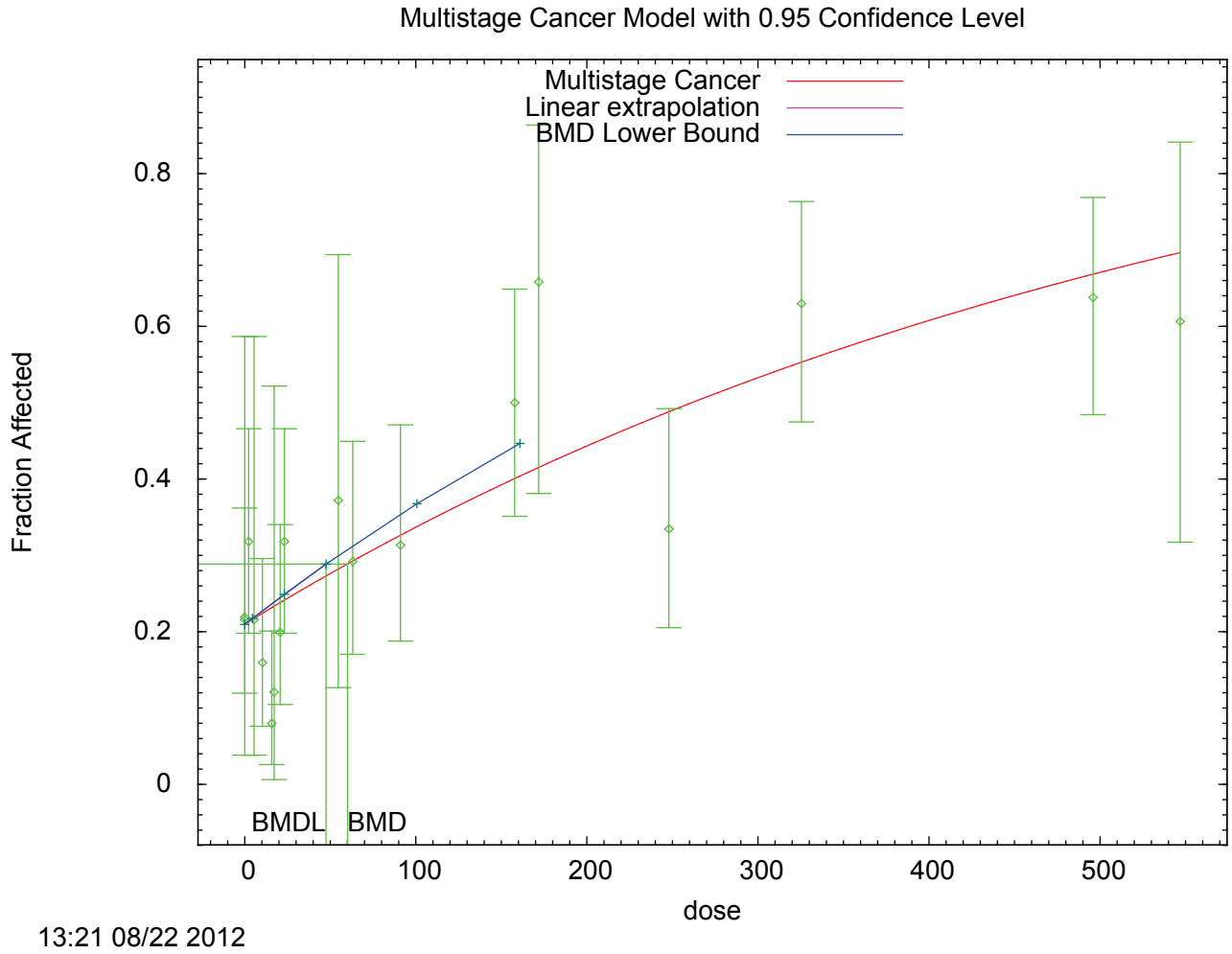
Run 139
Species: Mouse
Gender: Male
Organ: Liver
Response: Aden./Carc.
Study: NTP 1994 and Placke et al. 1996 Combined m = 3



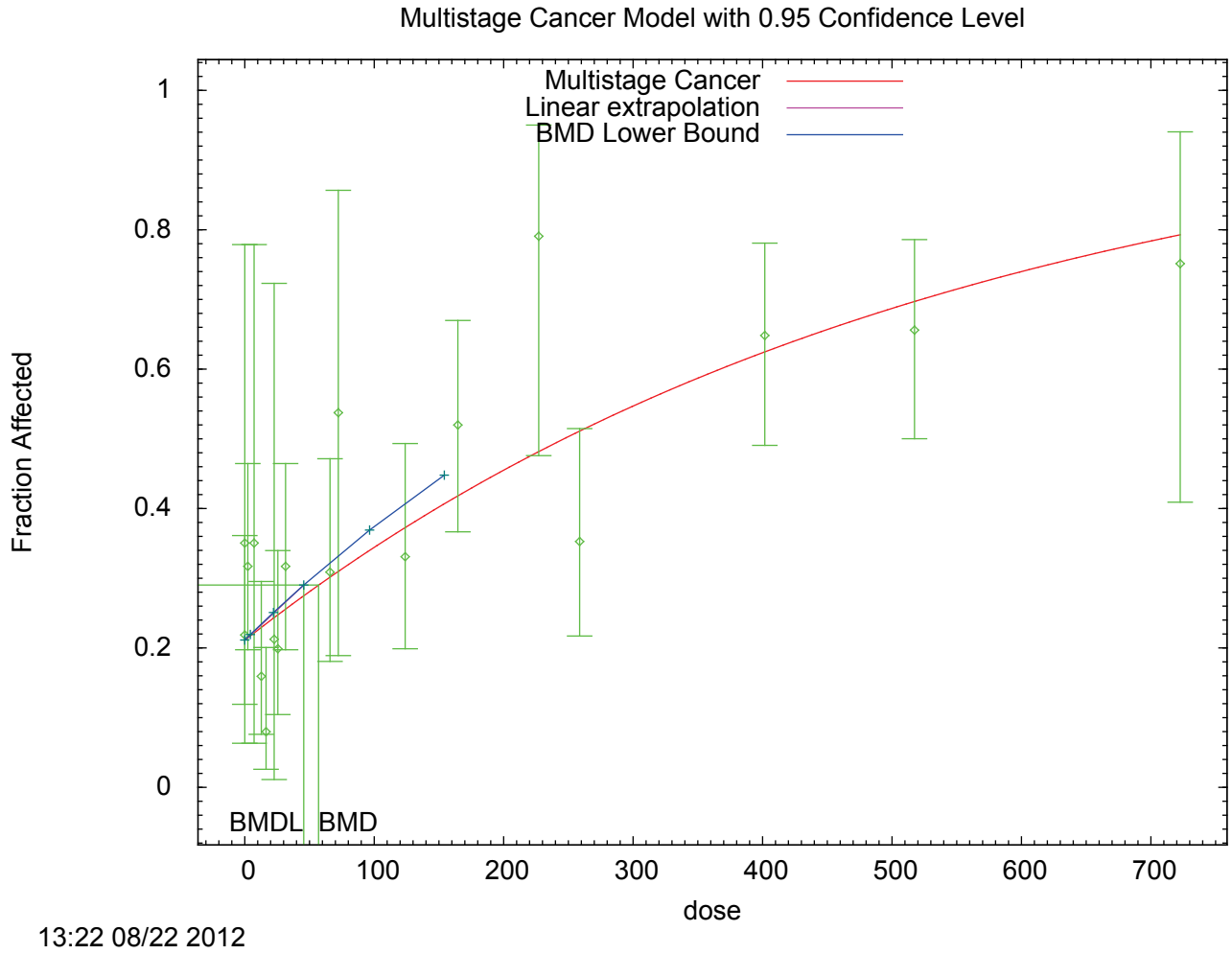
Run 140
Species: Mouse
Gender: Male
Organ: Lung
Response: Adenoma
Study: NTP 1994 and Placke et al. 1996 Combined m = 1



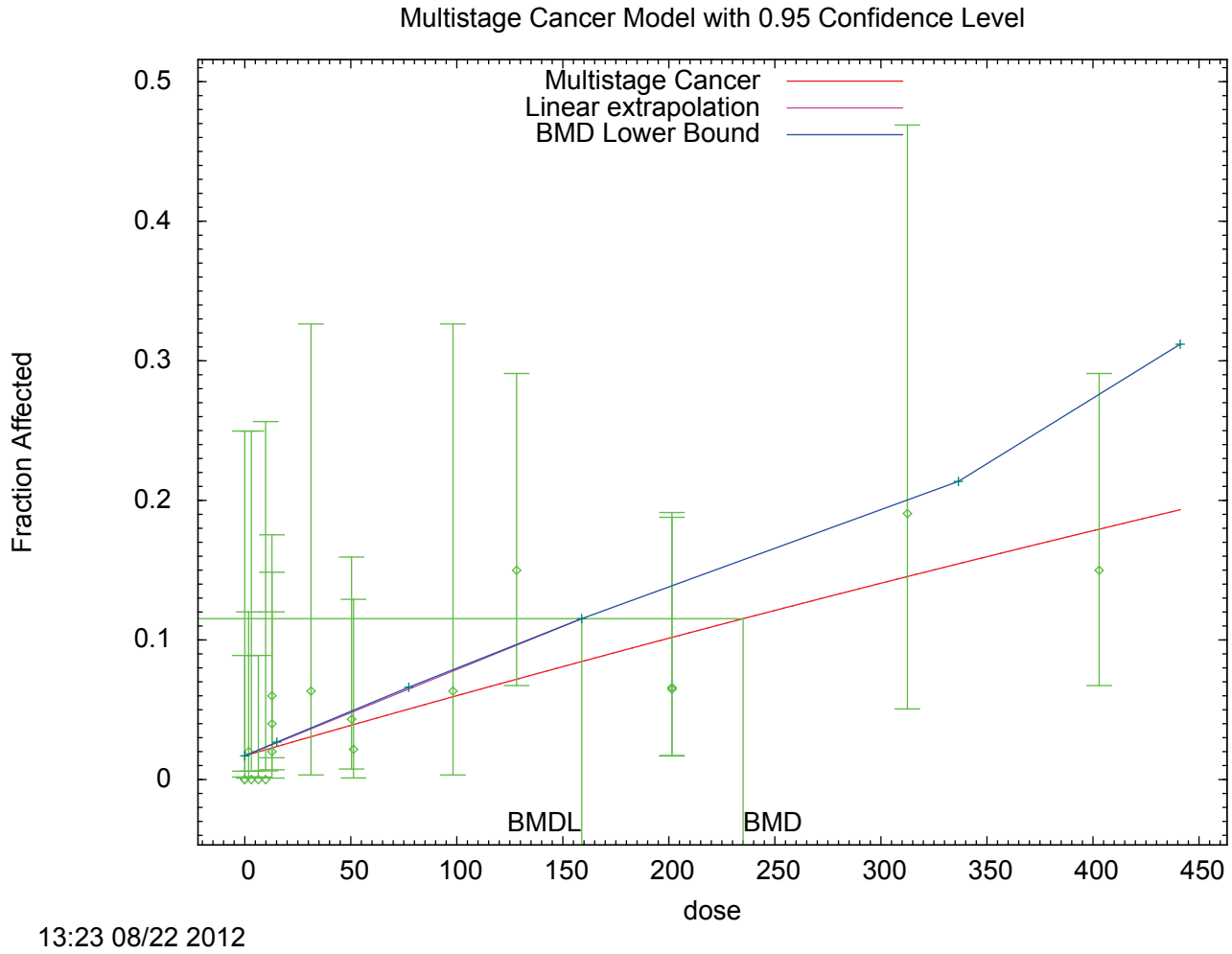
Run 141
Species: Mouse
Gender: Male
Organ: Lung
Response: Adenoma
Study: NTP 1994 and Placke et al. 1996 Combined m = 2



Run 142
Species: Mouse
Gender: Male
Organ: Lung
Response: Adenoma
Study: NTP 1994 and Placke et al. 1996 Combined m = 3

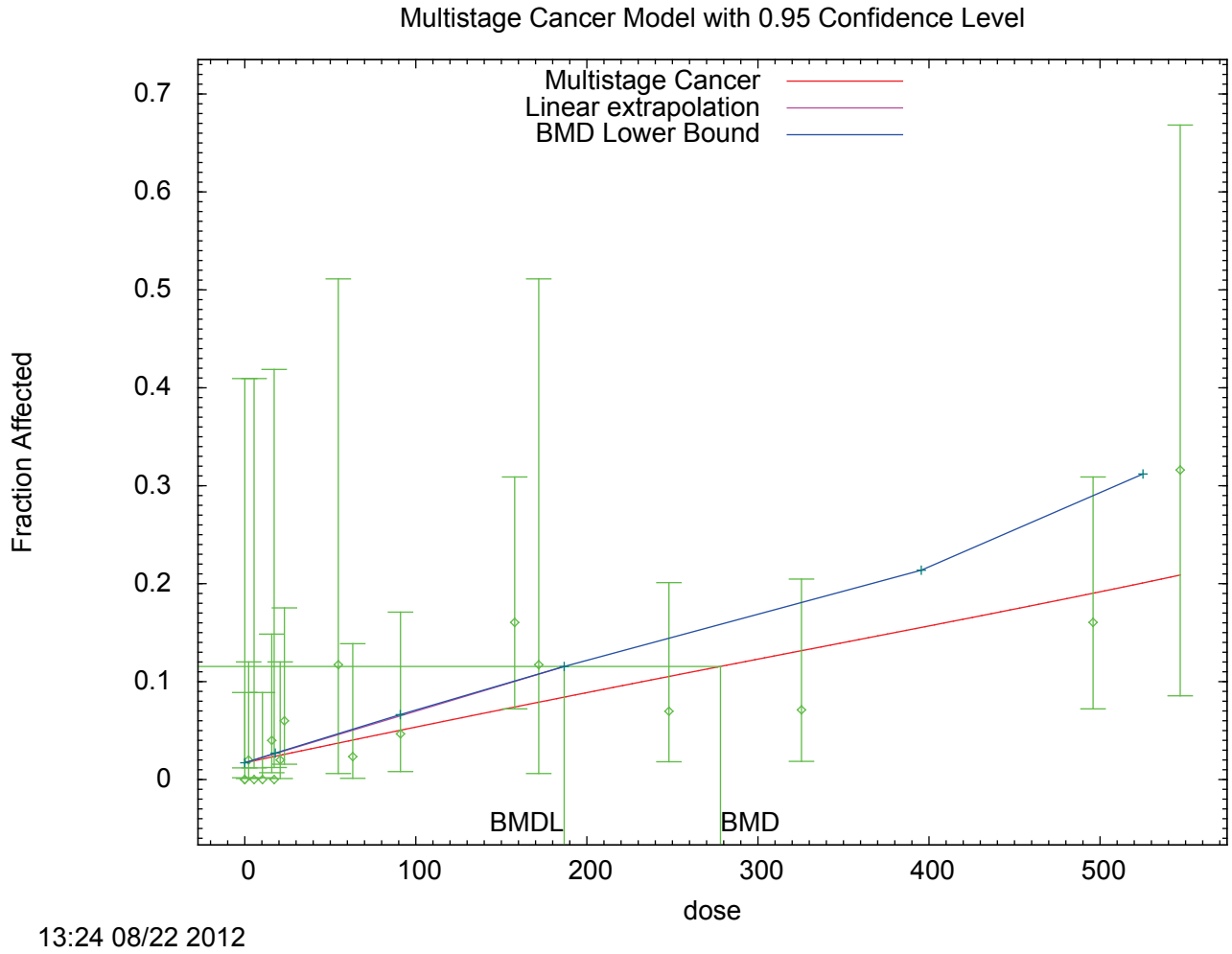


Run 143
Species: Mouse
Gender: Male
Organ: Lung
Response: Carcinoma
Study: NTP 1994 and Placke et al. 1996 Combined m = 1

