

Meta-analysis of venom toxicity of 167 most lethal ophidian species provides a basis for estimating human lethal doses

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Research

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Abstract

Background: This is the first meta-analysis to characterize intra-ophidian-species variation in whole venom. The largest possible meta-analysis possible at this time, it encompasses all known records of animal lethality studies over the past 100 years. These results are not artifacts of resistant test-animal-species, and show orders of magnitude beyond the 1.6 logs (40 fold change) range of lethal dose documented in literature between amphibians, lizards and mice.

Methods: 1198 lethal dose study results for 167 of the most lethal venomous ophidian species in the world are analyzed.

Results: LDLo does not differentiate from LD50 across studies, indicating the true range of toxicity is probably larger. The belief that for route of inoculation, IC<IV<IP<IM<SC has good support ($R^2 = 0.90$). However, 5% of ICs were the highest dose, and 7% of SC inoculations were the lowest dose. Within the mouse test species, for one route of inoculation, the widest LD range is 3 logs (1000 fold change, $N = 14$). Within mouse, for multiple routes of inoculation, the widest LD range is 3.6 logs (4,150 fold change), $N = 20$, SC/IM. The strongest correlate for range of lethal dose results is the number of studies ($R^2 = 0.56$); followed by the number of test-animal-species ($R^2 = 0.55$); then by the number of routes of inoculation ($R^2 = 0.43$).

Conclusions: Scientists working with humans should use combined LDLo and LD50 meta-datasets for all data and calculate: mean, median, minimum, range, and standard deviations. Standard deviation multiples will provide desired coverage. For estimating LD50 range and minimum lethal dose for species with little data, I recommend curating a meta-dataset of related snakes, and computational research to strengthen this.

Introduction

Human lethal dose-response to ophidian venom is a thorny problem, as it is impossible to perform ethical lethal dose studies on humans[1]. This forces the use of animal studies, despite the problem that human dose-response may be different than other animals, including monkeys. When LD50 for a different species is used to guess at the human lethal dose, this is typically calculated by choosing a study and multiplying a value by the mass of a typical human. This procedure is based on common assumptions that will be discussed below. Anecdotally, scientists and physicians have a bias toward more recent studies.

When selecting a study, some variation is expected between test species for the same ophidian species, but not a great deal within the same test species.

A common assumption is that lethal dose toxicity varies by method of inoculation from most toxic to least toxic as: intracerebral (IC), intravenous (IV), intraperitoneal (IP), intramuscular (IM) and subcutaneous (SC). Regarding inoculation methods, there is also a view that comparison of results between inoculation methods varies radically, like “apples and rocks”[2], but no formal meta-analysis backs up either assumption. These assumptions imply that as long as the route of inoculation is constant within the same test species, then toxicity reports across studies should be reasonably close, which makes selection of a study to use fairly arbitrary.

Every scientist in the field whom I asked (data not shown), believed that the LDLo value should be lower than LD50, and that this should hold across studies. This assumption regarding LDLo is also implicit in the publication of LDLo values at all, because if they were not meaningful then they would not be defined as something worthy to capture and record. By definition, LDLo should be lower than or equal to LD50.

Variation in venom and prey (test animal) susceptibility

There has been literature suggesting the common assumptions of lethal dose should be reconsidered for some time, with recent authors documenting variation in venom components. Variation of venom by season, and between related species and subspecies has been a consideration for antivenom preparation [3–6]. Geographic area and predator-prey evolutionary relationships are another factor in venom variation [7–15]. In addition to geographic differences, ontogeny can affect venom component variance [6, 16], which is presumed to be an adaptation to differing prey species during development. Differences may also be linked to the sex of the ophidian[17].

Quite recently, some degree of plasticity of venom in *S. m. barbouri* in response to prey species was documented, which should create some intra-test-species modification to LD50 [18]. It was also shown that *C. simus* modifies its venom with miRNA to achieve small variation based on prey species, which implies some unquantified degree of intra-test-species variation in LD50 for this ophidian species [19]. And recently, intraspecific variation in rattlesnake venom neurotoxin was identified as significant within a geographic area, ranging from near zero neurotoxin, to a large fraction. This implies an unquantified degree of intra-test-species LD50 variation by an unspecified mechanism [20, 21].

Generally, as long as the test animals are not species that have adaptations to venom such as the western ground squirrel (*Spermophilus beecheyi*)[22], some opossums (*Didelphidae*), hedgehogs (*Erinaceidae*), mongooses (*Herpestidae*), weasels (*Mustelidae*), some skunks (*Mephitidae*)[23], or the honey-badger (*Mellivora capensis*)[24], then test species is not documented to make major a difference. Except for the ground squirrel, these are predator species that prey on venomous snakes.

Among prey species, literature shows that lizards probably require roughly 4X the venom dose that mice do as demonstrated by the relative dose delivered by *C. concolor* [25]. Frogs are an order of magnitude (10X) more resistant to *Sistrurus* venom than lizards, while in mice, the variation in toxicity of *Sistrurus* venoms correlates with mammals in the snake's diet [26]. (Mouse variance was unspecified, but assumed less than an order of magnitude since it was not specified in the same paper discussing frog resistance.)

Multiplying the single order of magnitude of frogs by the 4X of lizards gives us a total 40 fold change currently documented between test-animal-species, which is 1.6 logs. Thus, differences between test-animal-species are expected, but based on current literature, with the exception of frogs, these differences should be less than one order of magnitude.

Characterization Of Data

This meta-analysis includes every known study for the top 264 venomous ophidian species winnowed to the 167 species with 2 or more studies. These data were primarily extracted from the Steinhoff database, and secondarily from the Drugfuture database[27, 28]. The data for this meta-analysis had several factors that could be examined: Ophidian species, test-animal-species, and route of inoculation.

Curation

The dataset was extracted in May of 2017 and curated. Curation included identification of each reference and bringing it into the table, as more than half of the Steinhoff database entries were pointers to the Drugfuture database which contained the literature reference. Apparent duplicates were removed. Of these 264 venomous ophidian species, 167 species with 2 or more DB entries were accepted, for a total of 1198 lethal dose (LD) DB entries

Of those 167 species records, 94 ophidian species had more than one DB entry for the same inoculation route in the same test-animal-species. There are 17 test species in the dataset plus 2 that are generic “species,” mammal and bird, for a total of 19 test-animal-species categories. Most of the data is either mouse (70%) or rat (5%).

The maximum number of test-animal-species for one ophidian species is 12. Test-animal-species is sometimes a loose term in this context, as there are many species of monkey, duck or frog, and there might be some venom component sensitivity variances. However, no literature indicates variances of more than 1 order of magnitude between test-animal-species, and one would not expect exotic monkeys, ducks or frogs to be tested. For part of this analysis, “mammal” and “bird” are treated as separate species. In most cases the designation of mammal would be a rat or a mouse. And most birds would be pigeons or chickens. As will be seen, using only mouse data did not change results.

These studies were mostly performed in the 20th century, with the peak from 1975 to 1979 (Fig. 1). This suggests that venom science may not believe that further studies are necessary or useful. As will be seen, this does not appear to be the case.

Methods

The analysis had several questions. First, does ophidian venom variance fall within the literature documented parameter of 1.6 logs (40X fold change) between mice and frogs test-animal-species? Second, to what extent did the assumption that $IC < IV < IP < IM < SC$ hold? Third, what correlates are there to dose range, and how much of a contribution could be assigned to each factor?

