Part I

Hit and Lead Profiling. Edited by Bernard Faller and Laszlo Urban

ISBN: 978-3-527-32331-9

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Process Logistics, Testing Strategies and Automation Aspects

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1.1
Introduction

This introductory chapter tries to step outside the details of any particular measurement type, in order to review the organizational landscape in which ADME/Tox experiments are conducted and the different approaches to delivering high-quality, decision-ready data to drug discovery teams. In particular, attention is paid to: (i) the many different groups of scientists involved in the overall process from request to data; (ii) different models for converting data to decisions; and (iii) themes that challenge the process, particularly increasing demand for more and more data; and then (iv) a framework is described for improving the process that should be applicable in any organizational context, following the well trod path of root cause analysis; and (v) finally examples are given of three types of effort to organize the overall process through automation and software.

The authors hope that the overview provided here will help many laboratories organize their talent, technology and people in such a way as to maximize the availability and impact of ADME/Tox data throughout the drug discovery enterprise. With respect to the specific choices of technology, we hope that the discussion of root cause analysis and different organizational models enables groups to develop long-term plans that build toward efficient use of talent and laboratory space through both hardware and software.

1.2
The Process from Raw Ingredients to Data

The overall workflow of ADME/Tox characterization of lead compounds is typically distributed across multiple departments or functional groups within pharmaceutical companies, often with specialized groups for different assays, analysis and interpretation. A representation of the overall workflow is provided in Figure 1.1.

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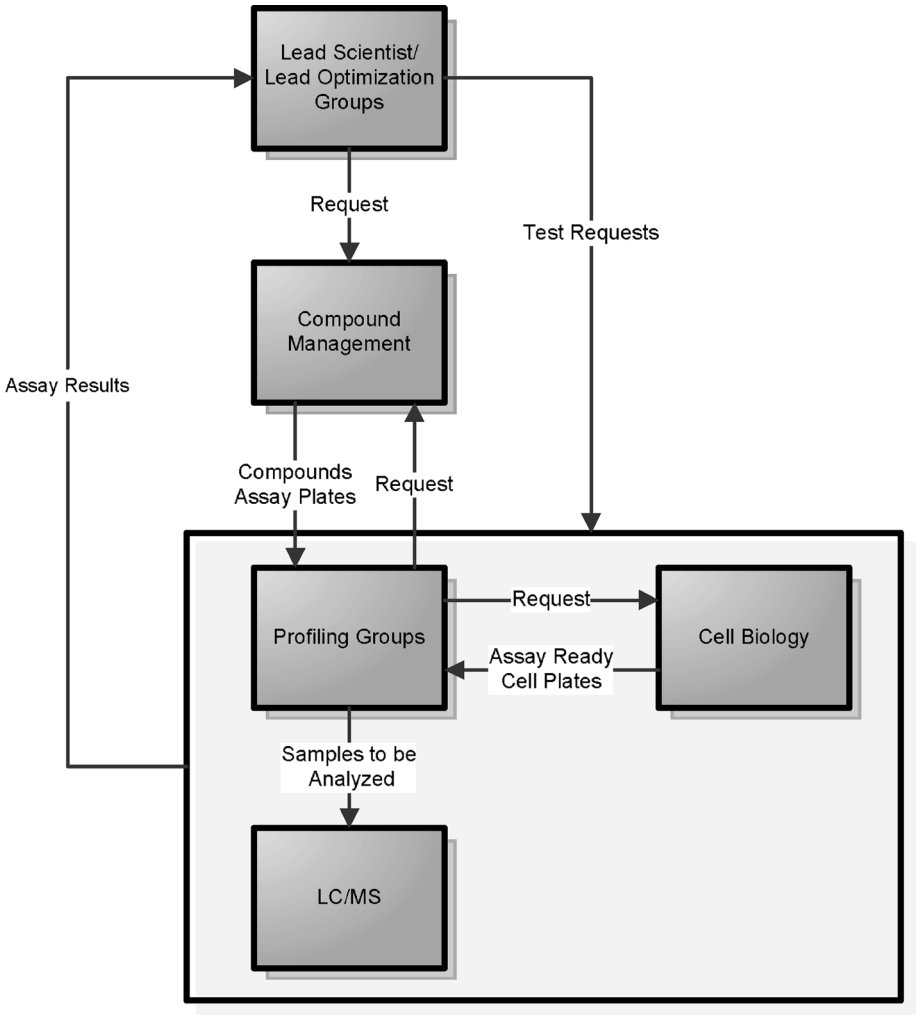

Figure 1.1 A typical DMPK workflow. Requests typically come from the lead optimization group for a set of compounds to be tested in a number of ADME/Tox assays. These could be according to predefined campaign strategy or selected a la carte. Quite often the profiling group initiates the activities of compound management and coordinate the preparation of biological material from cell biology. This may require one to three weeks lead time to get materials to the profiling laboratory. Once materials arrive the testing can commence. Aliquots of the compounds may be sent to the LC/MS for purity and ID confirmation. After completion of the ADME assays by the lead profiling group the results are collected, quality controlled and sent back to the lead optimization group for detailed review as input for subsequent synthesis/optimization cycles.

While the departmental structure varies from company to company and often from site to site, the workload of getting compounds through this process typically breaks down into a few defined areas of functional specialization. Each of these groups have

challenges unique to their responsibilities, that impact the overall effectiveness of moving raw materials through to data. Some aspects of these challenges are briefly framed below.

1.2.1 Compound Management

This group manages large chemical libraries containing up to millions of samples (often in different formats) and maintains a complex database of sample inventory. This group typically fills orders received from various screening groups and scientists for thousands to millions of samples. Because rapid order turn around is critical to fuelling materials for the drug discovery process, major investments have been made to enable the compound management group to cope with their essential and demanding role. They are often the most automated group with large storage and sample retrieval systems where samples are typically stored frozen at −20 °C in large rooms or expandable compartments and retrieved with industrial robotics tolerant of the harsh atmosphere. Once samples are retrieved these groups also have dedicated systems for cherry picking, re-arraying, thawing/freezing and repackaging. When dealing with massive numbers of samples, efficient software is key for inventory management and order fulfillment.

