A Computer Experiment Model to Investigate the Effects of Drug Dosage in Animals, for Use in Pharmacological Education and Research

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Summary — The ACD-IDEA database, which was originally developed by the authors in 2004, is an ongoing compilation of existing data on the *in vivo* doses of compounds at which various responses in certain animal species have been observed. It can provide an infrastructure for various research/educational efforts, and creates a synergy for new applications. In this paper, some of these applications are described. Specific interfaces within the database are designed for users who are not computer specialists. Users can search the database to find the answer to a query, or they can design a simple virtual animal experiment. In the second case, the interface is used to undertake a dialogue with the system, in order to test the user's knowledge regarding an experiment under consideration, and to allow the user to glean additional information on better experimental planning. The use of this virtual experimental tool should lead to savings in time, animals, materials, and monetary costs, while the effective learning outcomes of pharmacological experiments are maintained or enhanced.

Key words: *computer experiment, drug-dosage data, educational tools, laboratory animal experiment.*

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Introduction

The ACD-IDEA database, originally developed by the authors in 2004 (1, 2), is an ongoing compilation of data on the *in vivo* doses of compounds at which various responses in certain animal species have been observed. This kind of collation and review of existing data is very important for all sciences. In the fields of fundamental and applied experimental science, the amount of data is immense, having been obtained over a long period of time. There are also enormous process and material costs associated with the acquisition of such a volume of data. Hence, improved processes are necessary to extract the precise data and the knowledge we need from them. Computer tools can assist in solving these types of problems. When considering a large amount of data, database applications can be implemented that are capable of storing, maintaining, distributing, and sharing the data (3, 4). One of the most important advantages of using appropriately designed computer database applications for such a large amount of data, is that one can obtain new data from a limited number of additional experiments, or sometimes even with no additional experiments at all. Some of these computer databases permit the application of expert systems (4), and give the user the capability of conducting virtual experiments based on the data within them. The availability of databases for use in new drug research and development is also important, and this importance will increase in the future, as the costs and complexities involved in drug discovery and development continue to increase (5). Indeed, in order to enhance and augment classical laboratory research, it is foreseen that many computer-aided applications will need to be developed for use in the field of pharmacological research (6).

Pharmacological research and education covers a wide range of considerations. One of them is the *in vivo* determination of drug-dosages for application to different experimental animals. Many types of drugs are applied to a range of experimental animals via different administration routes, at various dosages, producing basic exposure data for the individual parameters that are tested. In many instances, these experiments produce very different and contradictory results. It is imperative that such data should be registered, published and shared among those involved in the field, to avoid unnecessary duplication of efforts and repetition of experiments. Often, similar experiments producing the same data are repeated continuously throughout the world, at least for educational or research purposes.

The number of registered and published pharmacological experiments is extensive, and covers a period of almost 100 years (7, 8). The ACD-IDEA database was developed for the collation of many of

these experimental data. By using this database, it is possible to acquire new information and insight about the existing experiments. Also, by using the database, it is possible to maintain records of these data and to share them with a wide range of relevant educational institutions and other pharmacological institutions throughout the world. In this way, extensive savings can be achieved in pharmacological experimentation, when the data produced are maintained in an appropriate database. The main advantage of using this kind of database is the saving of animals, materials and time by avoiding the unnecessary repetition of experiments. A second advantage is the opportunity to use computer technology and software to conduct virtual (computer-based) animal experiments. To date, many virtual experimental applications have been developed in the fundamental and applied science fields (for example, see Hwee *et al.* [9] and Camillan [10]).