For this analysis, null hypotheses conform to what is available in published literature. To make data comparable between ophidian species, normalization was performed based on the range of values present. These normalization steps created fractions of the total range, or the fold change of the highest value over the lowest value.

Null hypotheses

The minimum lethal dose for single ophidian species has a factor of 40 or less fold change variation (log of 1.6). This statement captures the idea that across test-animal-species and inoculation methods, while there is variance, it should not exceed what has been reported in literature. (e.g. 4X and 10X, for a total of 40X)

Within a single test-animal-species for a single ophidian species, there is a factor of 2 or less range of lethal doses between studies (log approximately 0.3).

LDLo values cluster below LD50 values such that the mean of the respective fractions of the range differ from each other by more than one standard deviation, or, barring this, by standard error.

LDLo values will not be maximum lethal doses reported across studies.

The lethal dose varies by route of inoculation such that $IC < IV < IP < IM < SC$ for 90% or more of venomous ophidian species across studies.

Primary views of data

This meta-analysis examined 3 primary views of the data with subset views: 1.) Minimum lethal dose (LDmin) and maximum lethal dose (LDmax) for each ophidian species; 2.) The range fold changes for: all data (LD-ADrf), single test species (LD-SSrf), single test species – single route of administration (LD-SRrf), for each ophidian species; 3.) The

fraction of the range within one ophidian species that each DB entry represented, expressed as a percentage, explained below.

Range fold change is $R_{max} \div R_{min}$ where R_{max} is the highest LD for the species, and R_{min} is the lowest LD for that ophidian species. (LD can be LDLo or LD50.)

LDmin is the lowest lethal dose reported for an ophidian species. LDmin can be either an LD50 or an LDLo. LDmax is the highest lethal dose reported across ophidian species, and similarly, can be either an LD50 or an LDLo.

The lethal dose range fold change is the LDmax divided by the LDmin ($LD_{max} \div LD_{min}$) for an ophidian species. The fraction of the range for an entry is $LD_{entry} \div LD_{range}$, where $LD_{range} = LD_{max} - LD_{min}$.

There are three LD range fold changes: All data (LD-ADrf), single species (LD-SSrf), and single species, single route (LD-SRrf). LD-ADrf means DB entries for all test-animal-species were used. LD-SSrf means that the widest range fold change within one test-animal-species for an ophidian species was used. LD-SRrf means that the widest range fold change within one test-animal-species that was administered by the same route was used. (e.g. use the highest and lowest LD for IC, IV, IP, IM, and SC, and take the largest range multiple for one of the routes of inoculation.)

After curation, the data was sorted by species and then by lethal dose. It was further segregated by species family, finding 1 Atractaspid, 1 Homalopsid, 8 Colubrids, 69 Elapids, and 88 Viperids. Having only 8 Colubrid species in the dataset made those results questionable to indicate a significant difference, although it may exist. (Data not shown.) The LD-SRrf difference between Elapids and Viperids did not appear to be significant. (Not shown.)

The coefficient of determination, R^2 , is the primary measure of significance used in this meta-analysis. Best fit regressions were exponential curves of the form $y = C \cdot x^m$, with one exception, which was a linear fit.

Analysis

LDLo does not differentiate from LD50 across studies.

64 out of 167 ophidian species had at least one LDLo value. These 64 ophidian species had 243 LDLo values, and 532 LD50 values. In the meta-dataset, 26 of these 64 ophidian species (~ 40%) had an LDmin that was, indeed, an LDLo. However, 24 of the 64 ophidian species (~ 37.5%) had LDLo values that were the meta-study LDmax, which was against expectations.

$$\frac{\overline{LD}}{R_{max} - R_{min}} \quad (1)$$

Where: \overline{LD} is the mean comprised of the LDLo or LD50 values within one ophidian species.

For Figs. 2 and 3, the range fraction is determined by Eq. 1. This way, relative toxicity between ophidian species is normalized, and comparison of variation can be done between ophidian species. In Fig. 2, the mean of the LDLo fractions is 28.3%, and the mean of the LD50 fractions is 27.2%. The standard deviation of the LDLo fractions mean is 29.4%, and standard error is 3.7%. The standard deviation of the LD50 fractions mean is 22.4%, and standard error is 2.8%. Even by the less stringent method of standard error, this is insignificant. Thus, the mean of the LDLo and LD50 range fractions for the 64 out of 167 are not meaningfully differentiated.

However, one might argue that significant difference might be seen in LDLo values for single test-animal-species, as shown in Fig. 3. Here, as above, the data does not show this. Instead it shows that for 4 out of 11 test-animal-species,

mean LDLo is higher than LD50, and exceeds standard error. (Mouse, guinea pig, dog, frog. Monkey was excluded due to low N for LD50.) Note that this also occurs for the highest N dataset (mouse). There is only 1 test-animal-species (rabbit) where mean LD50 is higher than LDLo and exceeds standard error as expected. However, rabbit has a low N and this disappears when median is used. (Standard error not shown for median.) Only the mouse test-animal-species exceeds standard error if median is used, and it still shows LDLo higher than LD50.

Strengthening this point, for 10 ophidian species, both LDmin and LDmax were LDLo's. The median number of DB entries for an ophidian species with one or more LDLo values was 4.

Consequently, because LDLo did not differentiate significantly from LD50 when examined across studies, LDLo designations were categorized together with LD50 for the rest of this meta-analysis.

Route of inoculation minimum and maximum lethal dose

In Fig. 4, there is good support for the concept that IC (intra-cerebral) < IV (intra-venous) < IP (intra-peritoneal) < IM (intra-muscular) < SC (subcutaneous). This is the only hypothesis not falsified in this analysis. However there are contradictory instances.

Out of 29 intracerebral injections (IC), 16 were LDmin values, which is as expected. So, approximately half the time, an IC injection was the minimum, and the N should be meaningful at 29. Using a synthetic x-axis the 0.9062 R² coefficient of determination suggests that approximately 91% of the distribution fits the assumption that route of inoculation varies as IC < IV < IP < IM < SC. The curve fit for LDmax shows the opposite trend, with a good R² suggesting 75% of results can be attributed to route of inoculation distributed in this manner. This latter R² being lower agrees with LDmax having higher variance (not shown).

However, 13 out of 249 of the subcutaneous injections were LDmin values, which makes this, unexpectedly the route of highest toxicity for 7.8% of the 167 ophidian species. Out of these 13, there were 4 venoms with strong hemotoxic or nephrotoxic effects, the other 9 were neurotoxic.

Of the intracerebral inoculations, 2 were LDmax, the opposite of expectations, (*Notechis scutatus* and *Naja atra*), which is 1.2% of the 167 ophidian species. *Notechis scutatus* and *Naja atra* contain both pre and post synaptic neurotoxins. *Notechis scutatus* had 26 DB entries and *Naja atra* had 17, so this should probably not be an artifact of a low number of studies performed for each. The percentages of these paradoxical SC and IC inverted cases are about the same, at 7% and 5% of their respective route of inoculation.

Venom toxicity range fold change

The range of venom toxicity per ophidian species within the mouse test species has a mean average of 2.22 logs (168 fold change) within a single test-animal-species, as shown in Fig. 5. This is 4.2 times the 1.6 logs (40 fold change) documented in literature for toxicity difference between test-animal-species, as discussed above. For all test-animal-species together, the mean range is 3.2 logs (1597 fold change), which is 40 times what current literature indicates.