In addition to having tools to aid in the tracking of sample location for retrieval, it is important to monitor sample volumes and to trigger notification when they are critically low. For example, some departments implement consumption-triggered logistics to switch to a "rationing mode" to limit their consumption. Feedback is required to request more samples to replenish their stock when larger supplies exist or can be re-synthesized.

Critical to the effective management of compounds is ensuring the quality or integrity of the samples submitted for testing. For example, compounds that have precipitated or degraded due to water absorption or too many freeze/thaw cycles will confound the results of assays. Often the long-term stability of compounds is not known and samples may be submitted to profiling groups without an integrity check. In these cases, it is up to the profiling group to do a purity and ID confirmation.

Another challenge the compound management group faces is the migration from legacy compound management systems in the face of changing strategies/technologies in screening. Older systems inherently pose limitations in the range of sample volumes and formats in which compounds can be delivered for testing. With the latest in assay technologies trending toward more cell-based assays [1] and miniaturization, additional reformatting is left to occur further down the line. Typically, there is no efficient means of dealing with the valuable excess samples which often end up being wasted. The latest in compound management equipment has greater flexibility in this respect and can even offer samples in dilution series, however turnaround time from order to delivery may start being affected. Ideally the goal is to provide samples with zero waste, in a variety of formatted outputs to be directly consumed by screening facilities, all within a suitable turnaround time. In practice a balance must

be struck in each organization between flexible formatting, material conservation and the response time from request to delivery.

1.2.2 Cell Biology

The cell biology group must maintain a continuous culture of various cells, each with unique growth rates and culture conditions to supply cell suspensions or seeded cell plates for the upcoming ADME/Tox assays. It is critical that this group is able to balance all activities to produce cells and deliver "just in time" in order to maintain the optimal window of cell health and density required for the variety of assays performed by the profiling groups. To cope, cell biology groups have had to become adept at predicting demand and managing highly responsive materials supply logistics.

In addition to meeting a sometimes complex delivery schedule, the maintenance of living cells also poses some challenges for this group. Cells that have overgrown or that have had inconsistent feeding cycles can begin to die or differentiate resulting in assay variability and misleading results. Consistent sample processing is paramount. For example, a simple failure to maintain aseptic transfer techniques can result in cross-contamination of samples and a significant loss of time, materials and productivity. These problems require stringent quality control measures, strict sample tracking and sufficient frozen sample supply to ensure a quick recovery.

The vast majority of facilities maintain their cells manually, with several technicians working diligently in front of biological safety cabinets. Even with the best planning, this becomes difficult to scale when some cell-handling steps must occur over the weekend. Some facilities have turned to automation to maintain their standard cell lines; taking some of the routine burden off skilled technicians and effectively achieving 24/7 operation when fully functional.

An alternative approach for alleviating these logistical issues, that may be amendable to some assay, is to use assay-ready frozen stocks. Cells frozen at high concentration would be seeded into assay plates and used later that day or the next. The build-up of frozen stock reserves is then independent of current demand and can even be purchased directly from suppliers. At least one such supplier has taken a step further by also providing ready-to-use assay plates with cells frozen within. By simply adding media it is possible to revitalize the cells and run your assay within hours [2].

It is uncommon that the cell biology department is dedicated solely to providing standard cell lines for consumption by screening groups. With increased focus on cell-based screening there is pressure to constantly develop and modify cell lines to address the current business strategy. With a manual or semi-manual approach it may take several months to develop a suitable cell line that is ready for standard production. More complex and flexible research-scale automation is on the horizon, that may prove to be the key to optimizing cell culture conditions at small scales that are representative of large scale production [3]. This automation, once proven, will

allow a dramatic reduction of human resources for the development of culturing conditions with more systematic sampling of environmental parameters and shorter development cycles.

Because of their unique talents and skill sets, profiling groups may also become responsible for broader cell biology functions. One such example would be high content screening (HCS). HCS has proven to be a valuable tool in assays such as toxicology, allowing for more complex mechanistic cell or system responses to be measured, rather than the simple "yes/no" or "how much" type of answers typically afforded by conventional screening assays. With the development of standardized bioassays and consumables used in an automated fashion to enable throughput enhancements and labor reduction, these specialized assays may move out into the mainstream screening battery.

1.2.3 Lead Profiling

We typically find that there is no single laboratory known as the ADME/Tox or DMPK laboratory. In most cases several laboratories are involved in performing one portion or another of the absorption, distribution, metabolism, excretion and toxicology studies, each with their own specialty. Some assays require advanced instrumentation, others must be performed manually, and some require sterile environments for cell-based screens. In general we see manned workstations dedicated to one or perhaps two different assays depending on the overlap of instrumentation required to perform them. Assays such as metabolic stability and cytochrome P450 can usually be performed on the same workstation, whereas CACO-2 and permeability assays may have their own dedicated equipment. To improve consistency and throughput, assays are semi-automated with simple instruments such as bulk dispensers and plate washers, or full liquid handling workstations surrounded by instruments and storage devices.

Considering the success of the intensified focus on ADME testing (i.e., a substantial decrease in drug failure due to poor ADME properties) a continued increase in demand on the profiling groups is to be expected [4]. Where groups are already running at capacity, it is difficult to squeeze through any additional requests without moving to processes and technologies that scale well.

1.2.4 Liquid Chromatography/Mass Spectrometry

Of particular note is liquid chromatography/mass spectrometry (LC/MS) detection. LC/MS technology is a critical technique for DMPK studies due to its ability to analyze samples with very high sensitivity and specificity particularly within complex mixtures. It is not uncommon to find LC/MS based sample analysis residing within its own functional department due to the specialized facility requirements and technical skills of the operators. Additionally with LC/MS instrumentation becoming lower cost and simpler to operate, they are also becoming a workhorse

of the profiling groups for certain assays traditionally analyzed with plate readers, such as cytochrome p450 inhibition, PAMPA and solubility.

While sensitive, this technology typically poses some throughput challenges. Even with the relatively large number of instruments seen within the laboratories, LC/MS analysis often remains a bottleneck.