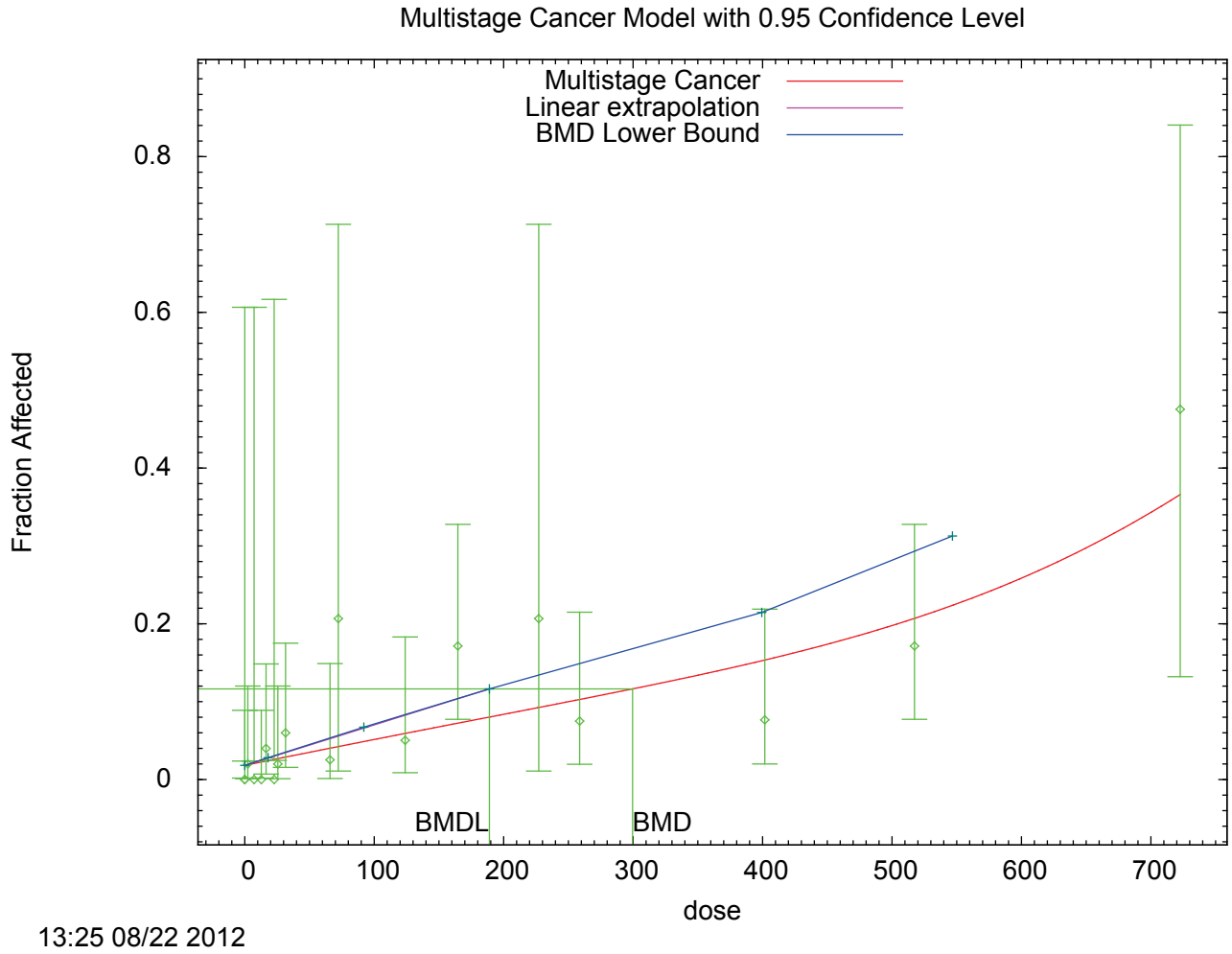


13:23 08/22 2012

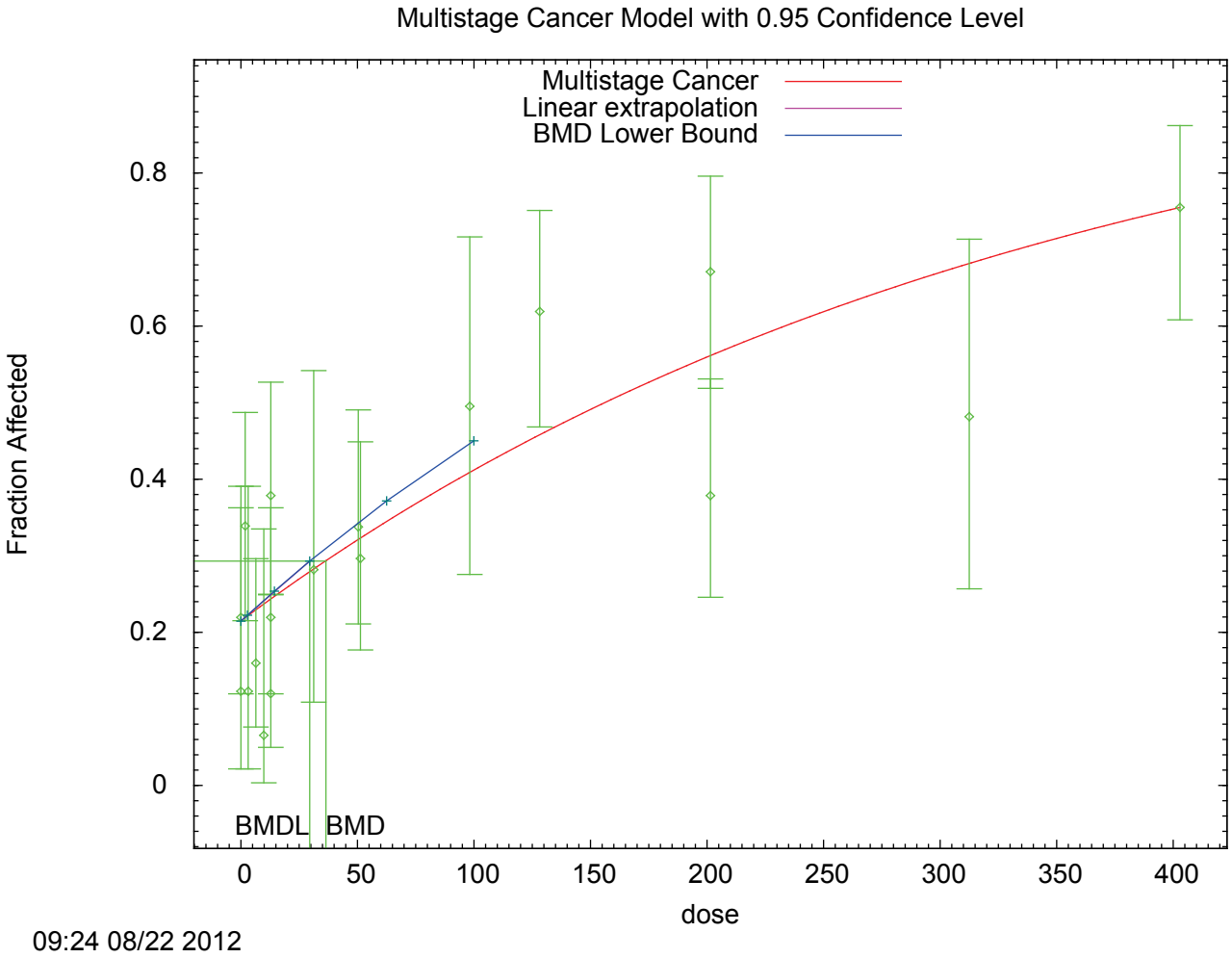
Run 144
Species: Mouse
Gender: Male
Organ: Lung
Response: Carcinoma
Study: NTP 1994 and Placke et al. 1996 Combined m = 2



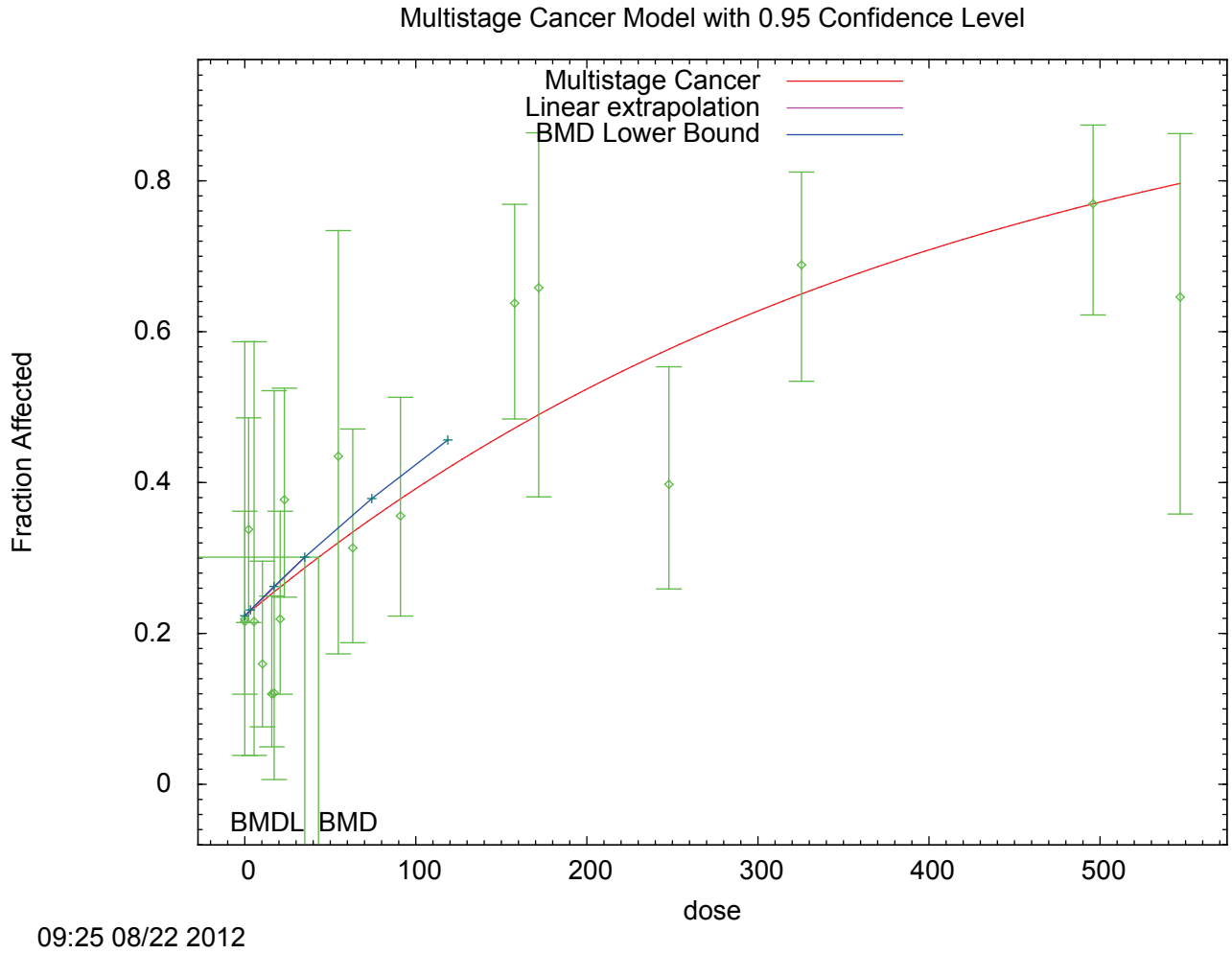
Run 145
Species: Mouse
Gender: Male
Organ: Lung
Response: Carcinoma
Study: NTP 1994 and Placke et al. 1996 Combined m = 3



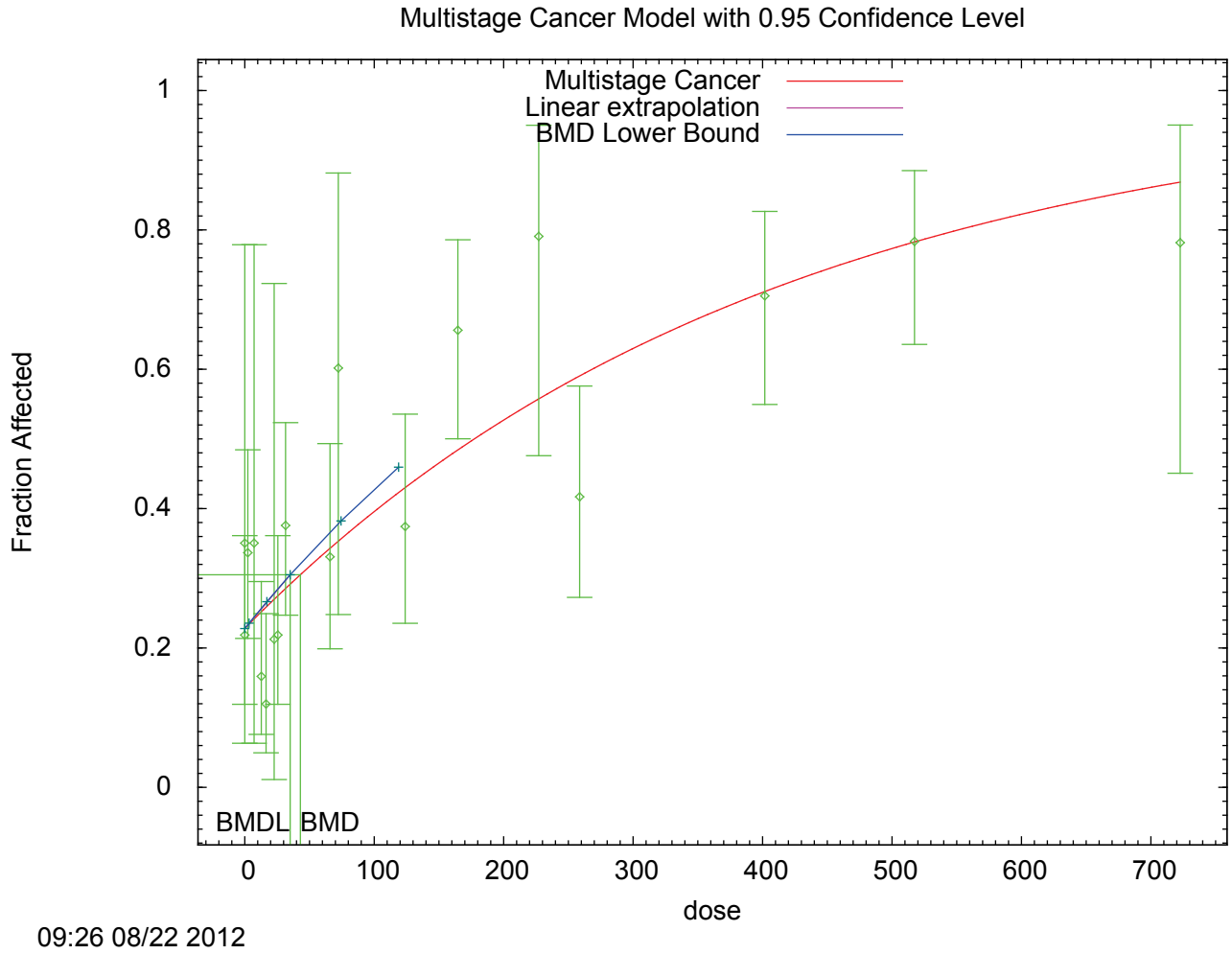
Run 146
Species: Mouse
Gender: Male
Organ: Lung
Response: Aden./Carc.
Study: NTP 1994 and Placke et al. 1996 Combined m = 1