The largest range fold change seen for an ophidian species tested in mouse for one route of inoculation is *Boiga irregularis*, 3 logs (1000 fold change), N(studies) = 14. The largest range fold change for an ophidian species tested only in mouse for all routes of inoculation is *Crotalus horridus*, 3.6 logs (4,150 fold change), N(studies) = 20, routes of inoculation: SC/IM. For one ophidian species data for all test-animal-species, including all routes of inoculation, the largest range fold change is *Naja nivea*, 4.46 logs (28,571 fold change), N (studies) = 22, N (test-animal-species) = 9, routes of inoculation: SC/IV between frog (LDmax) and rabbit (LDmin). These are among the highest N studies counts

for ophidian species. Note that a specific ophidian species mentioned does not mean this species has been determined to be the most venomous, or the widest range of all.

One might ask whether the range fold change increasing holds up when a single species and single route of inoculation is examined. We see this in Fig. 6, where the range fold change is plotted against the number of studies. No curve fit is shown because there is insufficient data for determination. However, by inspection, one can see that the range fold change does appear to increase as the number of different studies rises.

Figure 7, which looks at single test species for multiple routes of inoculation, shows a linear regression trend that reaches significance for the range fold change increasing as the N for number of studies gets larger. This graph appears to signal the same thing that a set of ecological diversity transects continuing to increase would. It indicates that to fully characterize ophidian venom lethal doses, probably requires more than 50 different studies.

Regressions of LDmin

Null hypotheses for minimum lethal dose

A.) Minimum lethal dose within each ophidian species varies by less than a factor of 2. B.) The minimum lethal dose does not correlate with the number of times the lethal dose has been tested (e.g. the number of LD DB entries).

Alternate hypothesis

Minimum lethal dose varies by more than a factor of 2, and minimum lethal dose correlates with the number of times dose has been tested.

Table 1
Regressions curve fit summary for LDmin, rounded.

Correlation examined	R ²
Number of routes of inoculation	0.30
Number of test species tested	0.31
Number of LD DB entries (Number of studies)	0.36

Number of routes of inoculation correlation to LDmin

In Fig. 8, as the number of routes of inoculation increases, the likelihood of having more test-animal-species for the ophidian species also increases. The fitted curve is probably determined by the probability of inclusion of a lower lethal dose value rising as the number of inoculation routes goes to 5, because, as was seen above, IC < IV < IP < IM < SC does tend to hold true.

The N for the number of routes of inoculation 1 to 5 are, respectively: 30, 36, 61, 33, and 7.

Number of test-animal-species per ophidian species correlation to LDmin

Here in Fig. 9 the apparent drop visible in the fitted curve is 1.5 logs, a fold change of 32X. Similarly to the above, it should be expected that lethal dose would drop some with larger numbers of test-animal-species, because literature

indicates that some animals are up to 1.6 logs (fold change of 40X) more susceptible to certain venoms than others, and there is some frog data in the dataset.

Additionally, the more test-animal-species there are for one ophidian species, the more likely it is that there will be more routes of inoculation. However, in the dataset, there are multiple instances of the same test-animal-species occupying LDmin and LDmax, and quite a few are very close to this state, which suggests that, indeed, the number of times an ophidian species is tested is a major factor.

Number of DB entries (studies reported) per ophidian species correlation to LDmin

In Fig. 10 the “All data LDmin” fitted curve does not control for different test species. To address this criticism, “Mouse LDmin” shows the same graph filtered for inoculation of mice only. The R^2 value of 0.27 is not as good as the 0.36 R^2 value is for all data, however, the N is lower, and by inspection, there is a nearly perfect match for the curves for the region where they both have data. If there were no correlate by number of studies per species, then the fitted curves should be flat, whereas, both fitted curves span over 2 logs, and have nearly identical exponents and quite close constants. Consequently, these data suggest that the primary correlate for lethality is the number of studies that have been performed.

Regressions on range fold change

The range fold change is $R_{max} \div R_{min}$. The R^2 values for these regressions are larger than what we see above. These data indicate that there is a correlation for all measures with number of DB entries for lethal dose (number of studies reported). The number of routes of inoculation has a meaningful correlation for all data, and for single test species. I do not show these graphs, as they are redundant.

Table 2: Regressions on all data for range fold changes of highest over lowest dose. All data (LD-ADrf), single species (LD-SSrf), single species, single route (LD-SRrf).

R^2 below 0.20 not shown. N = 167 ophidian species.

Data, by species	LD-ADrf R^2	LD-SSrf R^2	LD-SRrf R^2
Number of routes of inoculation	0.429	0.34	
Number of species tested	0.5464		
Number of LD DB entries	0.5622	0.4042	0.2271

Discussion Of Possible Confounders

There may be errors in database record entries, or some papers or books got the numbers wrong. However, Sascha Steinhoff has made strenuous efforts to validate the data entries as evidenced by the sourcing of each one, and I have discussed this with him in personal communications as well. I do not believe that this is a significant source of error.

Biomedical science in general has a reproducibility problem[29]. However, venom LD50 and LDLo studies are straightforward to perform and the reproducibility issues in bioscience tend to be in more complex work. Against that, after multiple discussions with animal handlers and scientists practiced at injections, some plausible sources of error emerged. It is conceivable that an intracerebral injection was performed incorrectly sometimes, as this procedure is

arguably more difficult than the others. It is plausible that injections into animals are more difficult to standardize than believed, particularly small animals. For instance, subcutaneous injections may be done in different locations on the animal, and some of these may be more effective spots than others. It could happen that a subcutaneous injection hits a vein more often than thought. Similarly, veins may be missed and either become subcutaneous injections or intramuscular injections. In animals such as mice, muscles can be missed.

However, for this study, there is no way to know what issues there may be, and rejecting data post hoc because it doesn't fit preconceptions, particularly when no injection method appears to fit those preconceptions better, is hard to support. I do not have a basis for quantifying the degree to which these data might represent a window into the rate of bench error in venom lethality studies nor the rate of such error.

Another plausible influence on the dataset could be that the ophidian species that kill humans get tested more, and so those species that do get tested more have a wider range fold change of $LD_{max} \div LD_{min}$. To test this hypothesis, several methods were used. The top 14 venomous snakes listed as a threat to humans were plotted relative to their ordinal in the lethality and the number of studies in the order of threat. Of these 14 ophidian species, 11 were in the bottom (more toxic) quartile of the 167 ophidian species included in this study, and 10 of the 14 were above the median of 5 studies, with a median of 10 studies among this set. So there may be some effect from studies being directed at snakes dangerous to humans.

Another confounder could be that the venom database contains subspecies lumped together and that this might affect the range fold change and minimum lethal dose. This hypothesis was tested by finding ophidian species that had called out multiple subspecies.

I found 22 out of the 167 species had subspecies listed. Only 7 out of those 22 could have had impact because the LD_{min} and LD_{max} were possibly different subspecies. Just 2 of these 7 ophidian species had a range fold change that changed by a factor of 2 or more, and just one changed by a factor of 20. The mean range fold change for the 7 that changed out of the 22 with listed subspecies was 0.88.

The mean LD_{min} fold change for the 7 species out of 22 was 5.29. For all 22 species, the mean LD_{min} fold change was 2.36. The impact of segregating named subspecies and treating them as different species was tiny, out in the 3rd and 4th decimal place of the fitted curve exponent. This suggests that unidentified subspecies might sometimes matter, however, overall it is not defensible as a meaningful contributor in this dataset.