A typical injection and analysis time for LC/MS may be somewhere between several seconds to a few minutes, depending on the complexity of the sample and the LC/MS technology used. More often than not the LC/MS is connected to an auto-sampler capable of handling several 96 or 384 well plates, allowing a high degree of walk-away time once the system is up and running. Only a handful of technicians may be required to manage several units. Technology is now in hand to make the cycle time shorter, further reducing the cost per sample, making the shift to LC/MS analysis more attractive [5].

1.3
DMPK Testing Strategies: the Process from Data to Decisions

Critical to the success of a DMPK testing strategy is the ability to efficiently make decisions that affect the overall drug discovery process. These decisions are made by stakeholders in multiple core disciplines in multiple departments and affect which compounds are carried on to combinatorial library expansion, medicinal chemistry optimization and further biological testing. Within the profiling department itself the ability to deliver critical data to the organization is largely gated by the ability to process and make informed decisions on the quality of data in a timely manner. It is at this stage of the process where significant opportunity exists for efficiency improvements to be made by many organizations.

A common problem experienced under the current automation paradigm, with the demand for greater results in shorter iteration cycles (1–2 weeks), is that screeners are required to collect data from several single assay workstations, often run on different software platforms. With the demand for results on more compounds per week, the screeners must process more samples through the assays, each with fewer data points and replicates. This trade off in quality for quantity can result in lower overall data fidelity.

The management of this screening workload distributed across multiple workstations can be rather labor-intensive and error prone without appropriate sample and data management tools. This places a large resource burden on screeners who could otherwise spend their time on higher-value activities such as more rigorous data evaluation.

As organizations have historically navigated the changing requirements for ADME/Tox testing different decision-making philosophies have evolved that can impact the effectiveness of screening approaches and their underlying logistics.

One common approach is the use of scoring criteria. Typical practice is to run all the compounds under investigation under a battery of assays in parallel without consideration for their interdependence. For example, 500 compounds will be run through several assays within a week or two. When the campaign is complete the data

is passed on to the researchers for a score card type of evaluation based on a summary of all the results. The selection of the best compounds is then based on a consultative evaluation of all the available data. This approach can have a few drawbacks depending on how it is implemented. Since all data on all compounds is required prior to decision making, the turn-around time for data evaluation and QC can sometimes be longer than desired for the next iteration of compound synthesis. Additionally, the volume of multivariate data that must be analyzed in order to make the decisions is also very high and can confound the selection process.

Another common approach is the use of cut-off criteria to define which compounds should be carried forward. Each assay in a campaign has a predefined limit for acceptable values and compounds that fail these criteria are abandoned. For example, compounds that fail the cut-off for solubility may be dropped from further consideration. This approach has strengths in promoting the discipline of only advancing the very best compounds and simplifying the decision making process by enforcing a "live with the outcome" culture. In practice some flexibility or relaxation in the strict criteria may be required in order to ensure that sufficient compounds can progress through the pipeline.

It is interesting to note that this approach can be implemented as either a parallel or hierarchical screening approach depending on whether or not the data on failed compounds is required Figure 1.2. For example, when screening a focused library for

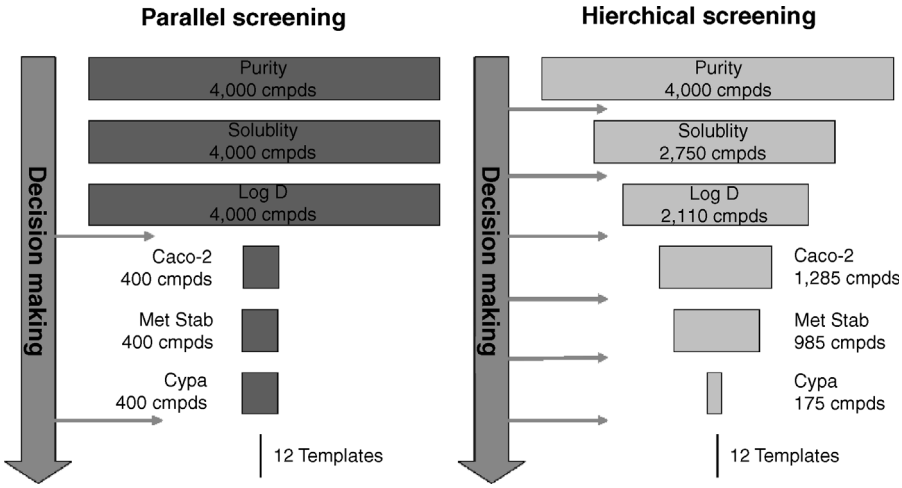

Figure 1.2 Comparison of parallel and hierarchical screening strategies.In the parallel screen the first three assays are run in parallel followed by a manual decision to reduce the candidate compounds down for the subsequent set of three assays. The use of real-time data QC steps and feedback in the hierarchical approach supports the filtering out of failed compounds prior to submission to the next assay. Both strategies depicted defer the lower throughput assays until the end of the campaign. The gradual filtering model in the hierarchical approach is a more informed process when compared to the large single elimination of 3600 compounds seen in the parallel approach. The resulting 12 templates from the hierarchical approach can be viewed as having a greater potential for a marketable drug.

structure–activity relationship (SAR) modeling, all data on failing compounds is of use. In this case running the assays in parallel would make the most sense if the screening capacity is available. However, where a larger number of compounds are to be evaluated, the decision to test the compounds hierarchically can produce a significant resource saving and throughput enhancement. In a hierarchical screen the assays would be conducted in a logical order that enabled the elimination of failing compounds from further testing. This approach defers the time consuming assays until a large number of compounds have been ruled out.

Ideally the ADME/Tox screening laboratory service would be set up to flexibly offer choice in the screening strategy that best fits the current campaign circumstances. It would also provide real-time data feedback both to enable researchers to use the critical information to make decisions on further testing and to reduce cycle times by eliminating compounds midcampaign [6].