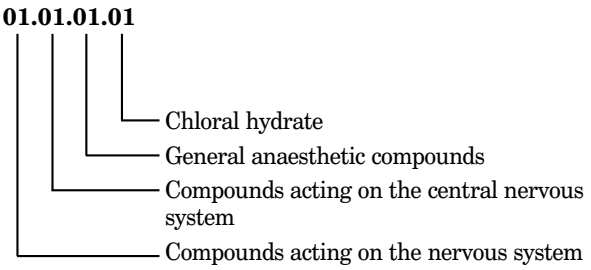
Methods

Database structure

The methods that were used to design the database, are readily available in the related literature (3, 4). A relational design pattern with three-layer architecture was selected for the database (3). To design the inner structure of the database, we examined a wide range of existing data from relevant experiments on laboratory animals, in two editions of *Drug Dosage in Laboratory Animals: A Handbook* (7, 8). As is well known, some drugs, such as aspirin, have a long history and are still in everyday use. However, the data on the experimental dosages associated with such drugs are not easily accessible, so in such cases we used related data from alternative known open sources (7, 8). Data were extracted from the two books (7, 8), as the first step in preparation for entering data into the database. Then, experimental dosage data were selected from articles that had been published in various international peer-review journals that were indexed in the Science Citation Index, and subsequently entered into the database. Since drug development is a dynamic process, resulting in the introduction of new drugs onto the world markets, this step of data entry will be an unending task. We envisage that the database will need to be continuously updated in the future, by its developers (or by its users themselves).

Compounds (drugs) were classified on the basis of therapeutic categories, and labelled according to a tree-like scheme. The scheme has four levels, as shown in Figure 1. The highest level contains a total of 16 nodes, where the 16th is 'Miscellaneous'. These 16 first-level nodes each have various numbers of second-level nodes, and this continues similarly through the third-level and fourth-level nodes. These data comprise the *Drugs* table. The relevant nodes at each level are chosen during the classification, according to the nature of the compound. The structure of the scheme can be better understood from the example shown in Figure 1. In this example, the code '01.01.01.01' refers specifically to chloral hydrate — i.e. it is in the drug category of '01. Compounds acting on the nervous system', specifically belonging to the class of '01. Compounds acting on the central nervous system', and it is an '01. General anaesthetic' compound. As another example, '01.01.01.02' refers to alpha-chloralose, which has the same higher levels of classification as chloral hydrate.

Figure 1: The four levels of the compound classification scheme



The levels used in the classification scheme are designated highest (left) to lowest (right).

A similar hierarchical scheme was designed to define the potential reactions to a drug in the *in vivo* situation, i.e. *Reactions*, although this scheme has only two levels. There are a total of 19 nodes in the first level, as listed in Figure 2. These 19 nodes contain different numbers of nodes at the second level. For example, the first-level node '01. Action on the central nervous system' has 69 second-level nodes, some of which are as follows:

01. Action on the central nervous system
    01.01 Hypnotic action
    01.02 Sedative action
    01.03 Potentiating chloral hydrate sleep-inducing effects
    01.04 Potentiating barbiturate sleep-inducing effects

The systematisation of the data by using the tree-like organisation results in the database having a clearer structure, reduces any repetition in data storage, and facilitates data access and modification.

A range of tables are available within the database, namely:

— *Drugs*: This table has 215 records, and represents drug code and drug name data. The drug name is an active pharmacological element of the related data. The main concept, the classification of drugs, was derived from the order used in *Drug Dosage in Laboratory Animals: A Handbook* (7, 8).

— *Animals*: This table contains data related to the experimental animal codes, which are indicative of the type of animal used in the experiments. There are seven records in the table: B (rabbit); C (cat); D (dog); M (mouse); N (monkey); P (guinea-pig); and R (rat).

— *Administration Routes*: This table is used to prevent the repetition of the data for animal names and administration routes. To do this, we established individual animal administration route codes: IM (intramuscular); IP (intraperitoneal); SC (subcutaneous); PO (per oral); and IV (intravenous). There are 35 records in this table (i.e. for each of the seven animal types, five different administration routes are described).

— *Reactions*: This table lists all of the known potential reactions to drugs in the *in vivo* situation. This table covers the reaction codes and reaction names. There are 249 different records in this table (the 19 first-level nodes are listed in Figure 2).

— *Resvivo*: This table contains the data from 'research *in vivo*', and this is the core data table in the system. It contains the experimental data from each published study that were analysed for inclusion in the database. Each record of the table contains the data for a single experiment: drug code; animal code; administration route code; apparent reaction code; applied drug dose (minimum and maximum levels); and a reference code related to the publication from which the information was taken. It should be noted that, if a single dose was used, both minimum and maximum dosages have the same value. There are 4273 records in this table.