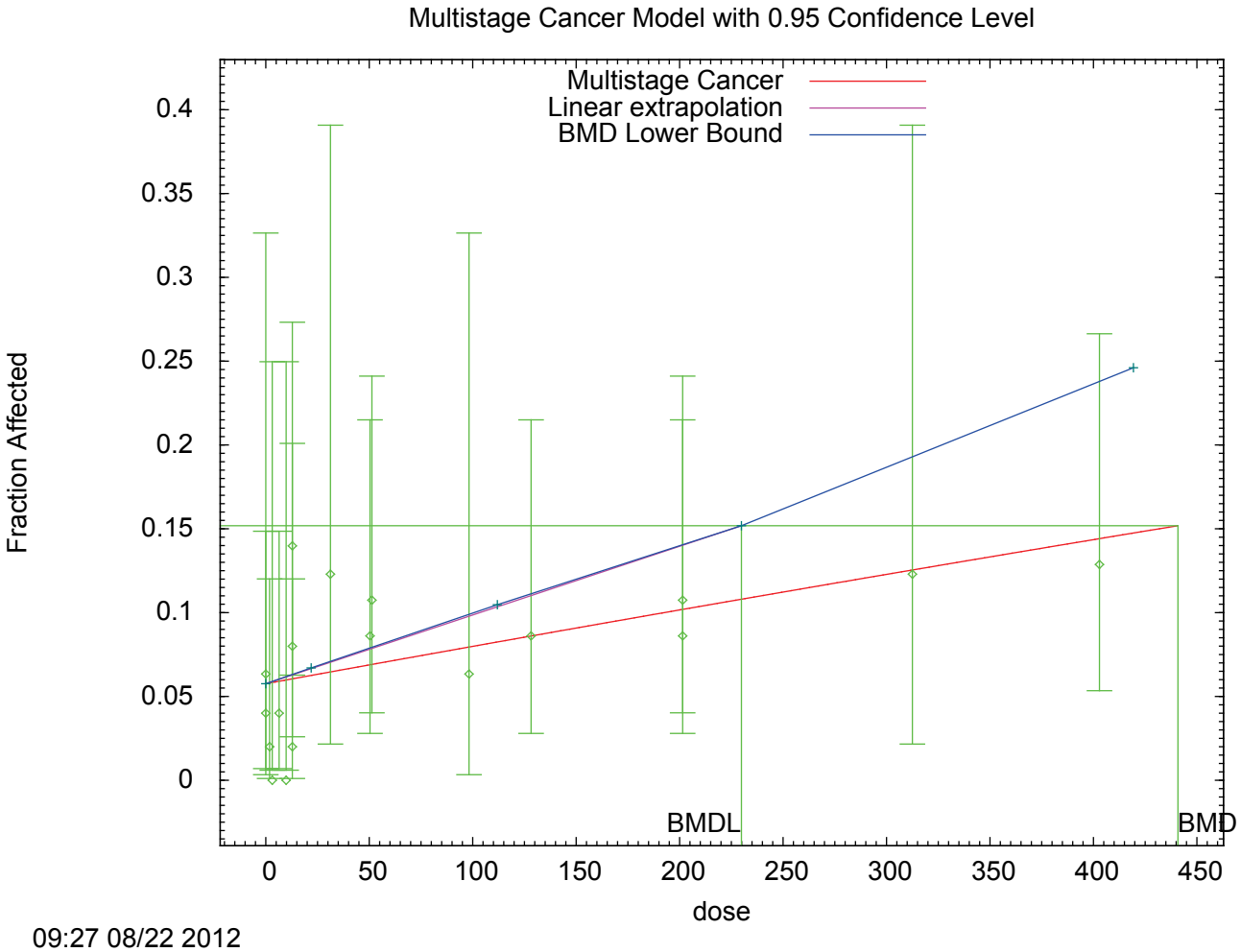
Run 147
Species: Mouse
Gender: Male
Organ: Lung
Response: Aden./Carc.
Study: NTP 1994 and Placke et al. 1996 Combined m = 2



Run 148
Species: Mouse
Gender: Male
Organ: Lung
Response: Aden./Carc.
Study: NTP 1994 and Placke et al. 1996 Combined m = 3

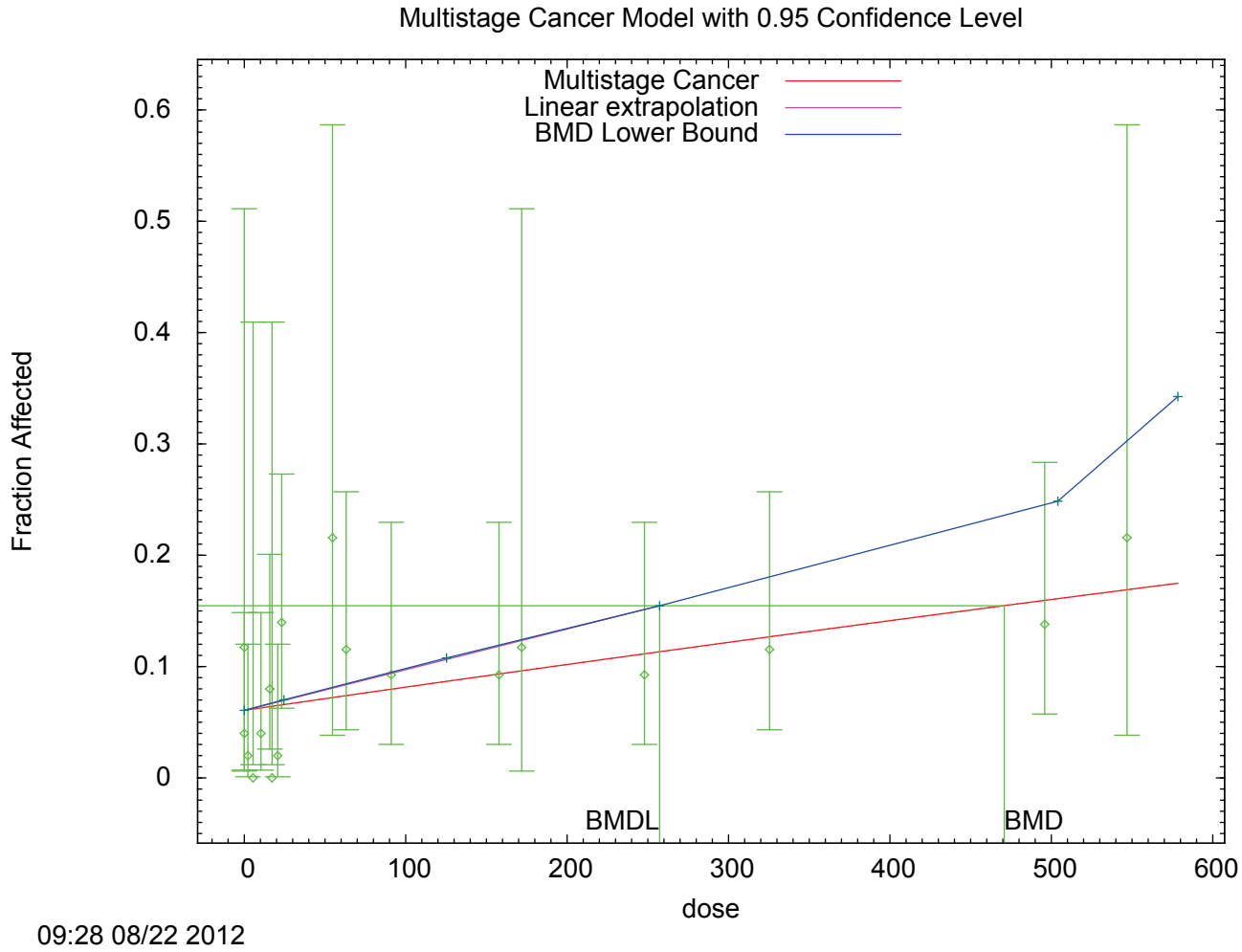


Run 149
Species: Mouse
Gender: Male
Organ: Hematopoietic
Response: any lymphoma
Study: NTP 1994 and Placke et al. 1996 Combined m = 1