Ecological Transects And Venom Variation

An ecological transect is a survey line of some length laid out in an area. Along that line, to some distance on each side, a survey is conducted to count the number of species. The transect is divided into segments, and each segment is a sampling of the species along the transect. As one progresses along the transect, the new species discovery rate will decline. Based on that slowing discovery rate, one can fit a curve, and using this, estimate the number of species in the area of the transect [30, 31].

These venom lethal dose data represent a kind of transect of the variation in potency of venom, where the transect is everywhere that scientists collect venomous snakes, and the discovery rate is some time period. However, I could not analyze this because there is simply not enough data on a per-test-species, per-inoculation-route basis to make it meaningful. The fact that $LDLo$ and $LD50$ do not differentiate suggests that we are so far from a proper sample size that we cannot currently make an estimate of where the limits are.

This venom diversity transect is filtered through the interactions with both the route of administration and the different test species used. This has implications for medicine, because human envenomation is a similar transect, where humans are the target species interacting with venom variance and dose delivered.

Conclusion

The import of this meta-analysis is several. First, the correlation between number of times a venomous ophidian's lethal dose is studied and the range of lethal dose indicates there is quite a bit of room for exploring lethal dose range, and that to properly characterize toxicity of whole ophidian venom is a large meta-project.

Second, the inability to differentiate between LDLo and LD50, and the preponderance of subsets where LDLo is higher than LD50, indicates that the N required to fully characterize venom is beyond what current studies have collected. This indicates, in turn, that the range of toxicity results for whole venom should be less reliable than they appear to be here, even for those ophidian species with the highest number of studies reported. In ecological parlance, the transect is at the beginning of its discoveries.

Beyond this, there are several areas that this analysis has bearing on: How to best estimate LD50 given current limitations; confirmations and caution relative to existing medical practice; and further research.

LD50 estimations

The correct way to define LD50 at this time is to use a meta-dataset. Treat LDLo the same as LD50, and provide LD mean, median, minimum, range, and standard deviation, along with the N for number of studies per test species used. Safety could be estimated by specifying from 1 up to 6 standard deviations (6 sigma) depending on desired safety margin. If an LDLo value is desired, this is simply the minimum lethal dose in the meta-dataset, and should be referred to that way (e.g. LDmin) to avoid confusion. This can be done separately for each route of inoculation.

Human LD50 estimations

The ethical problems of determining human dose-response force us to develop methods of estimation based on animal data. Yet, the human dose-response may be different than other animals, including monkeys.

It may be reasonable to consider exclusion of amphibian, bird and reptile data if there is sufficient N from mammals, where N is the number of studies conducted. However, from this meta-analysis it appears that a sufficient N is more than 50 different studies, and this is unlikely to happen soon. Also, there are multiple instances in larger ophidian datasets where non-mammal data is bracketed within mammalian data. Consequently, the most reasonable general course is to use aggregate data for all species, and compute as discussed above for the general case.

It has been argued that humans cannot receive IP or IC inoculations, except in the case of infants. However, there are cases of bites to the thorax in adults that appear to progress more quickly which may be similar enough to include it for that instance.

Should there be sufficient N for specific routes of inoculation, or if the IC inoculations appear to conform to the $IC < IP < IV < IM < SC$ model, then for human estimations, IC could be excluded.

LD50 estimations with little data, and computational research to support it

I recommend for estimating LD50 for an ophidian with little data, to use a customized meta-dataset for related species, and factor proportionally from the mean of the minimal known data. To do this, curate a related ophidian meta-dataset based on what is known of venom makeup and/or genetic distance, plus the prey species. For that meta-dataset, determine the LD mean, minimum, range, and standard deviation.

Estimation of margin of error for this proposed algorithm will require non-trivial development and validation against existing datasets such as the one used for this meta-analysis, and represents an area of computational research.

Human bite treatment: confirmations and caution

This meta-dataset tells us that, controlling for dose, envenomation effect can vary by over 28,000 times within one species. Adding uncontrolled venom dose into the equation indicates that medicine is probably dealing with effective dose ranges spanning up to 1 million times. Consequently, physicians treating patients with snakebite cannot presume that because they saw 10, or even 50 cases for one species, that this will necessarily tell them what will happen on the next bite. This is true even if they have gotten good at estimating the size of the animal from the distance between the fang puncture marks. This analysis confirms that snakebite treatment should always be treated symptomatically, and that this should be done aggressively, because sooner or later an outlier should appear.

These results also confirm that antivenom manufacturers should use mixtures from a variety of snakes of the same species for immunization of animals.

Research for venom toxicology

Ophidian venoms are a cocktail of many components. For a toxicologist working on individual venom components, whether there is significant variation in the lethality of venom components between snakes within the same species is an open question. There are tantalizing reports from India, for instance the practice of recreational cobra bites, that are suggestive of inbred strains [32, 33]. Researching this question will require many samples from wild snakes, optimally, with geolocation, size, estimated age, and sex recorded, with attention to sample sizes and control of test species. Gene sequencing of the whole genome and/or exome could also provide meaningful insight. Given that snakes migrate slowly relative to many other animals, genetic drift could plausibly generate some variation. This is a question that could take many years to characterize.

Abbreviations

DB

database. Here it refers to the Steinhoff database.

IC

intra-cerebral

IV

intravenous

IP

intraperitoneal

IM

intramuscular

LD

Lethal dose.

LD50

Lethal dose that kills 50% of the test animals

LDLo
Lowest lethal dose in a set.

LDmean
the mean average of the LD's

LDmin
lowest lethal dose within a set of lethal doses

LDmax
highest lethal dose within a set of lethal doses

LD-ADrf
lethal dose range fold change using all data

LD-SRrf
lethal dose range fold change using a single test species and a single route of inoculation.

LD-SSrf
the widest range fold change within one test-animal-species for an ophidian species was used. (e.g. within each test species use the highest and lowest LD, and take the largest range of the set.)

Range fold change
 $R_{max} \div R_{min}$

SC
subcutaneous

Declarations

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Availability of data and materials

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Not applicable.

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Figures

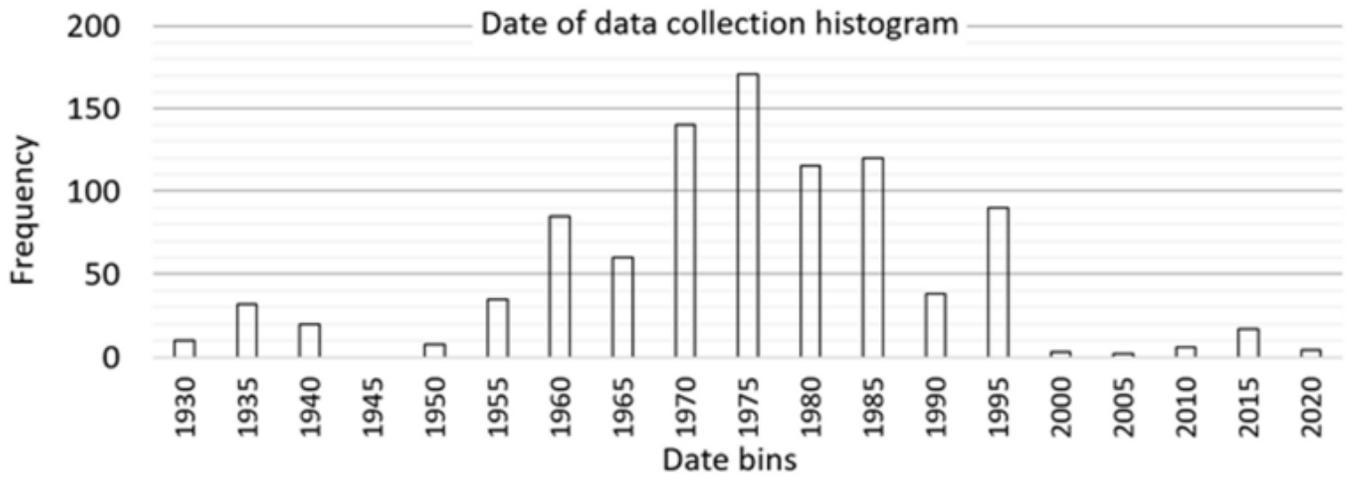


Figure 1

Time distribution of studies. Bins for date of report are the termination year for the bin. The generic mammal DB entries have dates from 1953 to 1985, with 24 of them in 1967. The unrecorded method of inoculation DB entries spanned 1958 to 1987, with 29 in 1967. This indicates a period when some researchers appeared to believe that the test-animal-species and route of inoculation was insignificant.