1.4 New Questions, New Assays and New Technologies Challenge the Process

In the wake of the impact of LC/MS on rapid bioanalytical method development, no compounds are advancing into first in man studies without explicit assessment of exposure levels in preclinical animal models. As a result, the clinical attrition rate due to poor DMPK has dropped dramatically [7]. Drug hunters are unsatisfied, however, by the arrival of bad news late in their programs: rather than killing compounds and killing programs, scientists would prefer to unravel cause and effect and design their series around the liabilities that increase risk of clinical failure. What has followed, therefore is an ever-expanding sequence of mechanistic assays probing passive and active phenomena for drug uptake, metabolism and elimination.

The main scientific drivers of clinical attrition remain toxicity (30% of failure) and efficacy (additional 30%) [8]. The latter is the domain of the burgeoning field of biomarkers, leading to promising notions of personalized medicines. Whereas the practical application of biomarkers in drug discovery and clinical development is challenged by many logistical and technological concerns, these are generally very closely related to the target under study within the research program, and tend to be handled outside of the ADME/Tox laboratory. Biomarkers that warn for the likelihood of mechanistic toxicities, however, have broad applicability. More and more these assays are invoked during lead selection and lead optimization programs. So, in addition to the biochemical and physicochemical assays exploring ADME phenomena, a new range of predictive Tox assays are growing in popularity [4].

These trends do more to challenge the preclinical profiling process with a growing menu of tests: they introduce new technological paradigms, which must be somehow knit into the scope of the laboratory workflow. Cell-based assays with readouts ranging from simple fluorescence to cell-based imaging to RNA extraction and quantitation by RT-PCR have come alongside solubility assays, PAMPA measurements and cytochrome P450 inhibition studies.

The range of cell types that must be prepared, cultured and manufactured on a just in time basis, the number of detection systems that must be accommodated, the complex scheduling of incubation periods, sample preparation and analysis procedures, the form and fashion of data and post-analytical processing all contribute to a very complex laboratory, balancing a complicated set of demands.

Another dimension of complexity must be layered on top of this description: the demand for these forms of data is growing as drug hunter teams become increasingly reliant on ADME/Tox feedback during the course of their lead optimization programs. Naturally, this growing demand for data is a welcome trend, as it indicates broader opportunity for impact, but of course increased demand exacerbates the complexity of the process.

An apt analogy may be the small intimate bistro restaurant, with a highly complex menu of offerings. With only ten tables the chef and sous chef can preside over each dish, artistically delivering perfect dishes in synchronicity for the customers at each table. The chef continues to invent new dishes, increasing the appeal of his menu, but also increasing the complexity of the process in the kitchen. Everything is fine with only ten tables. But word has gotten out, the bistro is good, and the manager has expanded the dining room. There are now 40 tables and somehow the chef has to figure out how to feed everyone to the same level of satisfaction at the same time. And the menu keeps getting bigger.

1.5 Organizational Models to Scale Up the Process

Like the chef, the laboratory manager has many constraints in moving forward: he cannot merely add staff and cost to the kitchen, he cannot begin to deliver inconsistent product, his responsiveness may not decrease, he cannot achieve quality without well qualified, well trained staff. Instead, he must identify real efficiencies that can be derived from scale.

Laboratories, just like restaurants, have adopted several different models for responding to increased demand: (i) the food court, (ii) the fast food restaurant and (iii) the family restaurant chain.

1.5.1 Food Court

In the food court, there are limited options – combos – to choose from, and each compound is subjected to a predefined battery of tests. This is akin to treating ADME/Tox experimentation as a form of secondary screening, eliminating or severely restricting à la carte testing options. Clear efficiencies can be gained per unit of data, and there are intellectual benefits for collecting wide arrays of information about many compounds, but there will also be a lot of data generated that will not be used. Economically, therefore, the best assays to include as a secondary screening panel are those that are broadly referenced and relatively inexpensive to produce,

such as basic physicochemical and biochemical endpoints (e.g., solubility, cytochrome P450 inhibition). Due to its predefined combo menu, the laboratory generally achieves medium to high throughputs at good efficiencies. More expensive or rare tests are disruptive to the workflow and are better handled outside of the generic test regime. Adaptation to changes and implementation of new assays are not easily accommodated by this set up.

1.5.1.1 The Fast Food Restaurant

The kitchen of a fast food restaurant is characterized by islands of automation, with well defined subprocesses focused on producing a certain kind of output, coordinated by a crew chief. The principal advantage of a fast food restaurant is consistency and fast delivery. The dedicated subunits are designed to perform a certain type of process (assay) at a high rate with very little room for change. Economically, this model is difficult to sustain unless each assay type has sufficient demand to justify the existence of dedicated space, equipment and personnel. It is also not as efficient as a secondary screening model. For assays that are routinely, but not always, requested then this model is very appropriate (e.g., CACO-2 permeability, microsomal stability). However, for the more costly and complex assays that are requested less often, the cost of dedicated people and equipment is hard to justify and as a result the assay has to come off the menu. This is why most fast food restaurants have a relatively limited menu, including mostly foods that are simple to prepare.

1.5.1.2 The Family Restaurant Chain

Dotting the landscape of suburban North America, the family restaurant chain lies somewhere between the bistro and the fast food restaurant. Menus are longer, the food preparation is more complex, the kitchen has multi-purpose stations dedicated to types of food and sophisticated systems for communication and tracking. By streamlining the logistics of managing the overall process and improving the duty cycle of kitchen equipment, kitchen staff and kitchen space, these restaurants are able to efficiently offer the restaurant's most popular items and more rarely ordered novelties. The food quality is consistent, the response time is fair and the price is relatively low.

It should be noted that among these three restaurant models, only the third offers the chef broad latitude in creating new recipes, extending and revising the menu, preserving a customer favorite and improving a staple item.

It is also very telling to consider where the investment is made in each scenario. In the secondary screening model (food court), investment is made to completely automate the experimental process, so that scale can be achieved at marginal incremental cost. Many specific engineering challenges are engaged to minimize the manual steps performed by laboratory staff. In the fast food model, investment is made in people, dedicated equipment and additional laboratory space, so that every type of assay can be supported in a timely fashion. In the family restaurant model, investment is made in managing the logistics of the laboratory workflow, so that tasks are not dropped as equipment and people switch from one assay to another.