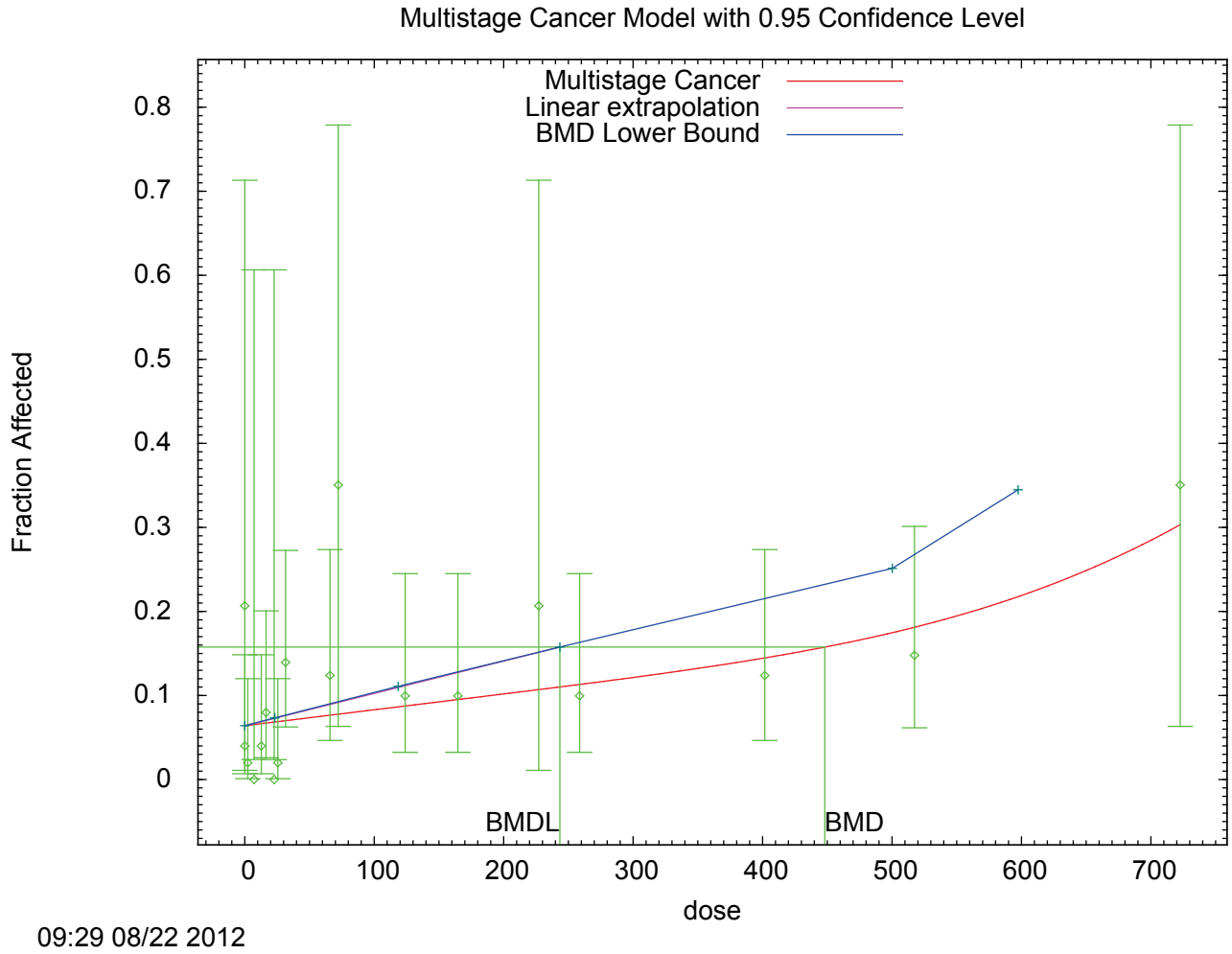


09:27 08/22 2012

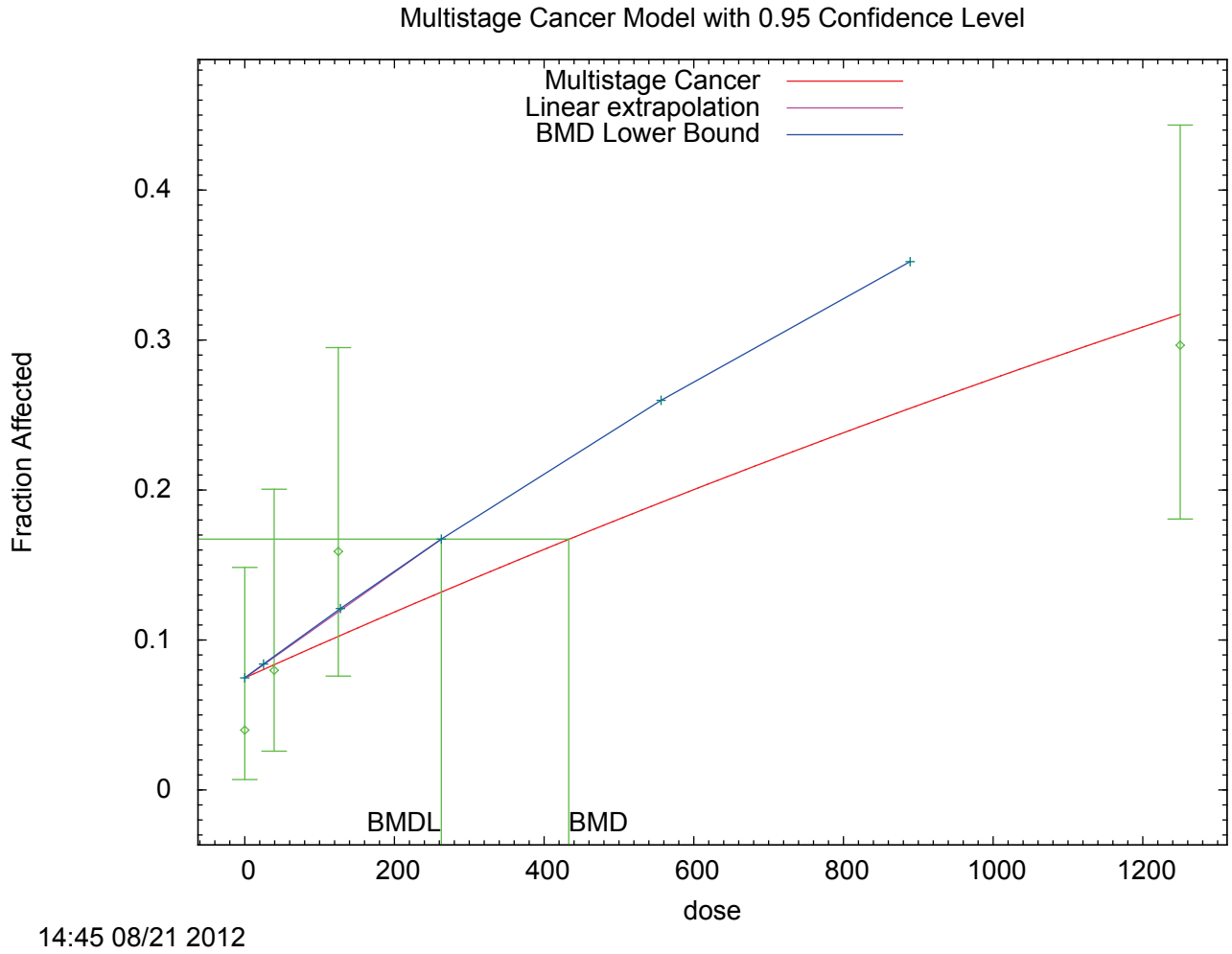
Run 150
Species: Mouse
Gender: Male
Organ: Hematopoietic
Response: any lymphoma
Study: NTP 1994 and Placke et al. 1996 Combined m = 2



Run 151
Species: Mouse
Gender: Male
Organ: Hematopoietic
Response: any lymphoma
Study: NTP 1994 and Placke et al. 1996 Combined m = 3

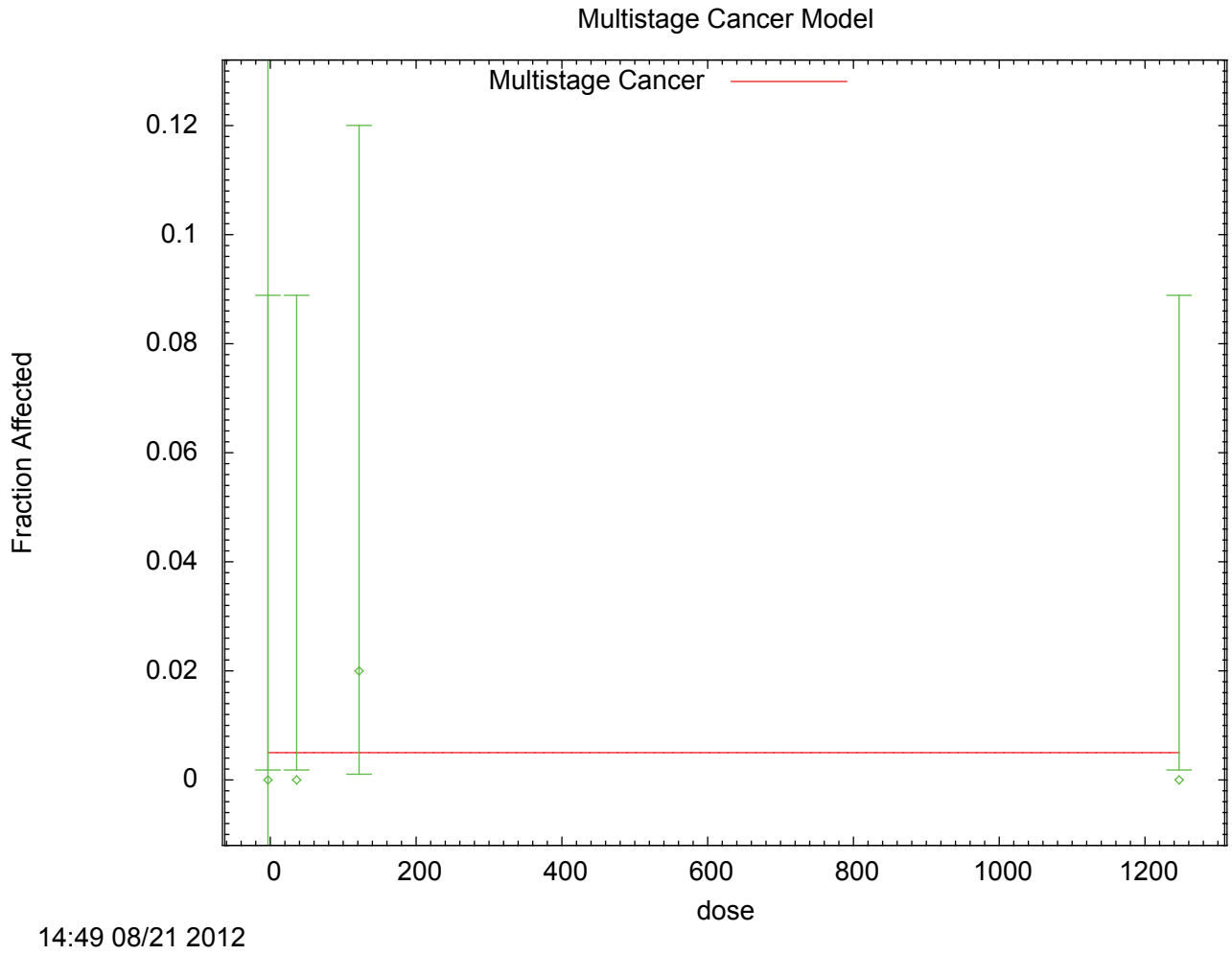


Run 152
Species: Rat
Gender: Male
Organ: Kidney
Response: Adenoma
Study: NTP 1999 m = 2

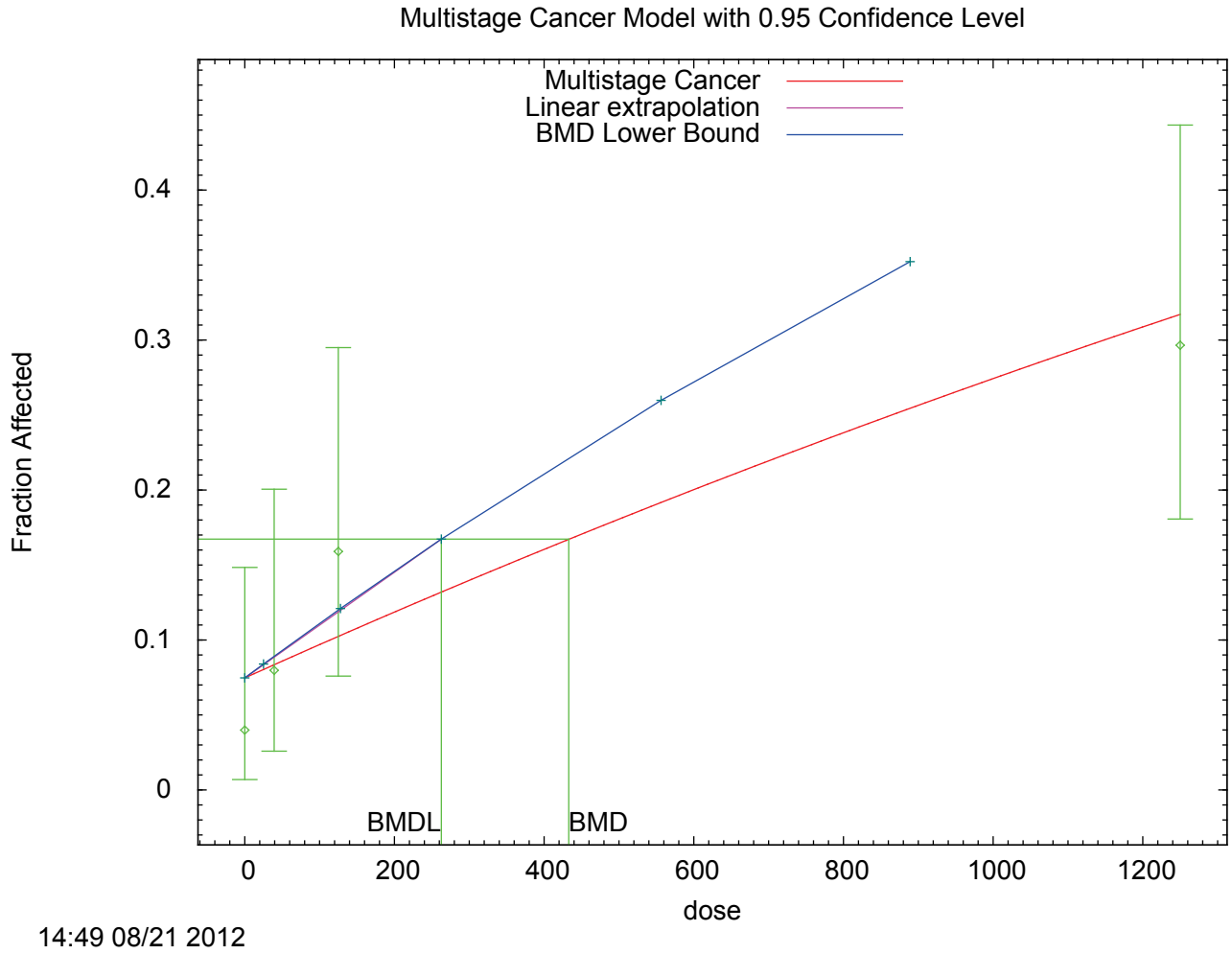


Run 153
Species: Rat
Gender: Male
Organ: Kidney
Response: Carcinoma
Study: NTP 1999 m = 2

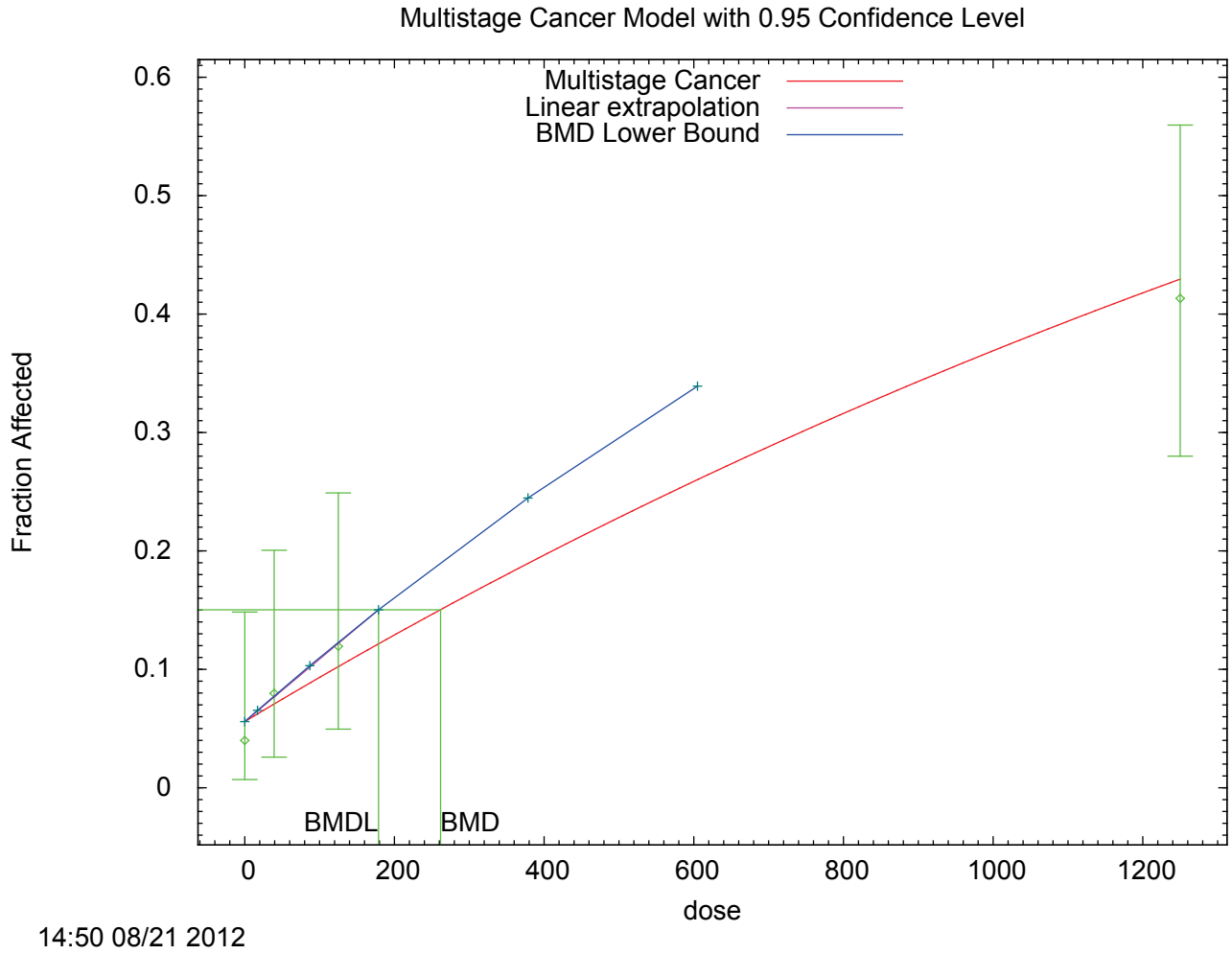
```
BMD computation failed. BMD is larger than three times maximum input doses.
```



Run 154
Species: Rat
Gender: Male
Organ: Kidney
Response: Adenoma/Carcinoma
Study: NTP 1999 m = 2