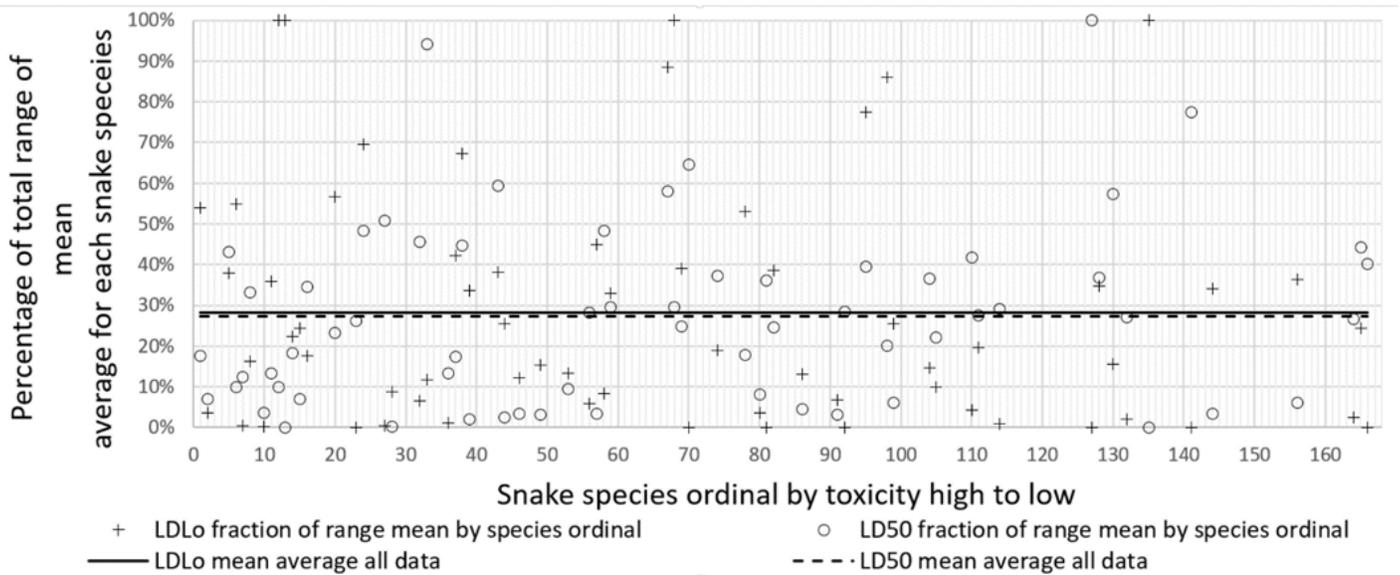


Figure 2

LDLo fraction of range compared to LD50 fractions of range. 64 out of 167 ophidian species had at least one LDLo value and at least one LD50 value. Average of the LDLo fractions is 28.3%, and the average of the LD50 fractions is 27.2%. They differ by 1.1%, or roughly 1/3rd of the standard error. One would expect LDLo to average considerably below LD50.

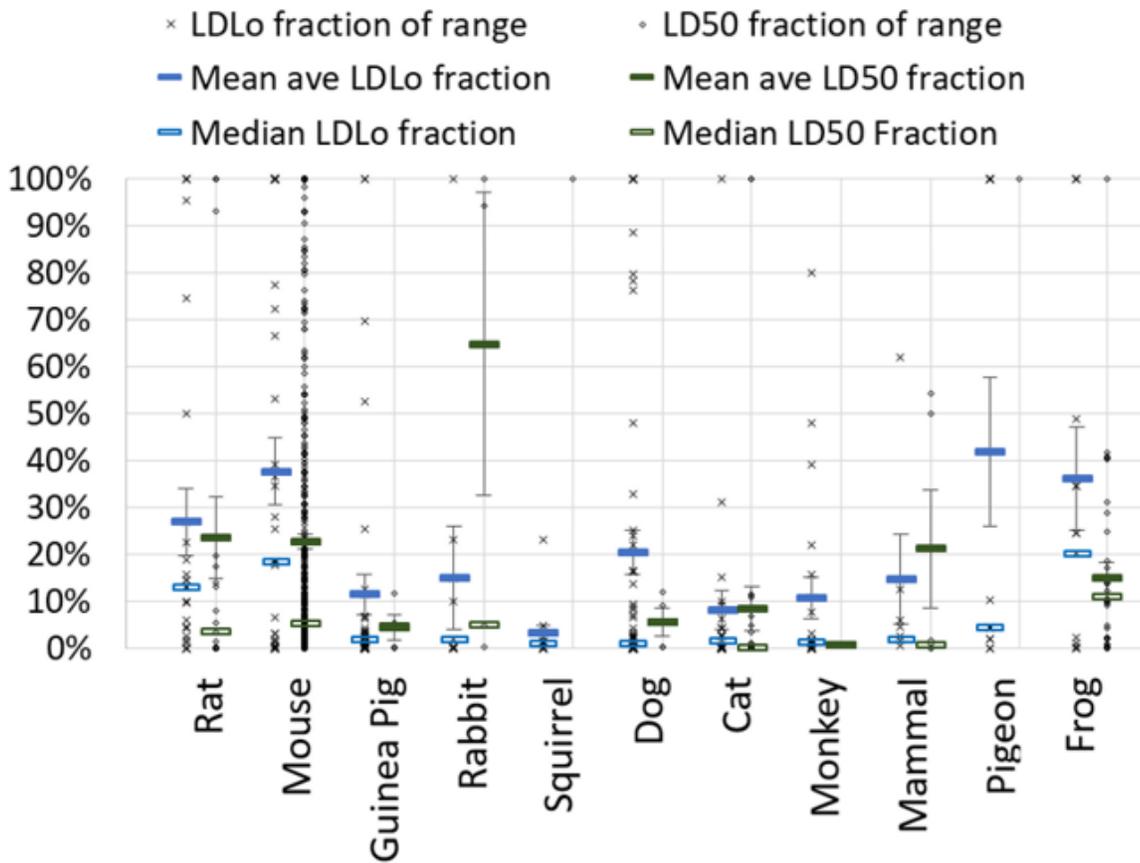


Figure 3

Fractions of range by test animal. Error bars show standard error. Only in rabbit is LD50 above LDLo with significance, however this disappears if we use median instead of mean. (hollow bars are median values) Higher N animal datasets like mouse, continue to show LDLo above LD50, and that this is significant.