Preclinical profiling laboratories generally begin as fast food restaurants, employing dedicated people, space and equipment for specific assays. As they grow in throughput and in the scope of assays offered, this proves to be the default model of laboratory growth, with incremental investment in more people, space and equipment to meet the growing demands of the organization. As these resources become more and more difficult to secure, laboratory managers would be well advised to invest in process management technology and to make the jump from fast food to family restaurant.

1.6
Critical Factors to Improve the Process

Notwithstanding the organizational model being pursued (or of course a hybrid of the above approaches), evolving the capabilities within the ADME/Tox laboratory is a complex process engineering exercise that involves detailed considerations of the roles, capabilities and limitations of all of the participating groups. Because each company has its own particular goals, organizational structure, size, scale and style, there are as many potential solutions as there are organizations. Nonetheless, there are common considerations that all groups must bear in mind when identifying opportunities to increase scope, scale, quality or efficiency. Stated in this fashion and viewed at the abstract level, we can see that the laboratory operation has a lot in common with a manufacturing operation and it may therefore be worthwhile to examine a well established methodology for manufacturing process improvement. Here we explore and adapt a typical and widely accepted approach to analyze processes and pinpoint process improvements: the fishbone model for root cause analysis.

Root cause analysis (RCA) is a class of problem solving methods aimed at identifying the root causes of problems or events. The practice of RCA is predicated on the belief that problems are best solved by attempting to correct or eliminate root causes, as opposed to merely addressing the immediately obvious symptoms. By directing corrective measures at root causes, it is hoped that the likelihood of problem recurrence will be minimized. However it is recognized that complete prevention of recurrence by a single intervention is not always possible. Thus RCA is often considered to be an iterative process and is frequently viewed as a tool of continuous improvement.

The following are basic elements the RCA would target in a generic production process.

- Materials
- Machine/Equipment
- Environment
- Management
- Methods

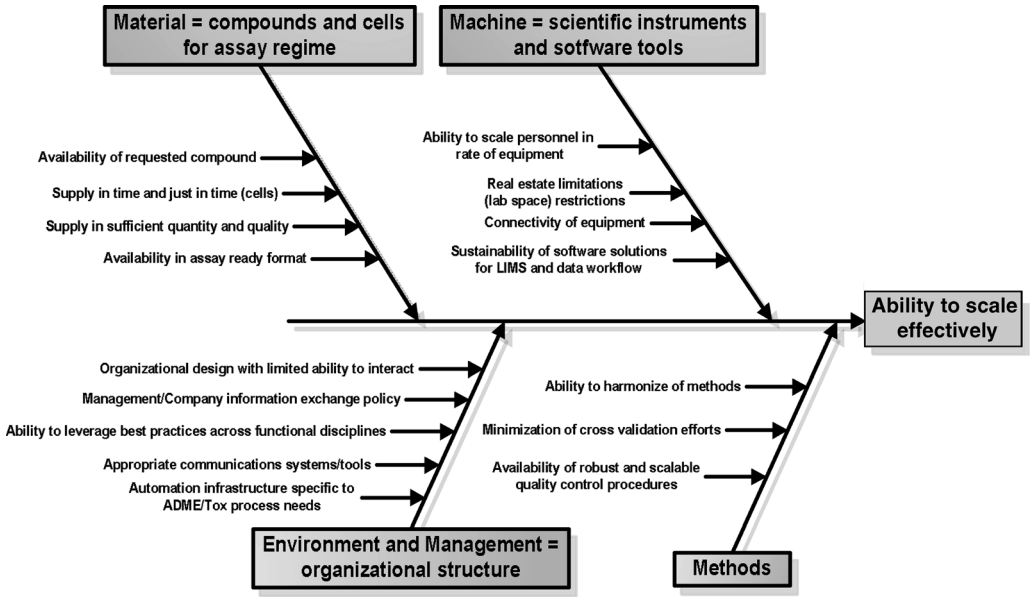

Figure 1.3 Fishbone diagram of ADME/Tox process elements. The scale up of the ADME/Tox screening laboratory requires careful consideration of all crucial elements involved in its process. The commonly accepted approach of route cause analysis has been applied to identify potential hurdles that should be reviewed when planning a significant increase in sample throughput. The importance of individual factors may vary due to the particular goals, organizational structure, size, scale and style of different organizations. The fishbone diagram tries to identify common areas of consideration when identifying opportunities to increase scope, scale, quality and efficiency of the testing process.

Many, though not all, of the factors in a generic production process have an analog within the ADME/Tox process (see Figure 1.3). The following section identifies process elements specific to ADME/Tox screening and potential hurdles an organization might face in scaling their operations.

1.7 Materials in ADME/Tox Screening

Materials in the ADME/Tox screening process relate mainly to consumables (plastic ware, tips, plates, reagents, etc.) and the raw materials the tests will be performed on, which are plates with compounds and cells. To simplify the analysis, it is assumed that access to consumables is not a major issue, since these supplies can easily be ordered through the supply chain and are generally available to the personnel performing the test assays (Figures 1.4 to 1.7).

The just in time supply of plates from the compound management and cell biology groups seems to be a more critical operational hurdle. The ADME/Tox screening

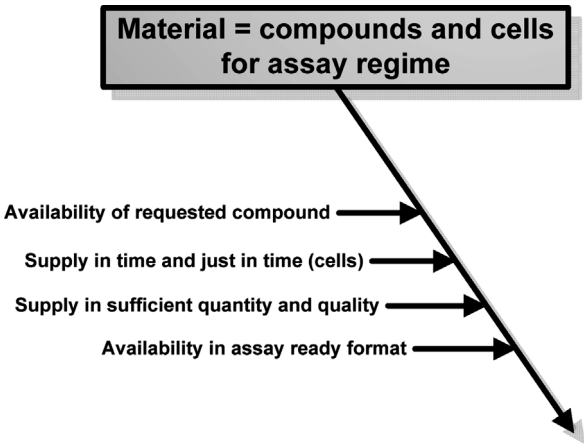

Figure 1.4 Fishbone element "Materials" in the ADME/Tox process.