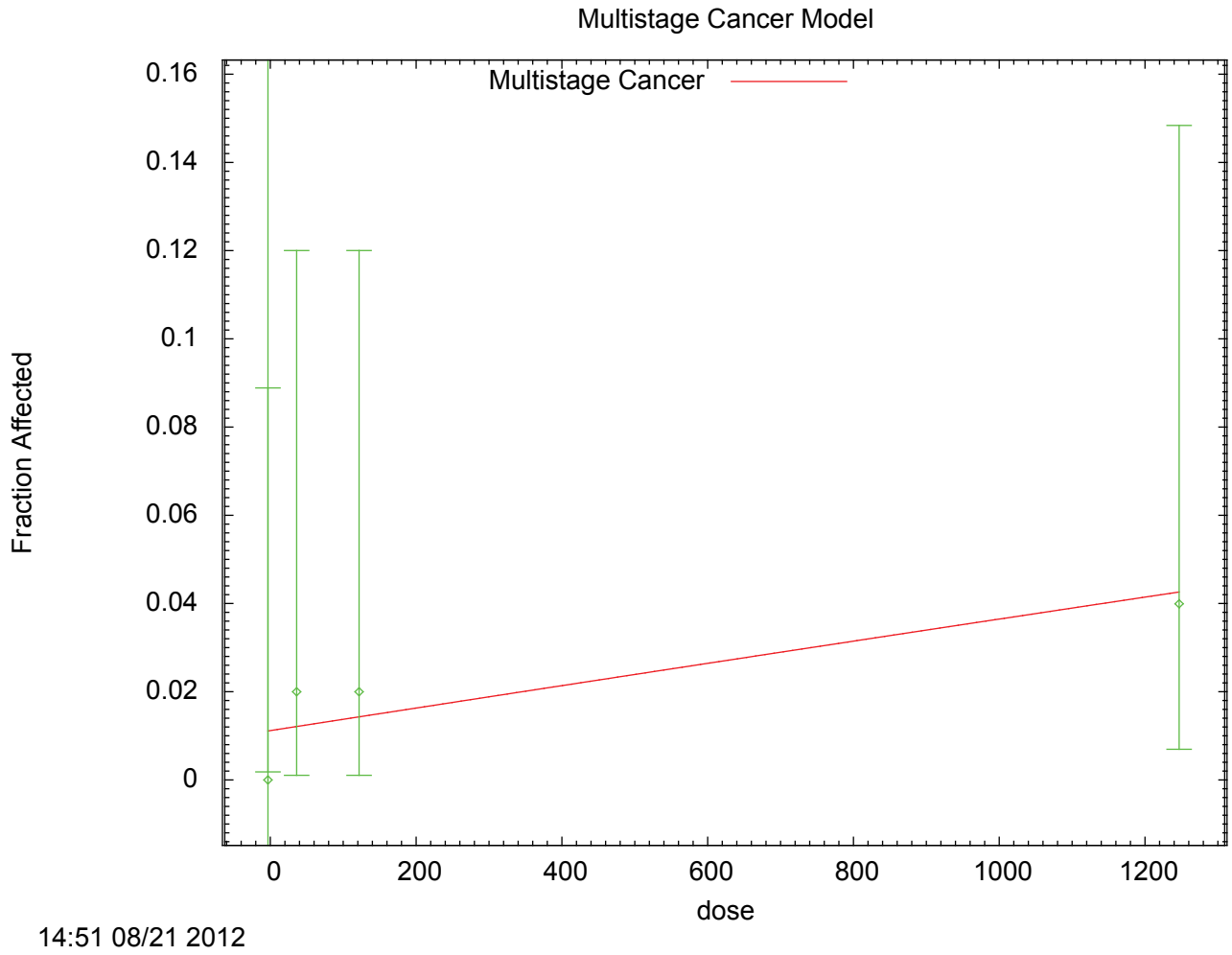


Run 155
Species: Rat
Gender: Male
Organ: Mammary gland
Response: Fibroadenoma
Study: NTP 1999 m = 2

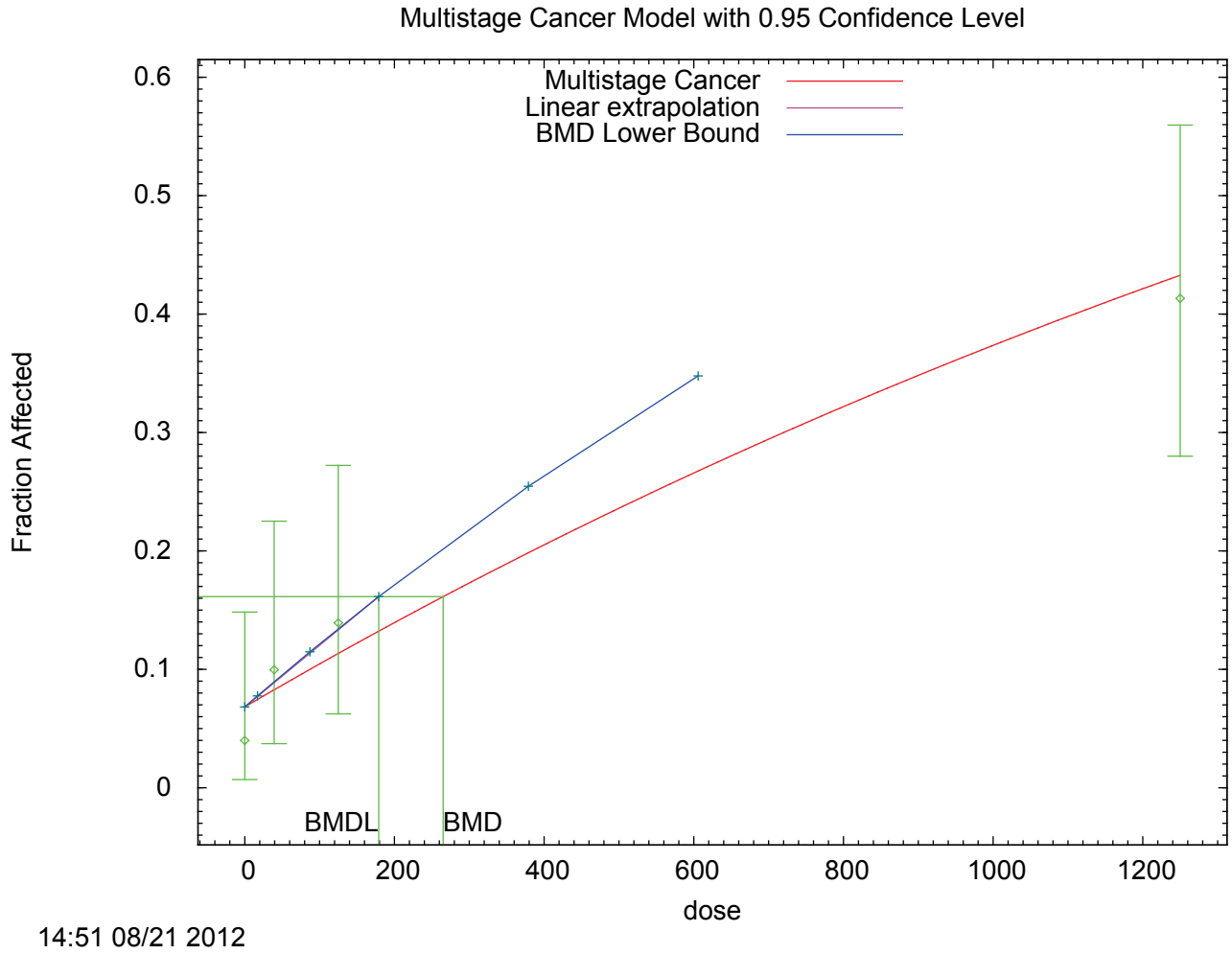


Run 156
Species: Rat
Gender: Male
Organ: Mammary gland
Response: Carcinoma
Study: NTP 1999 m = 2

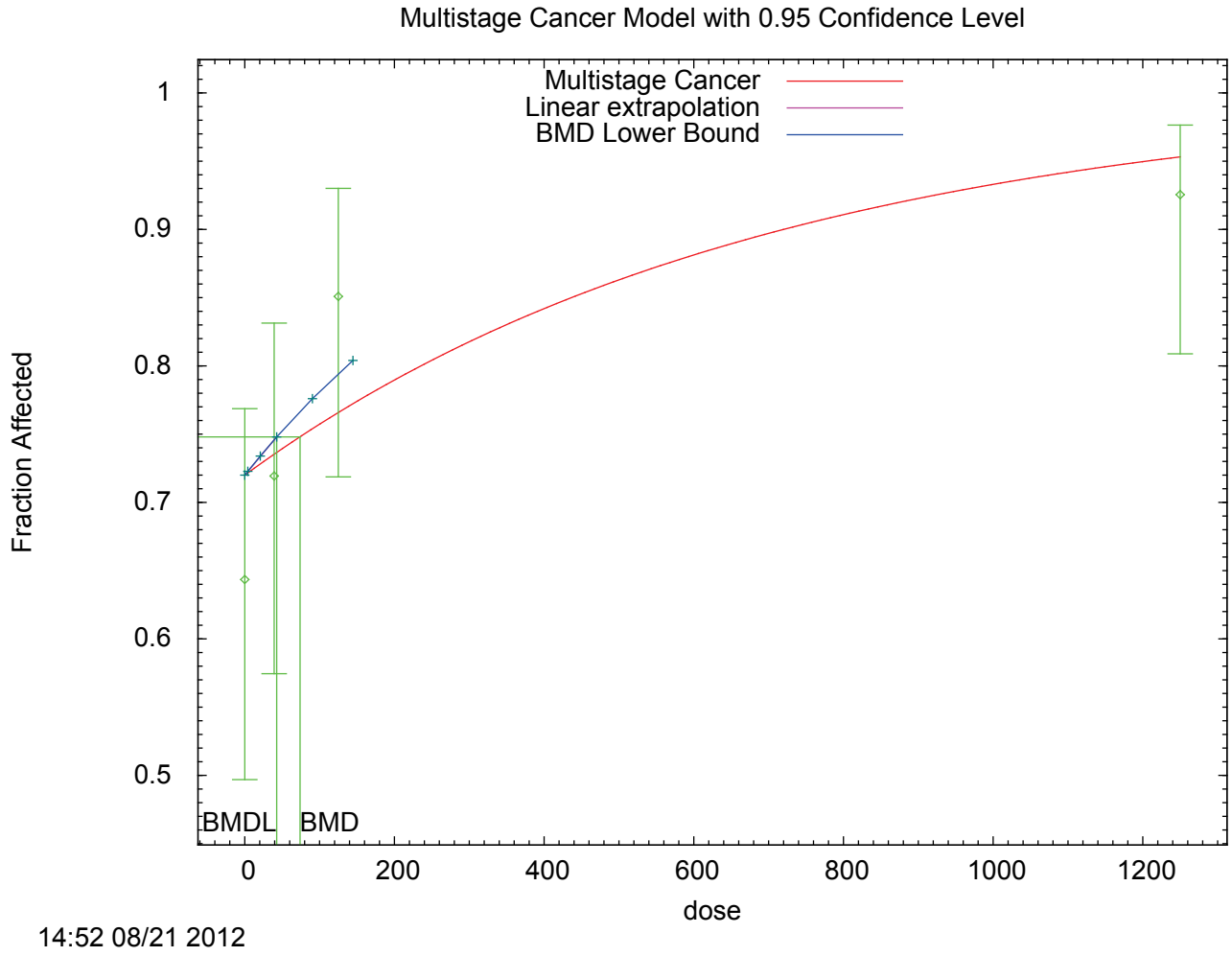
```
BMD computation failed. BMD is larger than three times maximum input doses.
```



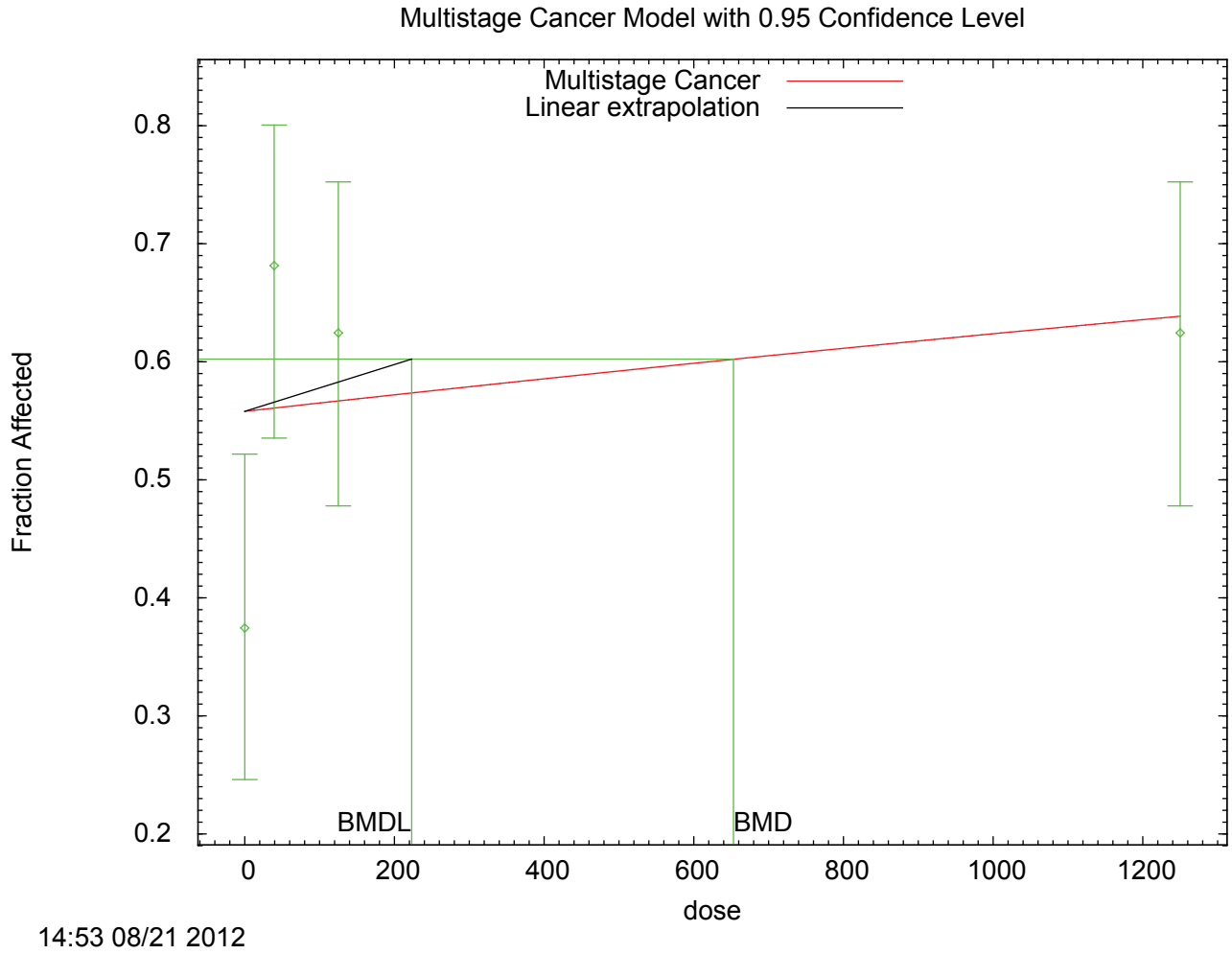
Run 157
Species: Rat
Gender: Male
Organ: Mammary gland
Response: Fibroadenoma/Carcinoma
Study: NTP 1999 m = 2