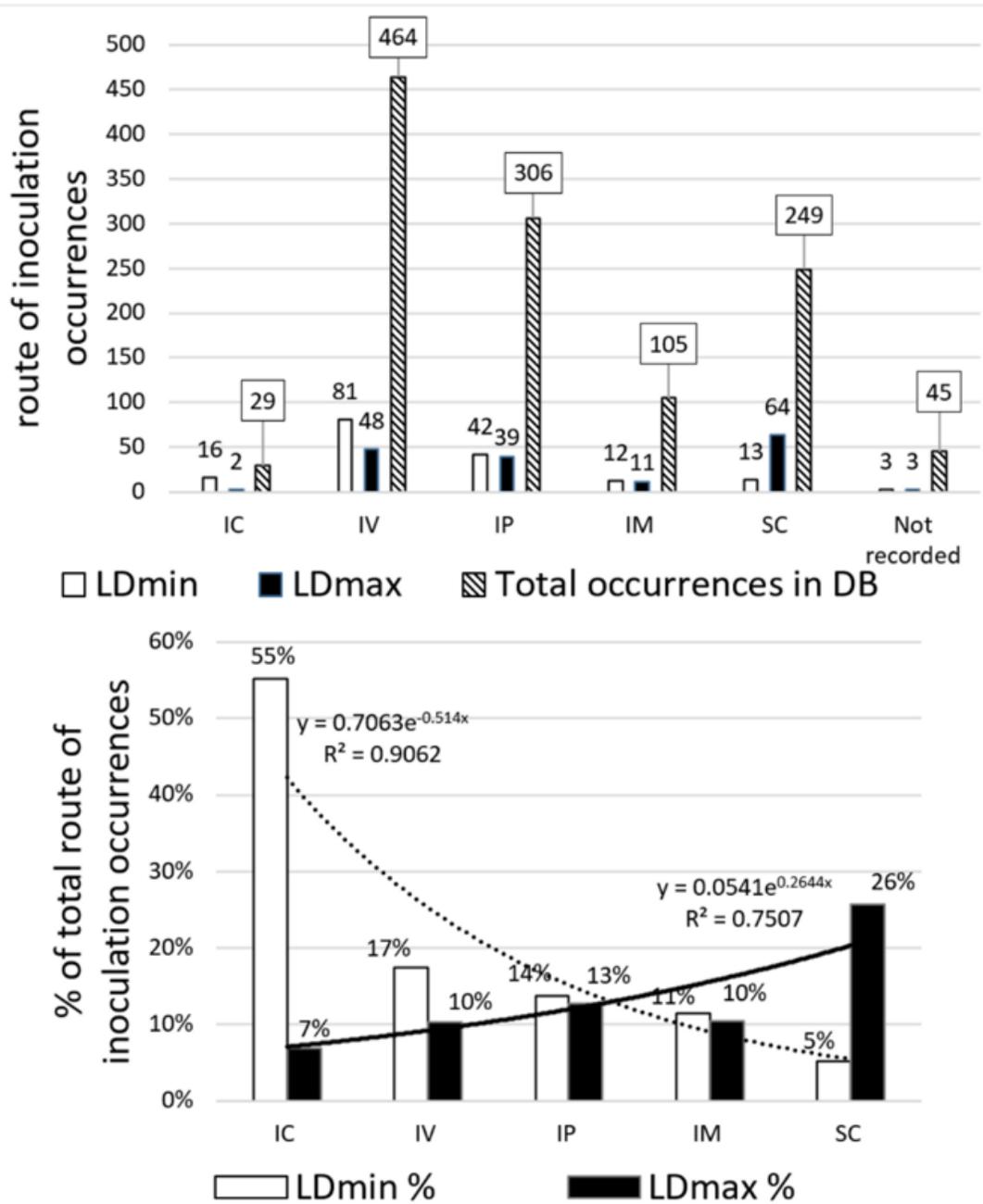


Figure 4

Route of inoculation distribution: minimum and maximum lethal doses.

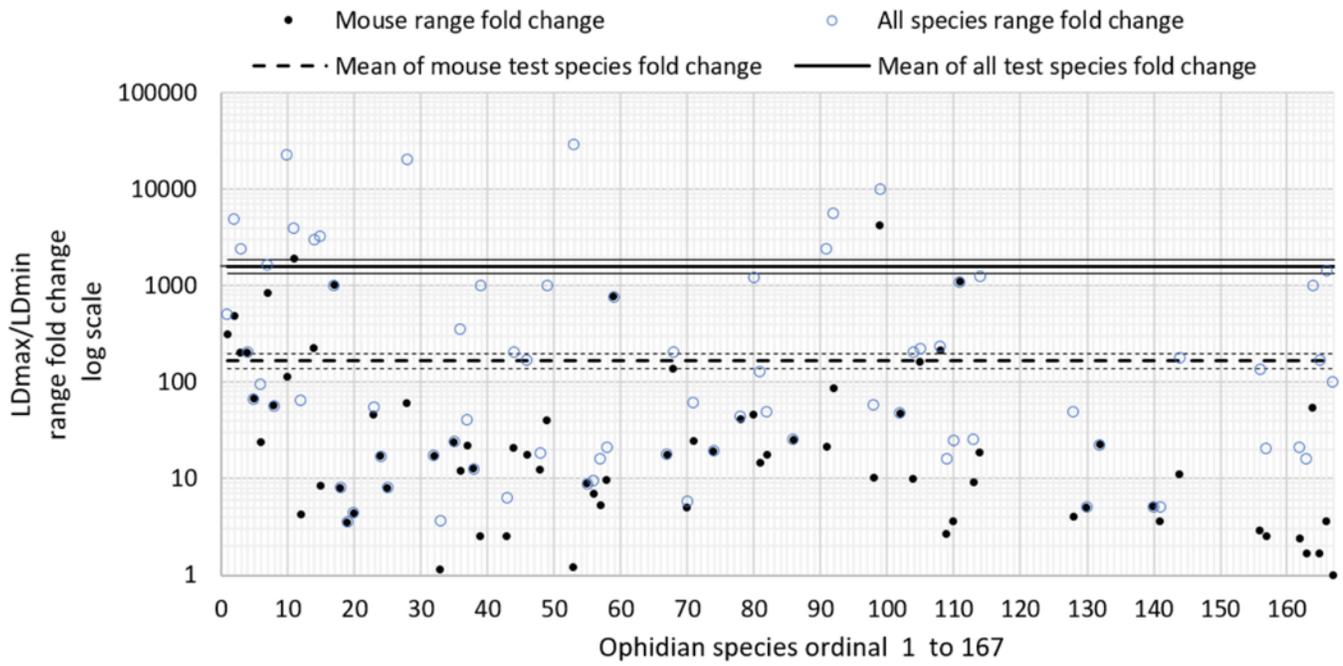


Figure 5

Mouse vs all test species LDmin and averages. Standard error of the means is shown as solid or dashed bars above and below mean average lines.