laboratories receive the compound and cell plates either in a standardized concentration (which usually requires subsequent manipulations to prepare them for the test assay), or in an "assay-ready" format for immediate consumption. Regardless of the delivery format, having the proper compounds and assay-ready plates available at the desired time and in sufficient quantity requires upfront planning and coordination with the compound management and cell biology groups. In our studies, we could identify two generic methods for the supply of compound plates: (i) as stock solutions in DMSO (usually 10 mM) or (ii) as test plates in ready to use form with compounds in appropriate dilution series and buffers, with wells reserved for standards and controls. In the case of compound plates supplied in DMSO, the local laboratory usually performs a reformatting step to prepare test

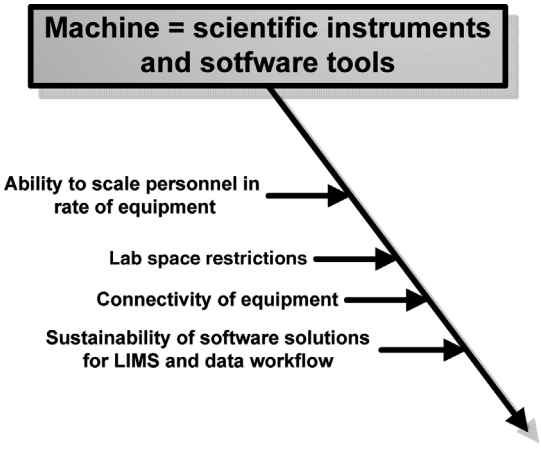

Figure 1.5 Fishbone element "Machine" in the ADME/Tox process.

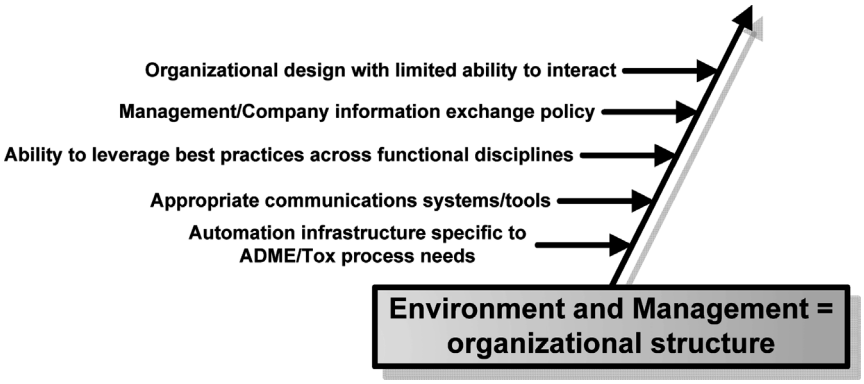

Figure 1.6 Fishbone element "Environment and Management".

plates in the proper pipetting format for subsequent testing. These reformatting steps are often rate limiting unless the local laboratory is equipped to quickly transform them into the desired test format. The reformatting procedures are usually done in a batch mode process that becomes the rate limiting step in the subsequent assay regime. Both the ordering scenarios and the standard format or assay-ready plates require tight linkages of demand and supply between ADME laboratories, compound management and biology groups. In many cases the research organizations put electronic ordering systems in place that allow synchronization between groups similar to supply management systems in production facilities. In most cases these systems prove to be effective, even though the time between request and delivery of compound may sometimes be as long as three weeks, even longer if a compound is in limited supply; in such situations, the library management group has to re-supply this compound from stock or powder solutions or place limitations on its use in ADME/Tox testing. These "long lead time items" determine the pace of research in the laboratory. A similar scenario

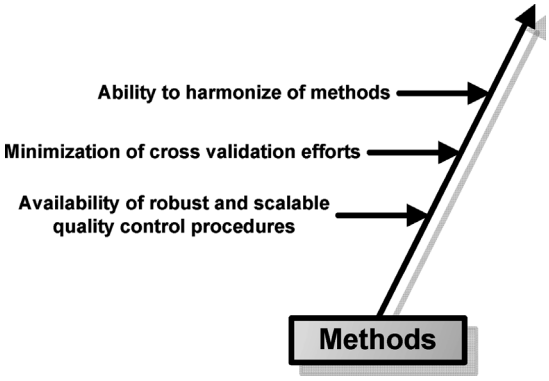

Figure 1.7 Fishbone element "Methods".

is seen with the receipt of assay-ready cell plates with the added complexity of dealing with living material.

1.8 Machines and Equipment in ADME/Tox Screening

The basic equipment in the ADME/Tox laboratory revolves around three major core technologies: (i) liquid handling; (ii) detection and analysis instrumentation; and (iii) software for data retrieval, analysis, interpretation and quality control.

1.8.1 Liquid Handlers

Liquid handling is a basic core function of all physiochemical, biochemical or cell-based assays performed in the ADME/Tox laboratory. Generic tools for these tasks are bench-top liquid handling workstations. The basic interaction with the equipment requires the loading of plates (test plates, assay plates, consumables, etc.), the programming/selection of test assay procedure and the transfer (unloading) of the prepared plates to the next instrument (typically a reader or MS analyzer). These workstations are initially ideal to increase throughput and capacity while gaining walk-away time for the scientist. However, when additional throughput is demanded from this infrastructure of isolated workstations, the laboratories are generally left with two options: (i) increase the number of workstations or (ii) alter the assay to run more compounds during the day.

The strategy of increasing the number of workstations is widely applied since a relatively minor incremental investment is required for each addition, which in theory results in a twofold increase in throughput for that particular assay. Perhaps four or five workstations are required for each overall twofold increase for all assays. Although not a 1 : 1 ratio, additional personnel is required to man the extra workstations. For a time this approach to scaling up works, however with the anticipated increases for ADME/Tox screening one can expect to see a limit reached relatively soon with the number of additional workstations and personnel that the facility can handle.

A complementary approach is to conduct the assays under high-throughput automated conditions. This can be either through the miniaturization of assays, that is, 96–384 plates and if possible 1536, or through the use of alternative assay technologies (e.g., microfluidics). Both scenarios require studies of equivalency testing and backwards compatibility with previous methods and results.