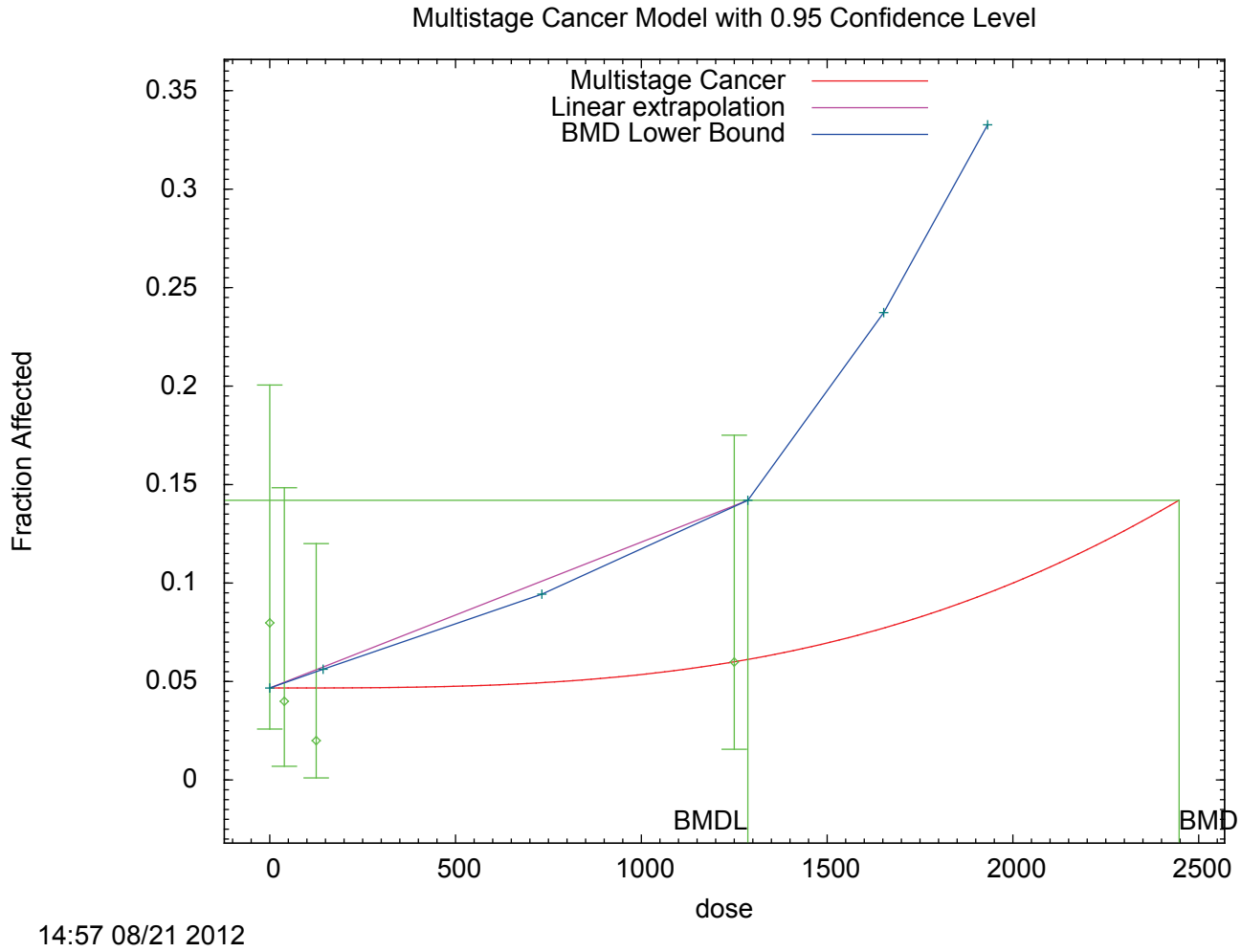
Run 158
Species: Rat
Gender: Male
Organ: Testis
Response: Adenoma
Study: NTP 1999 m = 2



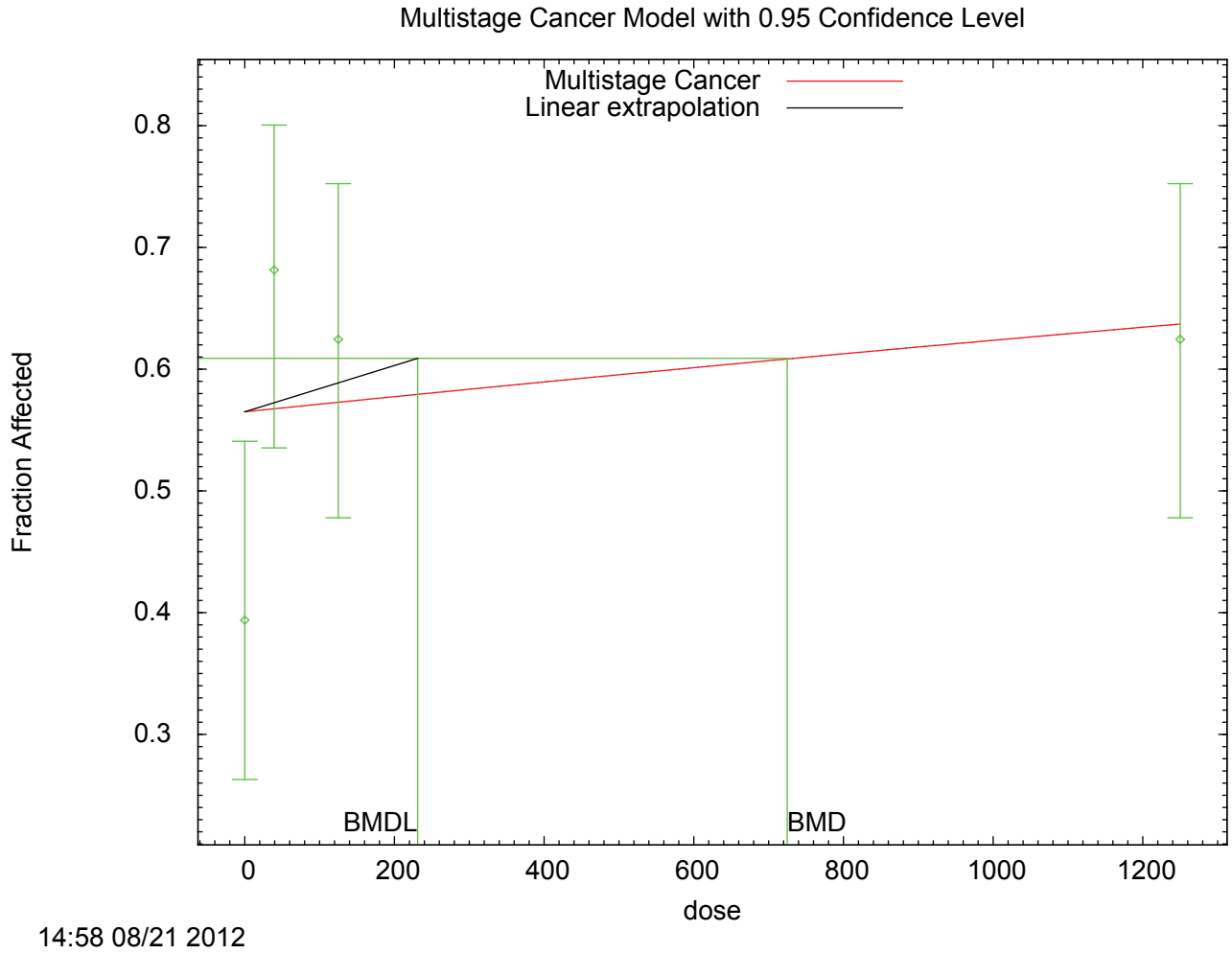
Run 159
Species: Rat
Gender: Female
Organ: Mammary gland
Response: Fibroadenoma
Study: NTP 1999 m = 2



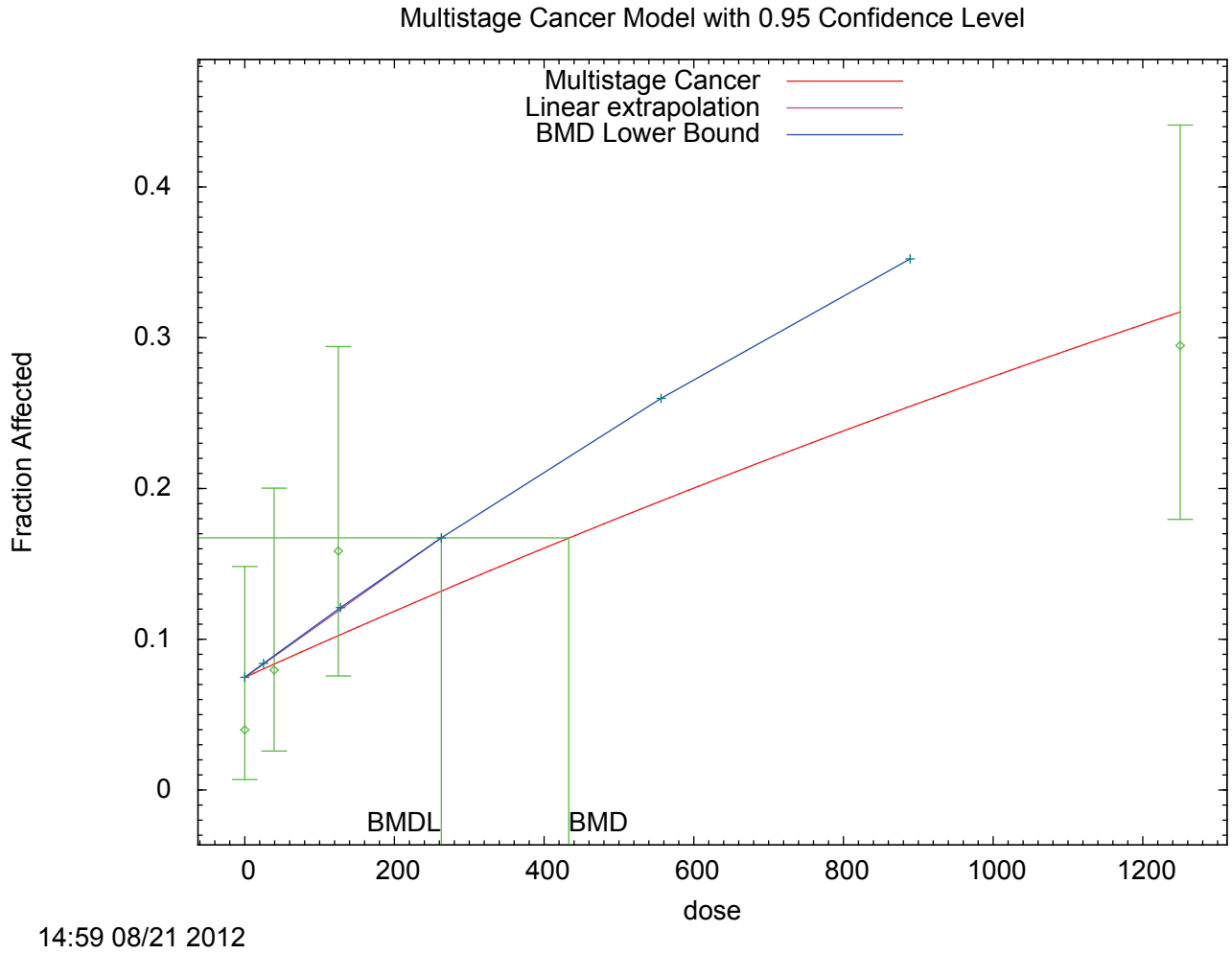
Run 160
Species: Rat
Gender: Female
Organ: Mammary gland
Response: Carcinoma
Study: NTP 1999 m = 2



Run 161
Species: Rat
Gender: Female
Organ: Mammary gland
Response: Fibroadenoma/Carcinoma
Study: NTP 1999 m = 2

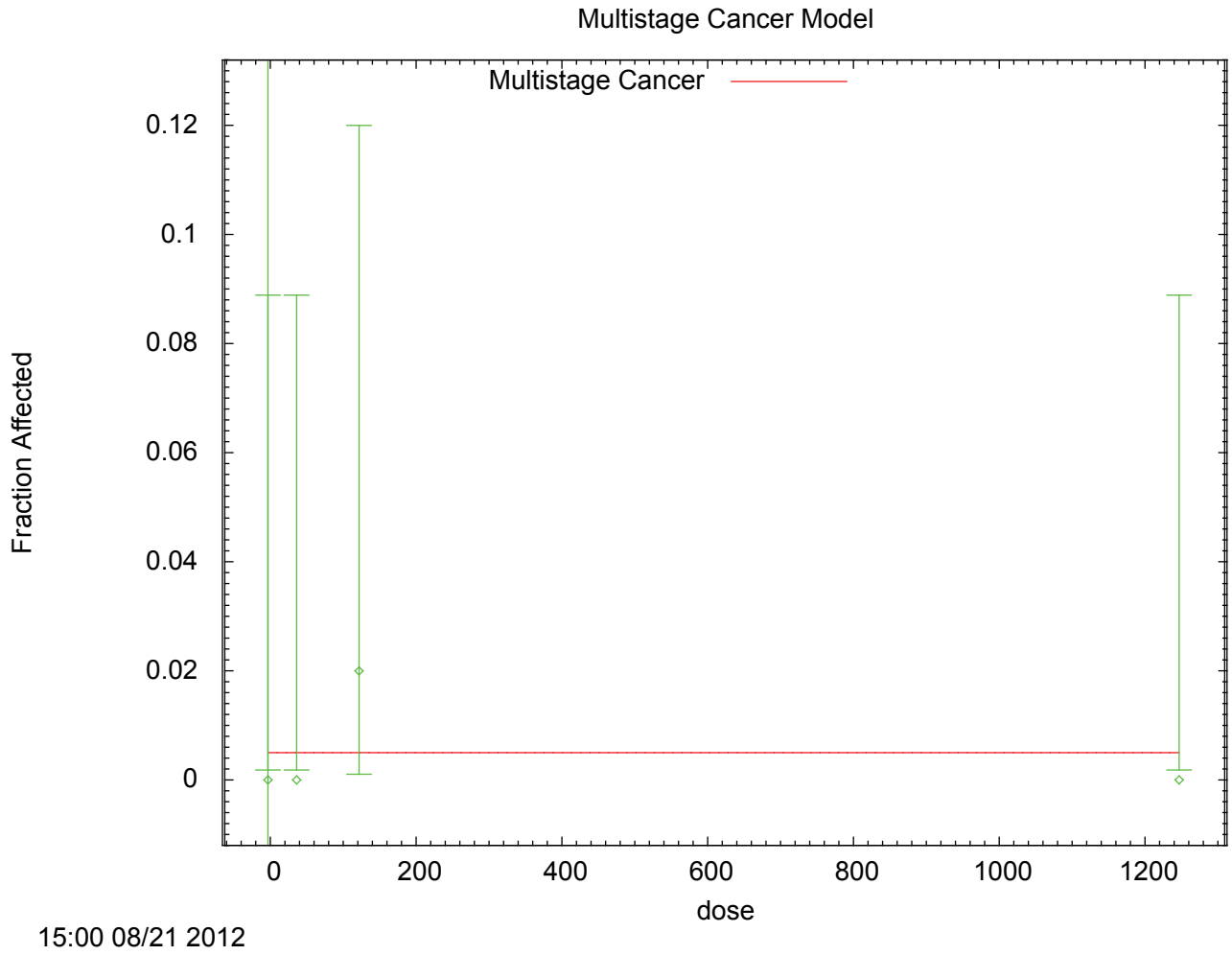


Run 162
Species: Rat
Gender: Male
Organ: Kidney
Response: Adenoma
Study: NTP 1999 m = 3