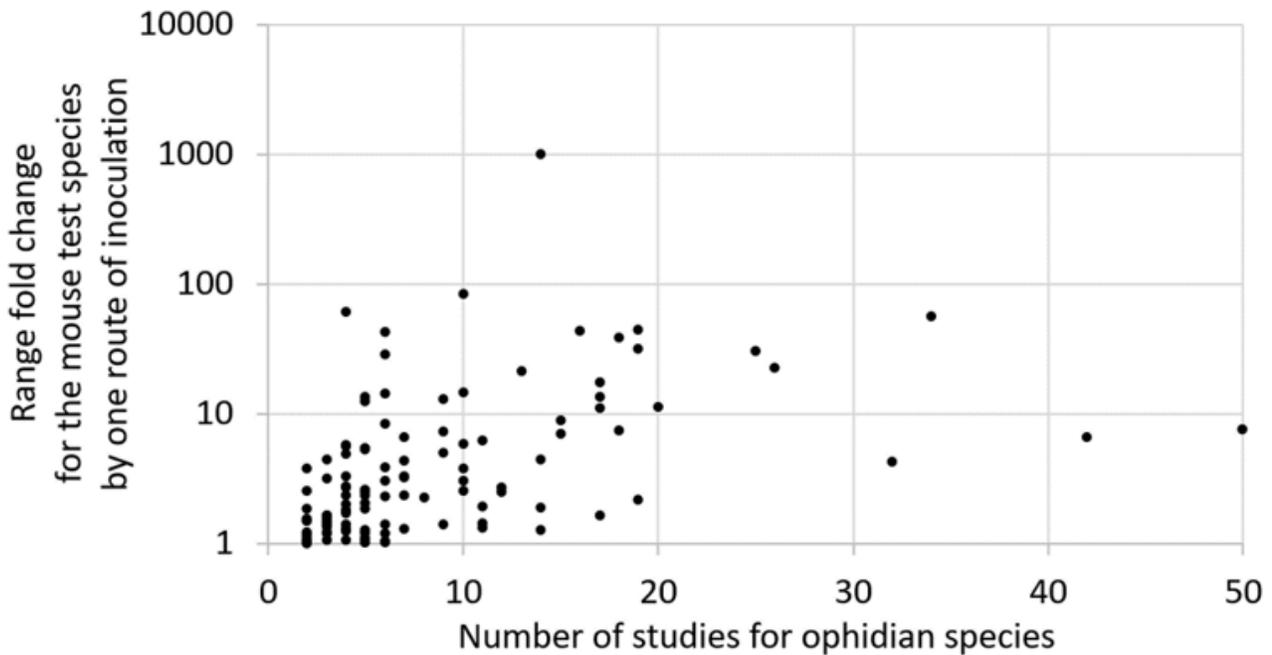


Figure 6

Range fold change (log scale) vs. number of studies for one test-animal-species and one route of inoculation. N = 116 ophidian species that have two or more studies for the same route of inoculation.

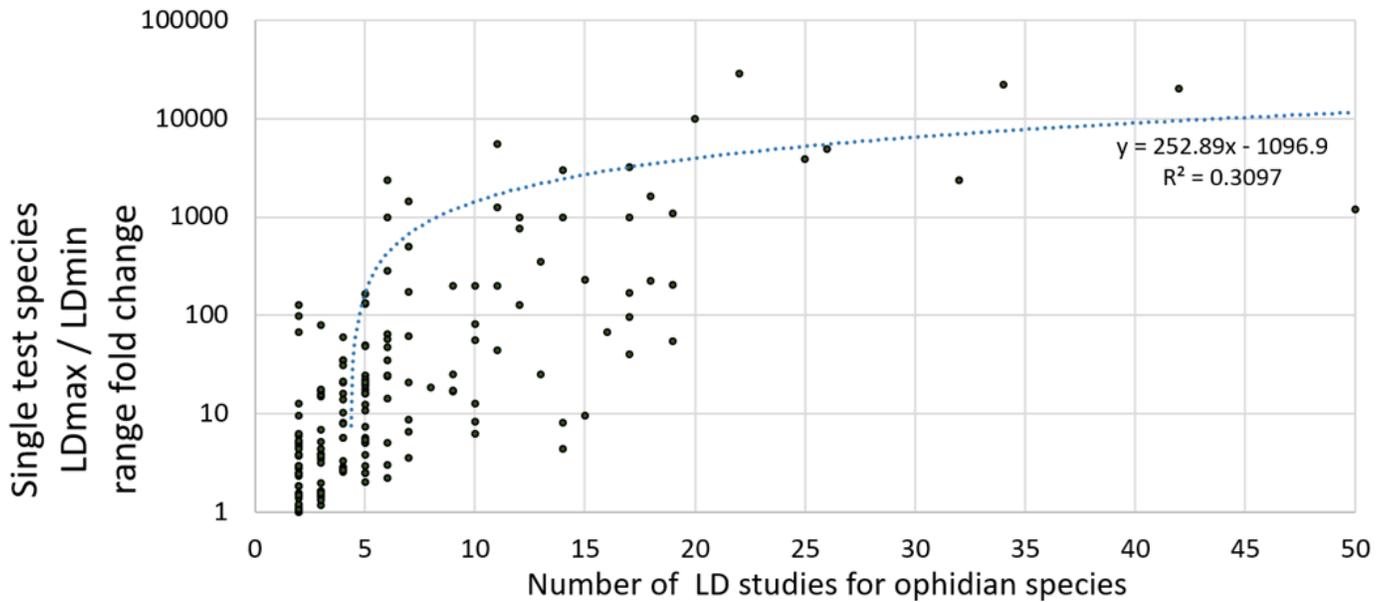


Figure 7

Venom range fold change for single test-animal-species and multiple routes of inoculation. N = 167 ophidian species with 2 or more reports for the same test species. There is a strong linear trend of increase in the range as the number of studies rises. In this graph, within each ophidian species, for test species with 2 or more entries, the test species with the largest fold change is shown.

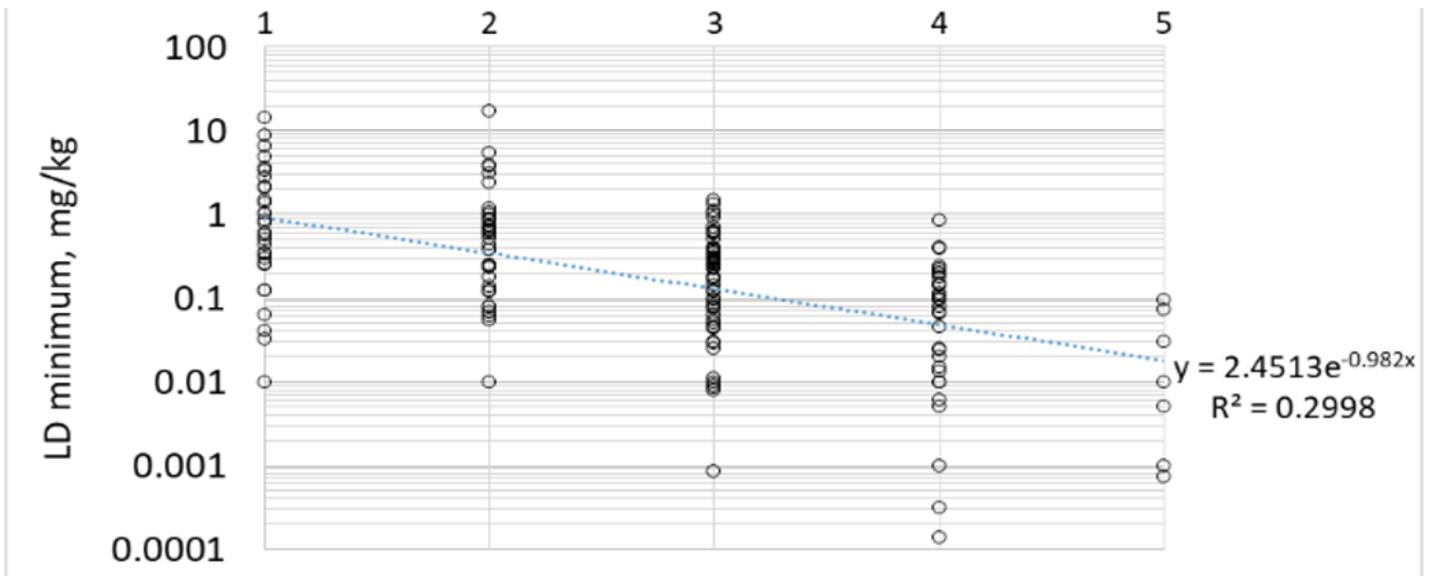


Figure 8

Minimum lethal dose vs number of routes of inoculation. (Table 1, first entry). The N for the number of routes of inoculation 1 to 5 are, respectively: 30, 36, 61, 33, and 7.