1.8.2 Detection and Analysis

Most detection and analysis is performed on either optical plate readers or mass spectrometers. While multimode plate readers are relatively compact, inexpensive

devices with parallel measurement capabilities (typically providing a fast read of a multiple samples in a 96 or 384 plate format in just a few minutes), LC/MS instruments analyze samples serially and are rate-limited by the chromatographic separation step, such that analysis of each well of a microplate can take several minutes, even with modern multiplexing approaches. In order to cope with the sample throughput demand, companies invest in multiple high-throughput LC/MS units to run analyses in parallel. Similar to the challenge in scaling liquid handling workstations, adding LC/MS analysis units also requires concomitant increase in laboratory space and personnel.

1.9
Software, Data Retrieval, Analysis, Manipulation and Interpretation

While the sample processing bottleneck is well on the way to being solved, the results analysis component still remains a challenge. A variety of software analysis tools exist to automatically analyze and reduce chromatographs to useful interpretive data. However even with automated analysis software, manual review of the data is often required, not only for situations where the chromatograph cannot be analyzed (poor resolution, inappropriate conditions, carryover, etc.), but for all results, where the human eye and experience can spot anomalies that the software simply misses. Much of a LC/MS technician's time is still spent hovering over a computer monitor with the mundane task of clicking chromatograph after chromatograph and rescreening the runs that have failed. With the shift towards the integration of LC/MS detection into automated systems will likely come the inherent benefits of deeper data integration and hopefully intelligent automated data QC algorithms in the sample processing workflow.

One of the major challenges in scaling up operations is the connectivity of instrumentation and the data/results they produce. Any increase in the number of instruments or instrument types also increases the number of necessary software bridges to enable tracking of samples and association of results with samples. Further, data analysis and QC operations often must be conducted using instrumental firmware, introducing further complexity into the overall workflow organization within the laboratory. Many pitfalls arise in this scenario: often instruments cannot be accessed remotely, or data file transfer is not in the inherent design of the instrument's firmware. This leaves scientists to perform tedious, relatively unproductive and error-prone tasks of copying and transferring data. It has been suggested [9] that about 70% of an ADME/Tox scientist's time is spent in data manipulation, interpretation and QC. Since limited commercial solutions for instrument and data interfacing are on the market, companies either produce their own systems (homebrew LIMS infrastructure) or add commercial solutions to their specific need (stitched together). Not only is such an undertaking a "tour de force" for IT groups, the resulting system is typically very difficult and costly to support, maintain and adapt. It is also quite common that a very small number of key

individuals have a detailed understanding of the system, leading to potential risks for the organization, in the case of turnover of key personnel.

1.10
Environment and Management = Organizational Structure in ADME/Tox Screening

While most of the environmental elements of the generic RCA model might not be directly applicable to the ADME/Tox screening laboratories, the job designs, layout of the work environment and the organizational design might present significant obstacles to a scale up.

Limitations to gaining efficiencies may include the inability to leverage existing talent and best practices across laboratories, departments and sites. Barriers to exchange are often the use of divergent tools or infrastructure and insufficient communication across departments.

In our experience with organizational designs, we have found instances of decentralized departments with little capacity nor desire to communicate to the department whose subsequent analysis is dependent on their results. This element links closely to the management philosophy and organizational environment they create. Organizing related functions and processes physically close to each other to encourage interaction between departments that depend on one another is an important step to improve interdisciplinary exchange and collaboration. It is understood that spatial closeness of related functions cannot always be achieved, especially when rapid scale up occurs. Advanced communication tools that facilitate intercompany exchange and relationship building can partially compensate for a limited ability of physical personnel interaction. Communication tools that allow scientists to post and discuss methods, best practices and results enable the scientists to better align their specific roles with their counterparts and create alignment. Fostering an environment that enables scale up without breaking crucial information links is largely dependent on the foundation that management has laid in its policies and encouragement for information exchange.

Many pharma organizations have chosen the path of laboratory automation with robotics and software to increase sample throughput. The industry realizes that those tools are most effective when combined with the appropriate in house support structure for implementation, operation and continuous improvement [10]. When in early 2000s the ADME/Tox testing laboratories were asked to provide a higher sample throughput, other areas in research process such as the primary HTS laboratories were already well equipped with robotics devices, automation and the appropriate personnel support structure. In contrast, automation tools and proven implementation strategies were relatively new to the ADME/Tox screening laboratories. Therefore management often looked to the HTS facilities to lend their expertise and potentially unused capacity to run automated ADME/Tox screening assays [11]. HTS systems are designed to process a high number of samples on a relatively limited set of assays. But, ADME/Tox testing deals with relatively small number of compounds

that are to be tested on a larger set of assays. Nevertheless, some ADME/Tox screens, mainly fluorescence or cell-based assays (e.g., cytochrome P450, PAMPA) did fit the HTS system designs and could easily be implemented into the testing regime of the primary screening groups.

While utilizing the HTS expertise and capacity might deliver the initial desired results, we recommend that serious considerations are given to the transfer of expertise and build up of a local automation and support infrastructure that is specific to the needs of ADME/Tox testing in order to understand crucial process steps, when a rapid scale up is required.

1.11 Methods in ADME/Tox Screening

Limited standardization of methods, procedures and equipment within a laboratory make it difficult to compare data generated at different points of time, and – within larger organizations – between departments and sites. Companies try to compensate for such incompatibilities through cross-validation efforts. Variability in the results is generally originated by different personnel conducting the experiment, due to different skill sets or deliberate seemingly "minor" changes to improve their own efficiency, variability of different types of equipment used to perform the same assay functions (different manufacturers) or differences in performing the assay manually as compared to an automated procedure. From a process efficiency point of view, cross-validations are wasted effort, since they represent re-work and do not enhance the resulting product (the result). In addition they are a burden in the sense that they have to be documented and maintained separately. Besides those efforts in re-work, cross-validations present a real obstacle to scale, since it isn't clear which process among the many alternatives will routinely deliver the most accurate and precise result. It seems obvious that in order to efficiently leverage scientific resources, equipment and processes a certain level of method standardization is required.

Even once a method is standardized, erroneous results can still be generated. As a result, it is critical to have robust quality control procedures in place. Here, careful attention should be paid to identify opportunity for in-process control measures such as internal standards, calibration, control plates, replicates and so on as opposed to post-processing data review steps. Inline QC approaches allow sources of error to be identified and remedied much more rapidly and help limit costly re-tests, or the possibility of erroneous data leaving the laboratory.