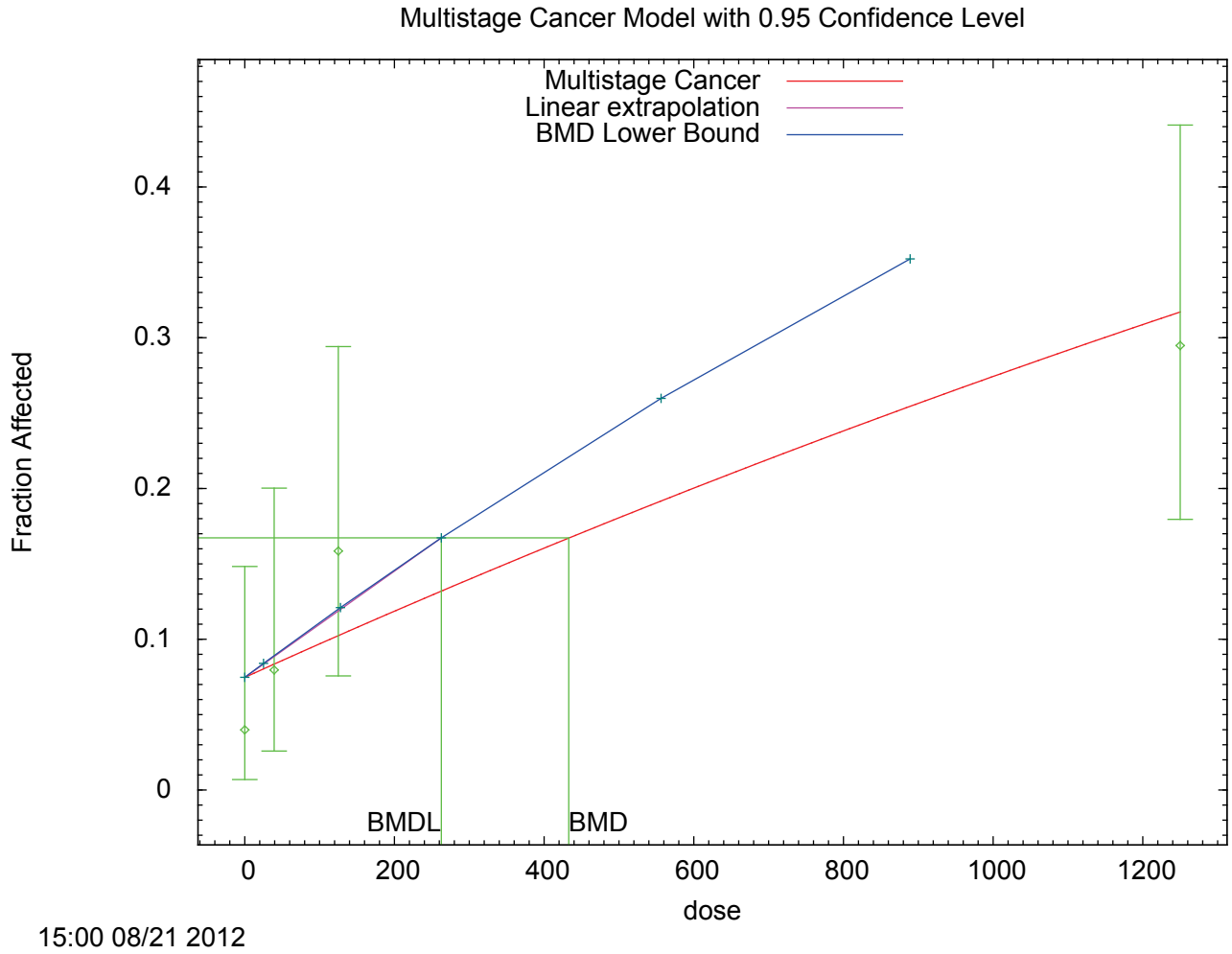


Run 163
Species: Rat
Gender: Male
Organ: Kidney
Response: Carcinoma
Study: NTP 1999 m = 3

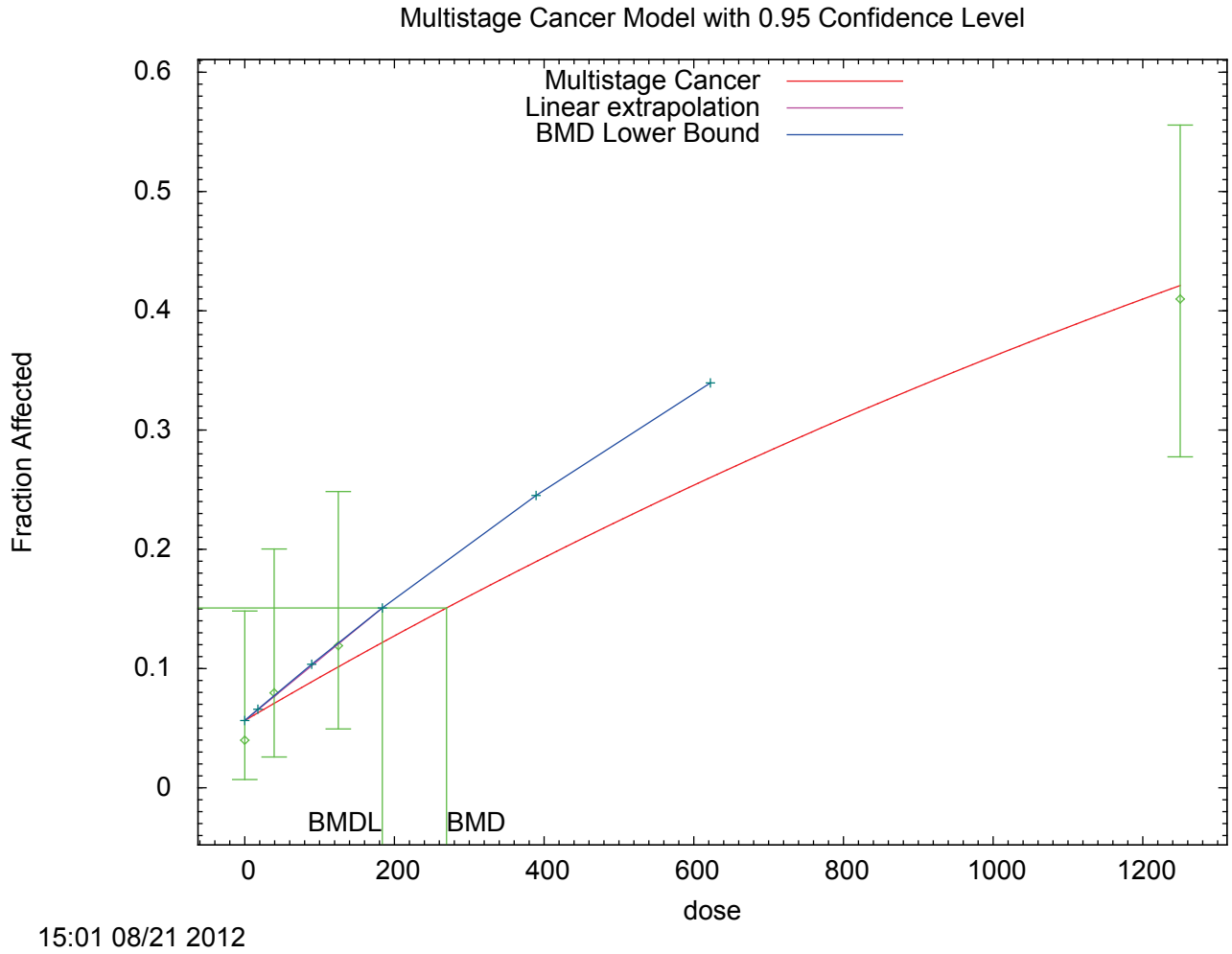
```
BMD computation failed. BMD is larger than three times maximum input doses.
```



Run 164
Species: Rat
Gender: Male
Organ: Kidney
Response: Adenoma/Carcinoma
Study: NTP 1999 m = 3

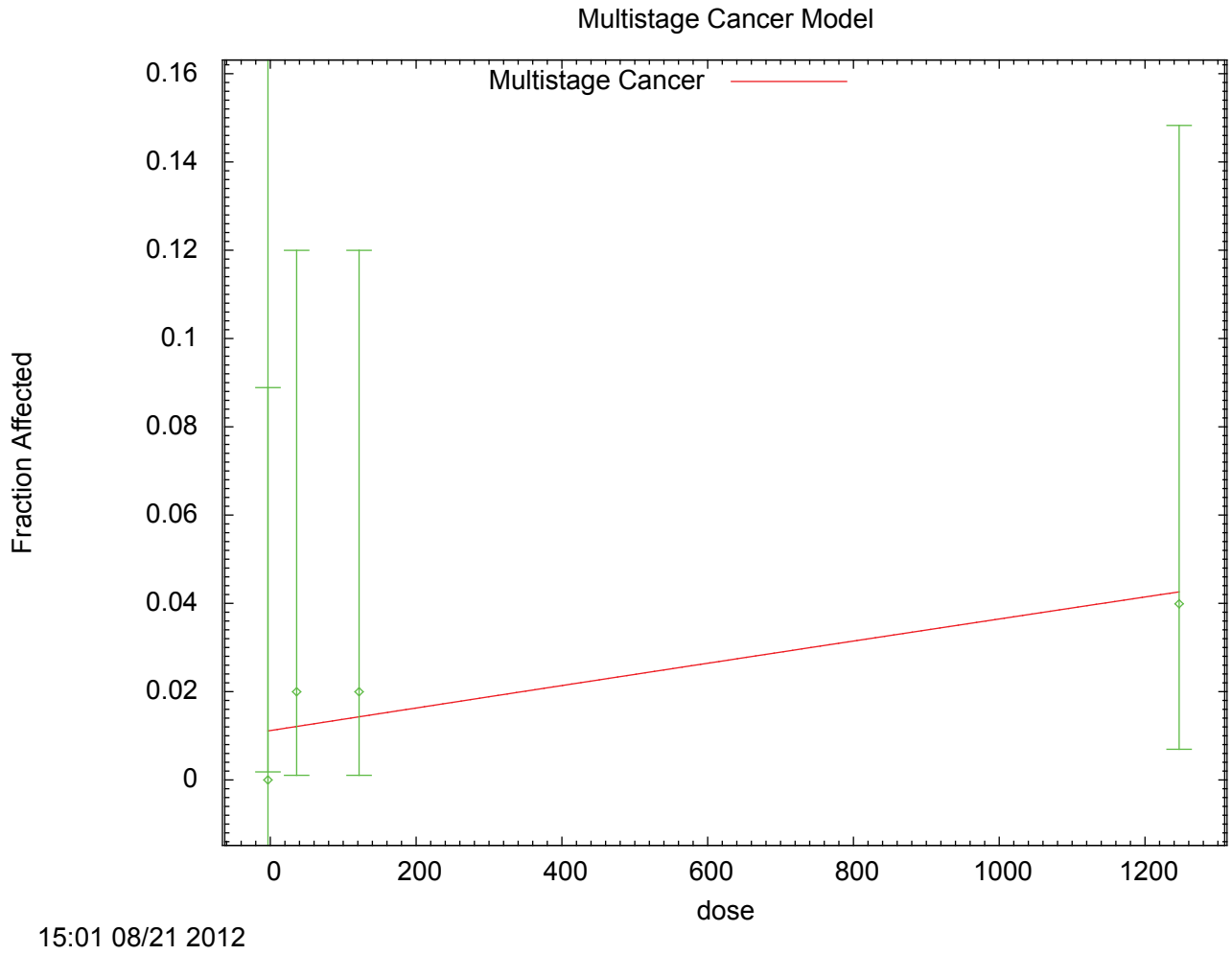


Run 165
Species: Rat
Gender: Male
Organ: Mammary gland
Response: Fibroadenoma
Study: NTP 1999 m = 3