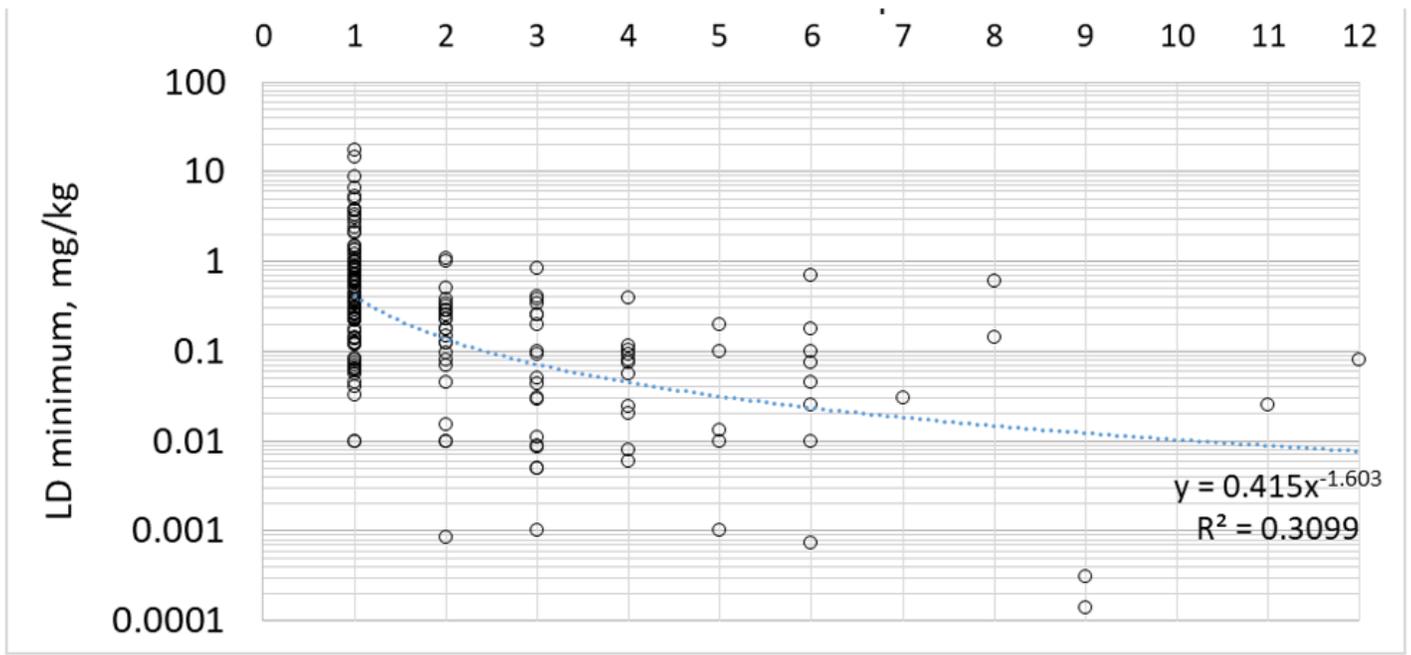


Figure 9

Minimum lethal dose vs number of test-animal-species. (Table 1, second entry.) X axis is number of test-animal-species.

Number of DB entries (studies reported) per ophidian species correlation to LDmin

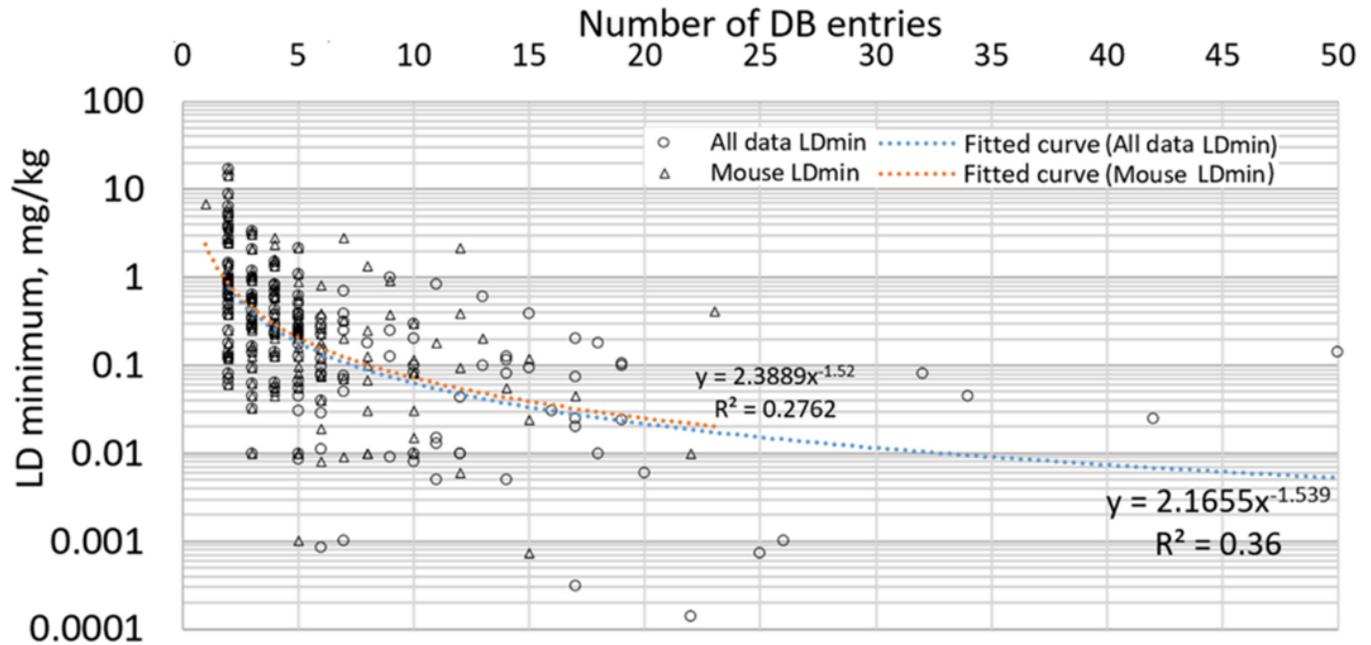


Figure 10

Comparison of LDmin for all data vs. mouse only data. Minimum lethal dose vs count of database entries per ophidian species. For all data, the fitted curve spans ~2.15 logs. For mouse data only, the fitted curve spans ~2.05 logs. (Ophidian species N = 167). What is visible by inspection is that when data is filtered to only include mouse

studies, the curve fit for mouse data is a near exact match, it is just truncated because of fewer ophidian species with higher number of DB entries.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SteinhoffsDBSelectedVenomscurateddatasetv1.xlsx](#)