Figure 2: First-level nodes in the *Reactions* table

01.	Action on the central nervous system
02.	Action on the autonomic nervous system
03.	Action on skeletal muscle function
04.	Action on the cardiovascular system
05.	Non-steroidal analgesic, antipyretic and anti-inflammatory action
06.	Action on blood haemodynamics
07.	Action on the kidneys
08.	Action on the gastrointestinal tract
09.	Action effective on the respiratory tract
10.	Action effective on the reproductive system
11.	Action effective on smooth muscle
12.	Action effective on the immune system
13.	Action effective on histamine, serotonin and bradykinin metabolism
14.	Action effective on liver function
15.	Action effective on metabolic function
16.	Action effective on cell proliferation and genes
17.	Antidote
18.	Toxic effects
19.	Miscellaneous

Structure of the model

The system is designed mainly for educational purposes. To support user–system relationships, a special set of user-friendly interface forms were created. Users can:

— extract required information from the database by entering a query containing different Drug–Animal–Route–Reaction combinations; and

— test their knowledge regarding an experiment under consideration and glean additional information on the topic of better experimental planning.

Three types of query can be put to the system: a Drug–Animal Query, a Drug–Animal–Route Query, or an Animal–Route–Reaction Query. Answers are displayed as a list of *Resvivo* records. A user might want to obtain further detailed information on the experiment displayed. This is possible by merely clicking on the *Reference code* field, at which the name of the source publication is displayed. The flowchart of a typical query put to the system is shown in Figure 3.

To design a new virtual experiment, the user initially defines proposed values of experimental inputs, i.e. the drug, animal, administration route, and drug dose. It is assumed that a user has a certain level of background knowledge on the topic. After entering the values, the system searches for record(s) in *Resvivo* that fit this input. If any records are found, then a multi-choice page of responses is displayed. Only one of the choices shown is a true answer. The user can select one of the answers, to ascertain their level of knowledge on the topic. If the choice is correct, then the relevant message and the list of fitted records are displayed. If the choice is incorrect, then a message is shown to indicate this and the possible error in the original input values appears. A user can go back and try other values, which makes this procedure valuable as a learning process.

Applications

Here are some simple examples to demonstrate the potential use of the system. The *Application Welcome Page* is shown in Figure 4. It contains a menu of four choices: three query choices to extract information from the database, and a fourth choice to activate a dialogue system for planning virtual experiments. This page also contains the names of the tables. The choice *Missing Data* can be used to send messages to the authors — for example, to highlight errors, new drugs for inclusion, and to give user-feedback and suggest proposals for improvements to the system, etc. Here are some examples of how the menu choices can be used:

1. *Drug–Animal Query*: The user would like to find out whether there is any information in the database on experiments in which chloral hydrate was administered to dogs. To put this query to the system, by using the top-down menus, the following fields would be selected, in this order: *Drug–Animal Query*; Compounds acting on the nervous system; Chloral hydrate; Dog. Then, after clicking *Submit*, a table with seven rows appears as the answer (Figure 5). This means that for these given data, the data-