1.11.1 Examples of Whole-Process Approaches

As can be seen through the lens of the root cause analysis discussion above, optimizing the laboratory process is a highly complex undertaking. In as many laboratories as the authors have visited, no specific solution has been seen twice. Here we showcase three general patterns that have arisen, each of which offers

distinct characteristics. While all three are drawn from the laboratories of large pharmaceutical companies, the learnings from each should be applicable at any scale.

1.11.1.1 Automation Islands with Manual Data Upload to a LIMS System

This is a common approach. A central LIMS system keeps track of the compounds, layout of plates supplied from compound management and the assays requested for each sample. Scientists track the mapping of samples though the preparation of test plates, sample preparation and analysis with the help of macros (usually programmed in Excel). At the conclusion of the experiment, data is uploaded back into the LIMS system for review and delivery to the requesting scientist.

Depending on the degree of automation, scientists may be preparing test plates and running the experiments manually, or operating preprogrammed liquid handler workstations. Depending on the degree of software integration, scientists may be manually entering data into Excel sheets (though this is rare nowadays), cutting and pasting results from one software package to another (this is very common, even from one Excel workbook to another), or using fully automated data upload macros (this is very rare).

The pros and cons of this approach depend on the degree of automation of the experimental and data analysis processes. When a great deal of manual pipetting and manual data manipulation is required, human error and fatigue can significantly compromise data quality. More automation of these steps can reduce these sources of random error, but may also hide systematic errors, unless the systems also include sophisticated capabilities to highlight deviation from expected performance. This is particularly true with LCMS analyses. Regardless, skilled scientists are spending a disproportionate amount of time performing manual steps.

This approach does have its merits, however, groups can evolve to this sort of system incrementally, automating experimental steps and data manipulations as they become burdensome, often using inhouse programming resources. Also, as no particular experiment format is "hard wired", changing methods is relatively straightforward. However, groups pursuing this approach should bear in mind that such flexibility comes at a cost: maintenance of a growing set of software "scripts" and macros can become unruly; it is not always possible to keep track of which macro version was applied to a specific piece of data, which makes trouble-shooting and retrospective comparison difficult; further it can become difficult to enforce standard operating procedures.

These cautions and the relatively high investment in laboratory staff, space and dedicated equipment make it difficult to scale this approach economically.

1.11.1.2 Complete Physical Integration and Automation

In our experience, very few ADME/Tox groups pursue a complete physical integration and automation strategy. Whereas this approach is very effective in accelerating high-throughput screening, it has proven rather difficult to adapt this to the ADME/Tox workload. Some elements of HTS technologies have been integrated into relevant stages of ADME screens, such as plate replication, sample preparation

and analysis running traditional *in vitro* ADME tests in a "HTS like fashion". HTS operates on the basis of campaigns, running a very large, fixed, compound collection through one specific assay as rapidly as possible, followed by re-configuration and another campaign and so on. ADME/Tox laboratories, in contrast, must provide real-time service for a different set of compounds each week, running each of them through a different panel of assays.

The closest example we have seen to a complete physical integration is the ALIAS system at Pfizer, Sandwich [4].

ALIAS is described as a robotic platform with integrated sample submission and LC/MS analytical systems. It consists of systems with centralized robotic arms that combine a series of modular assay workstations. As this example indicates, it is certainly possible to develop a highly integrated system for ADME/Tox application. However, due to their complexity, it is typically rather difficult to adapt such systems to changes in assay types/strategies and detection technologies, unless a fundamental integration infrastructure is designed with such flexibility in mind.

1.11.1.3 Federated Physical Automation with Software Integration

One attempt to build an automated ADME/Tox platform on top of such a flexibility-friendly integration infrastructure is our own work on the LeadStream system. The system is well documented elsewhere in the literature [12], so only a brief description is given here. LeadStream is a system of automated WorkCells, each with specific automation capabilities, tied together through a software system that manages all the data and sample flow through, from request to result (Orchestrator). One module, the Reformatter, receives sample plates from compound management and prepares assay-ready plates, including just those compounds that have been requested for each assay. The laboratory can include any number of ADME WorkCells that can be programmed to carry out any number of complex sample preparation experiments as well as optical readout. Additional LCMS WorkCells provide automated quantitation by LCMS. Both types of WorkCells automate the analysis of data and report results back to the Orchestrator software.

This approach provides certain operational advantages within the ADME/Tox laboratory, such as minimizing manual data and sample handing and improving overall throughput. The method also promises to avoid the main pitfall of more complete physical integration: difficulty in adapting to new assays or changes in experimental method. This platform is best suited for "greenfield" sites that establish a new laboratory infrastructure utilizing the benefits of an integrated approach to automation, sample and data workflow.

1.12 Conclusions

The demand for more ADME data has cascading effects that impact on several key groups within the pharmaceutical industry. It is likely in today's push for more and

more productivity that these groups are reaching or are already at capacity, with considerable limitations to cope with future needs. Due to the circular (re-circulating) workflow within ADME testing each group is dependent on the other in one way or another. The benefits in throughput gained through the typical approach of increasing personnel and instrumentation (with or without automation) will quickly reach a plateau without serious consideration for efficient workflow. This is achieved through clear understanding of the barriers that can prevent coordination of all activities and data results, and developing implementation plans that fit into one's current businesses mold.

Abbreviations

ADME/Tox	Absorption, distribution, metabolism, excretion/toxicology
CACO-2	Colonic adenocarcinoma 2 (human cell line)
DMPK	Drug metabolism and pharmacokineticss
DMSO	Dimethylsulfoxide
ID	Compound identification
IT	Information technology
HCS	High content screening
LC/MS	Liquid chromatography/mass spectrometry
LIMS	Laboratory information management system
PAMPA	Parallel artificial membrane permeability assay
QC	Quality control
RT-PCR	Reverse transcriptase–polymerase chain reaction
RNA	Ribonucleic acid
RCA	Root cause analysis
SAR	Structure–activity relationship

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