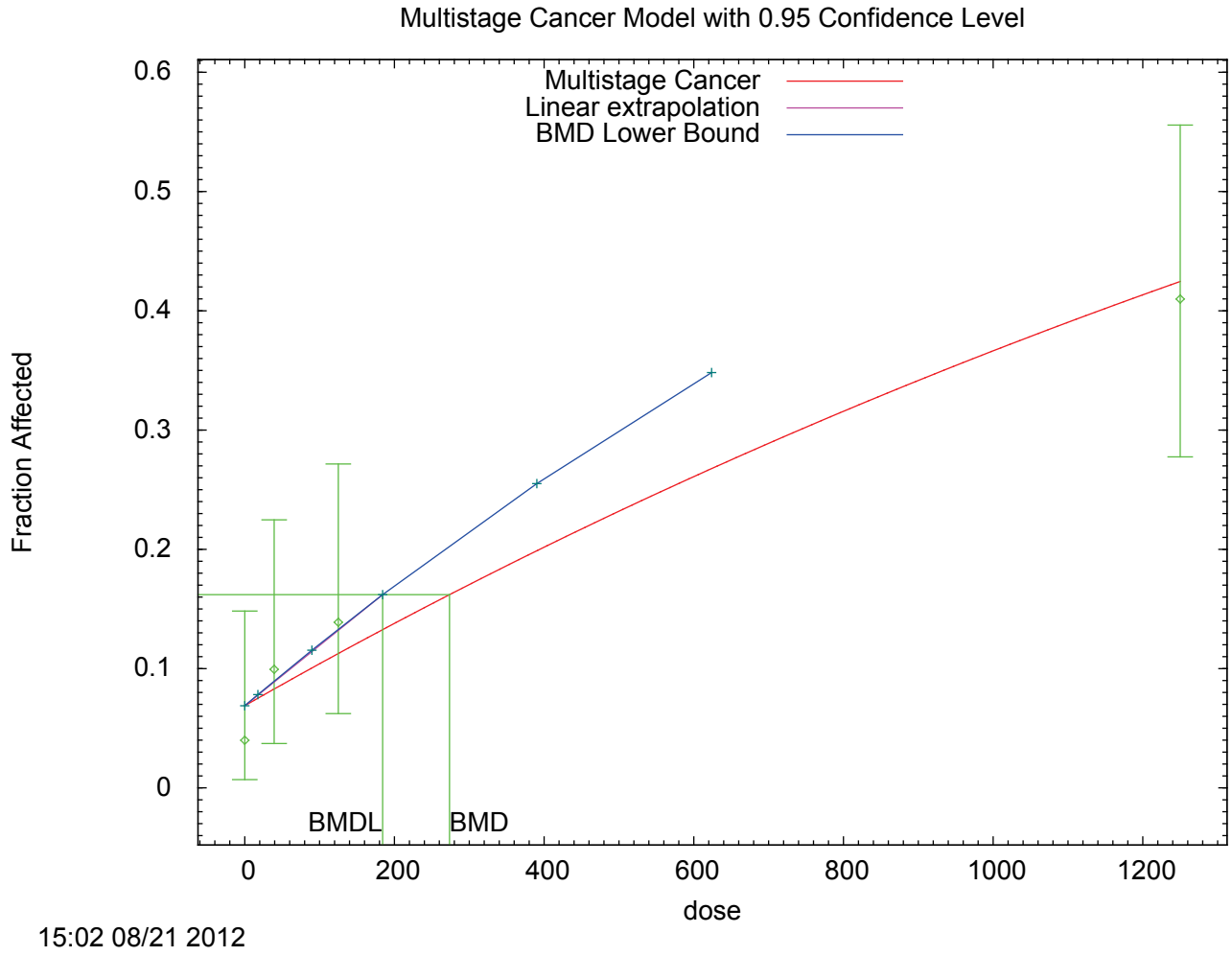


Run 166
Species: Rat
Gender: Male
Organ: Mammary gland
Response: Carcinoma
Study: NTP 1999 m = 3

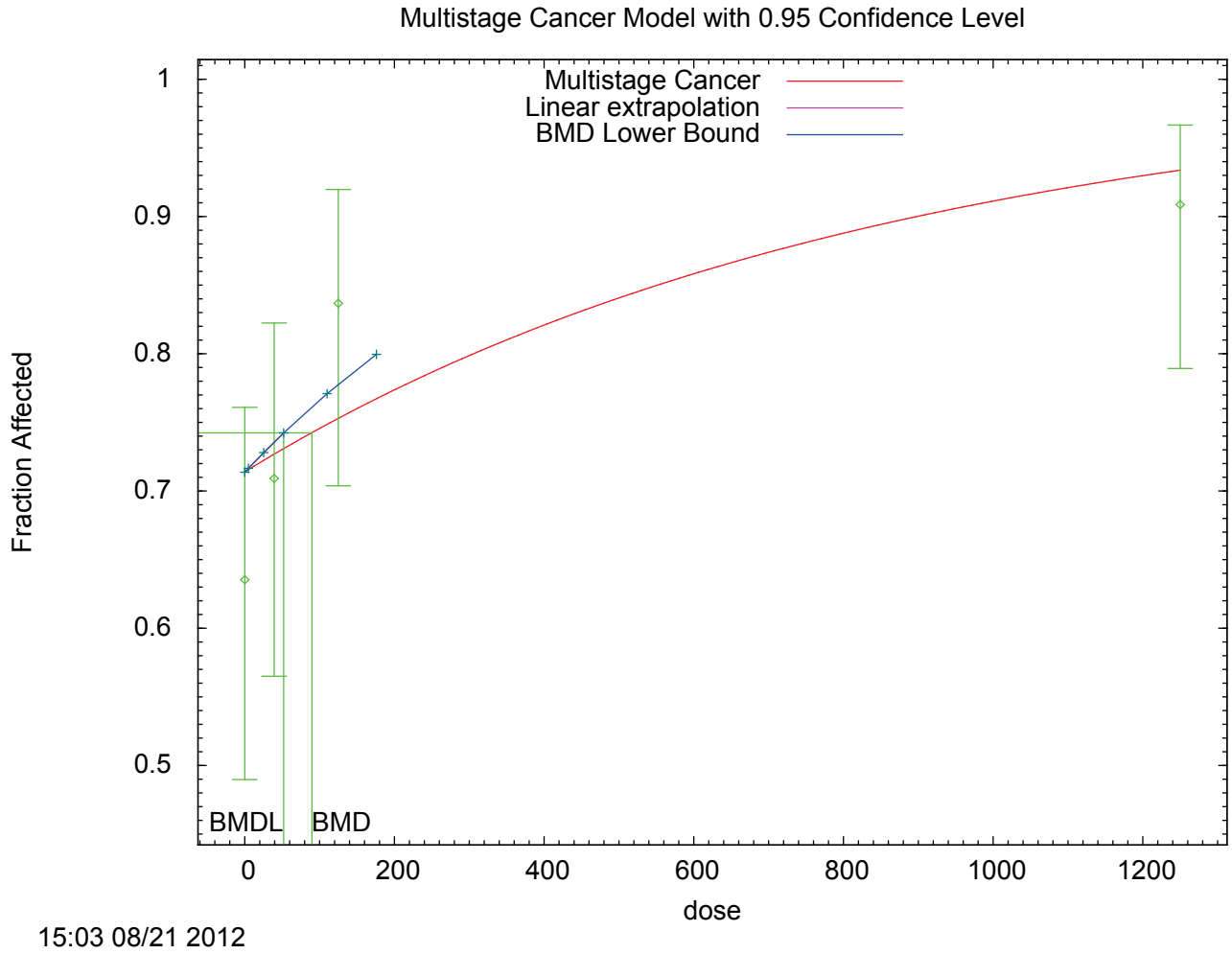
```
BMD computation failed. BMD is larger than three times maximum input doses.
```



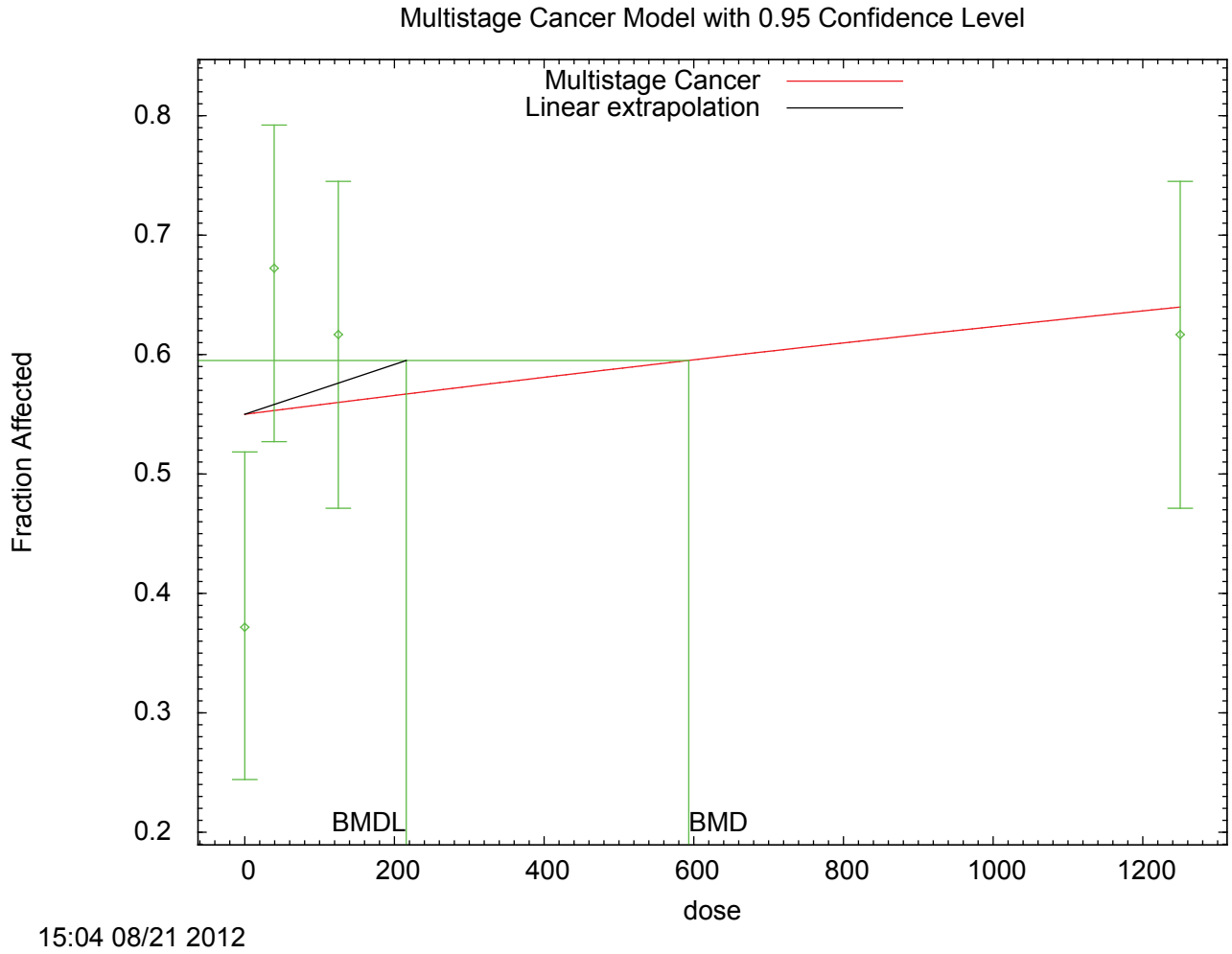
Run 167
Species: Rat
Gender: Male
Organ: Mammary gland
Response: Fibroadenoma/Carcinoma
Study: NTP 1999 m = 3



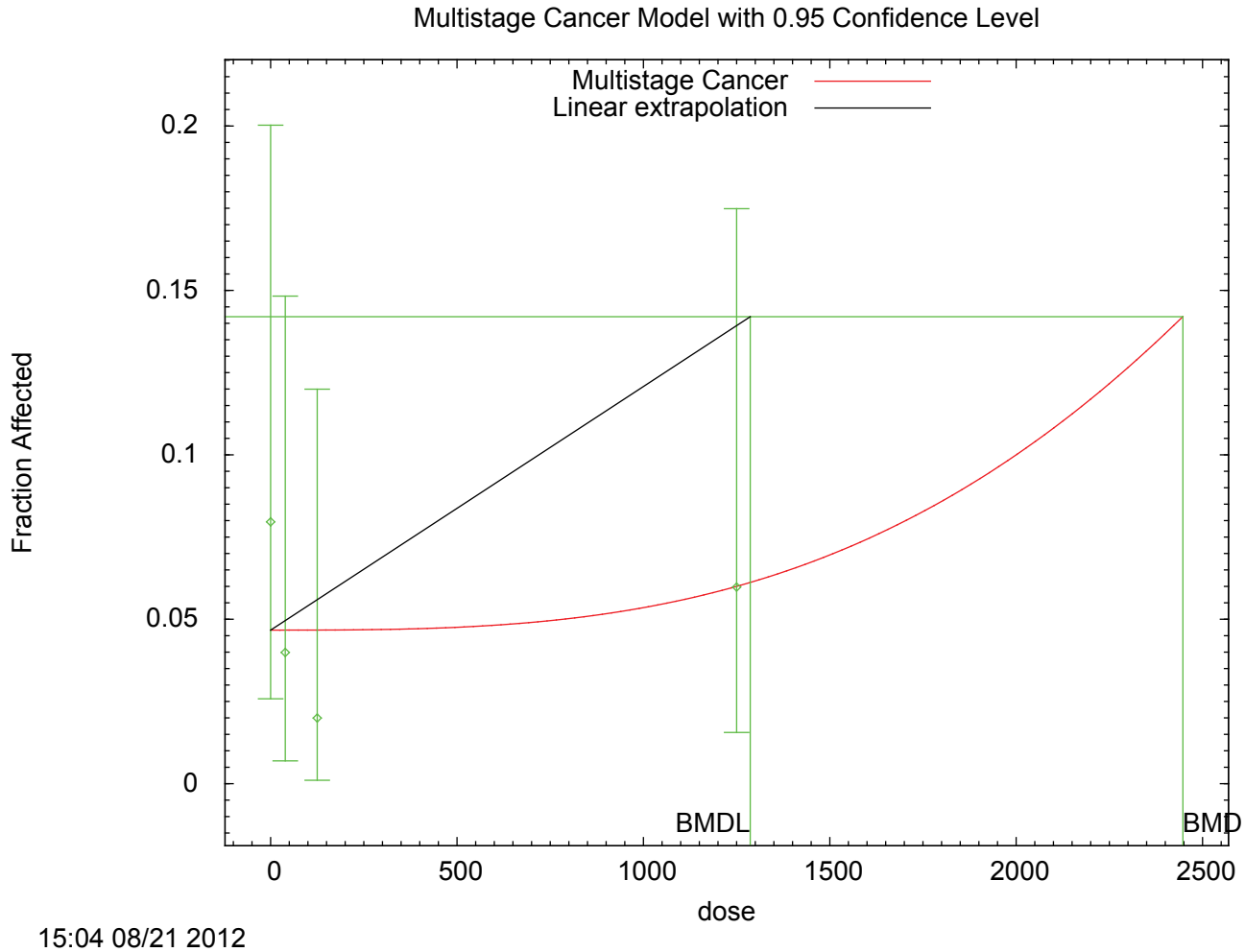
Run 168
Species: Rat
Gender: Male
Organ: Testis
Response: Adenoma
Study: NTP 1999 m = 3



Run 169
Species: Rat
Gender: Female
Organ: Mammary gland
Response: Fibroadenoma
Study: NTP 1999 m = 3



Run 170
Species: Rat
Gender: Female
Organ: Mammary gland
Response: Carcinoma
Study: NTP 1999 m = 3



Run 171
Species: Rat
Gender: Female
Organ: Mammary gland
Response: Fibroadenoma/Carcinoma
Study: NTP 1999 m = 3