Figure 3: A flowchart of a typical query put to the system

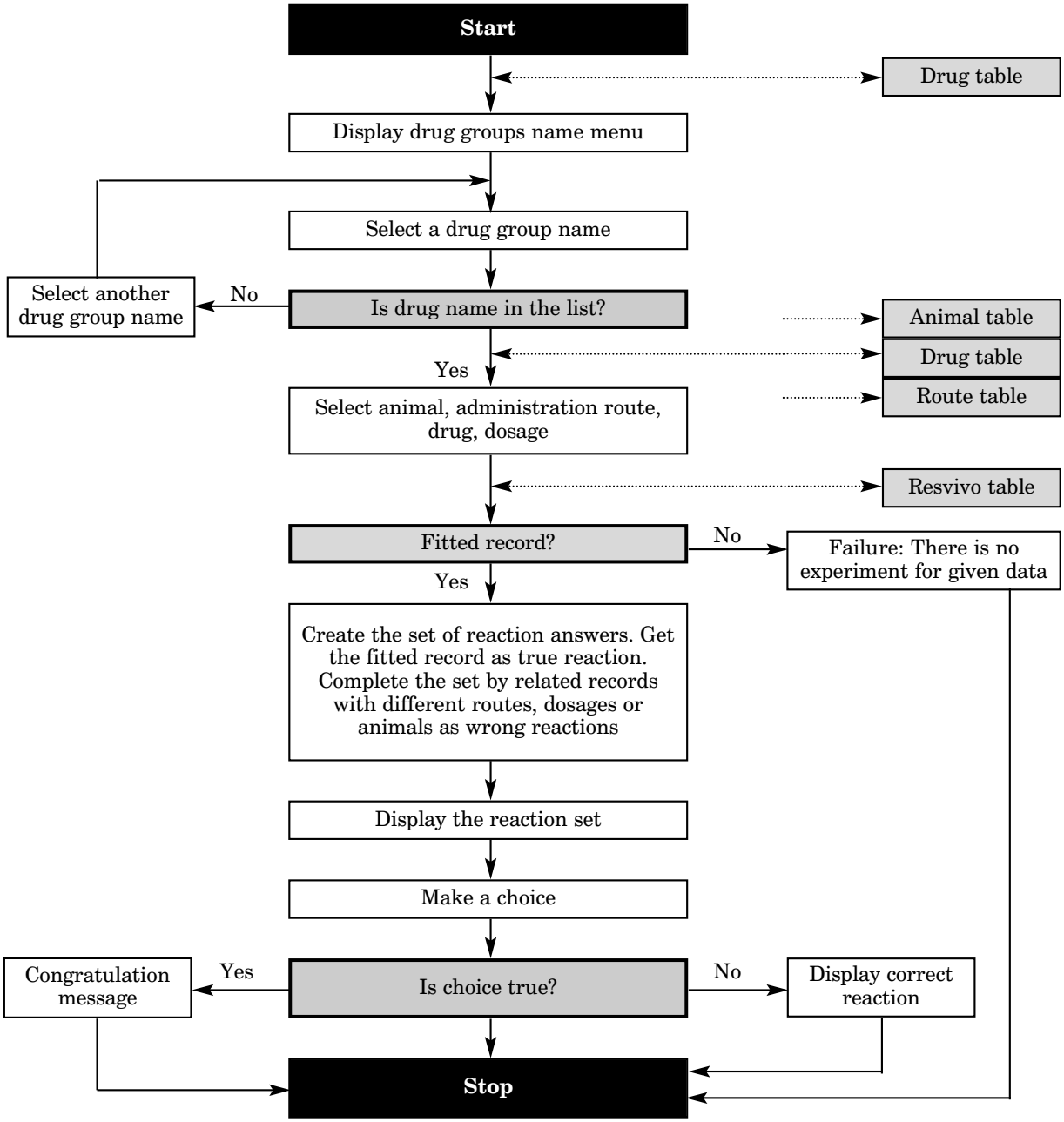
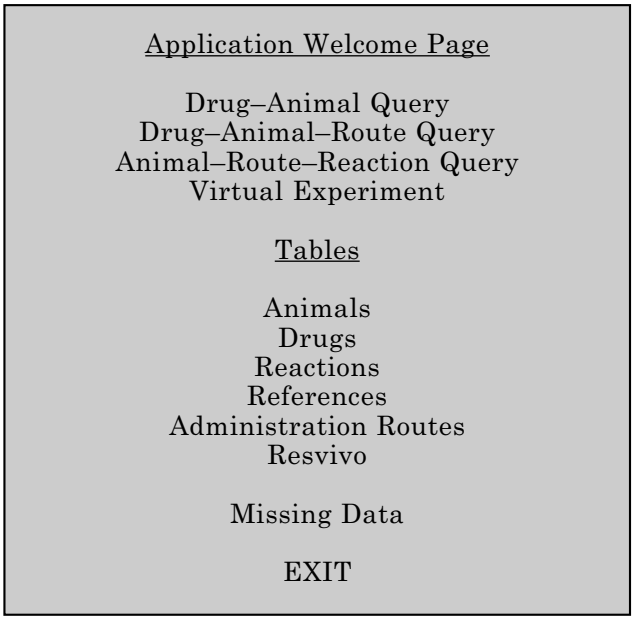


Figure 4: The *Application Welcome Page*



base contains information on seven individual experiments. In each of the rows, the main features of the experiment, i.e. the administration route, minimum and maximum values of the dose and the reaction are listed, as well as the identification code of the original publication. By clicking on this identification code, the source of further information on the experiment can be found.

By clicking *BACK*, it is possible to return to the previous page to try another query, for example:

2. *Drug–Animal–Route Query*: This is similar to the *Drug–Animal Query* described above. With this example, more-detailed data are inputted by the user: i.e. drug, animal, administration route.

3. *Animal–Route–Reaction Query*: This query is useful when the need is for information on drugs that were administered by a given route, to a given animal, or with a given response. For example: Ataxic action; Mouse; SC. The result of this particular query is shown in Figure 6.

Alternatively, a virtual experiment can be performed:

4. *Virtual Experiment*: Consider the following scenario: the effects of the drug, pentobarbital, on small animals are to be investigated. Of course, the user has some prior knowledge (although not complete) of such experiments, e.g. the dose range and the possible reaction when applying the drug to the skin of an animal. Firstly, the user wants to check this knowledge. To do so, the following data can be entered by using the top-down menus: *Virtual Experiment*; Compounds acting on the nervous system; Pentobarbital; Mouse; SC; 30.00. After clicking on *Apply Experiment*, a multi-choice table with four radio-buttons appears. Each radio-button is assigned to one of several drug reactions, and one of them is the true drug reaction for the given data — the user has to identify the correct one. If 'Ataxic action' is selected and *Submit* is clicked, then the answer is: 'Right. That is the expected reaction'. If the wrong radio-button is selected, the answer will be: 'Wrong. The expected reaction is: Ataxic action'.

If the experiment is repeated, but with the dose value of 35.00, the answer would be: 'No result. Possible error in dose'. In this case, other dose values could be tried.

Figure 5: An example result from a Drug–Animal Query

Chloral hydrate

Administration route	Reaction code	Dose lower limit	Dose upper limit	Reference code
IV	Respiratory depression	125	125	2281
IV	Changing behavioural activities	40	40	951
IV	Inducing EEG changes	40	40	951
IV	Anaesthetic action	125	125	2281
PO	Anaesthetic action	500	500	2281
PO	Having median lethal toxicity	1100	1100	203
SC	Anaesthetic action	150	150	2281

Figure 6: An example result from an Animal–Route–Reaction Query

Drug name	Administration route	Reaction code	Dose	Reference code
Pentobarbital	MSC	1.33	30	1075
Phenobarbital	MSC	1.33	69	1075

Discussion

The major purpose of the system is educational. However, it can also be used by researchers in pharmacology and related areas, and by stand-alone users, to extract information. The database contains refined data related to the *in vivo* pharmacological and toxicological effects of drugs currently in use. It contains information on drugs that is already known to the pharmacological community. The main data compiled in the present database comprise the *doses* of drugs given which produce *specific* in vivo *pharmacological effects* in *different experimental animal species*. Certainly, each dose producing a specific effect has an appropriate literature reference.

As a drug may cause different effects when administered to a certain animal species at different doses, via different routes, a type of virtual experiment is possible, based on the existing data within the database. This may facilitate the work of researchers, since information on the effects of a drug, which will quite possibly have been used in independent experiments at different doses, will be readily obtained without extensive surveys of bibliographical databases and/or without dose-scanning experiments having to be performed in the laboratory. However, quantitative dose–response relationships are not within the scope of the database.

The features of this database will support the *reduction* and *refinement* aspects of the Three Rs principles, as outlined by Russell and Burch (11), as the numbers of animals will be reduced through minimisation of dose-scanning experiments for known drugs. This database, through the virtual experiment facility, will also give an opportunity for researchers to explore the unexpected effects of a drug. This is also suitable for educational purposes. The number of user-friendly interfaces permit access to the necessary data without any major difficulties. This is particularly useful for users who are not computer specialists.

The system could be modified and improved in the future. For example, by adding specific knowledge types to the database, it could be converted into a Knowledge Base, and could then be used in designing an Expert System (12). Here, knowledge can be represented, for example, by using a rule-based approach in the form of *if–then* clauses, which would seem appropriate in this case. We are keenly awaiting user-feedback and suggested proposals for improvements to the system as it stands.

At present, users can access this database on the Internet at http://ue.aydin.edu.tr/experiment (*User name* = drug; *Password* = animal). We have made the database available as a non-profit product on the Internet, to allow free access for all interested researchers and students.

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