What determines if a compound has hormonal activity and are all hormonal compounds highly potent?

What determines whether or not a compound demonstrates hormonal activity?

The question of whether or not a compound demonstrates hormonal activity is complex and cannot be addressed by any simple assay or set of assays. To fully test the potential of a compound to demonstrate hormonal activity, it must undergo extensive studies that can take long periods of time (up to years) to complete. The United States Environmental Protection Agency (USEPA) has an extensive endocrine disruptor screening program. This program is an example of the batteries of tests, *in vitro* and *in vivo*, that a compound would go through in order to establish whether or not it demonstrates hormonal activity. Even then, the results are sometimes debated and there is often not a clear yes or no answer. For some compounds, whether or not hormonal activity is demonstrated, has been hotly debated for years.

Molecular structure alone does not determine hormonal activity

The molecular structure of a molecule, by itself, is not a good indicator of whether or not the molecule has hormonal activity. An excellent example of this is BPA (bis(hydroxyphenyl)propane, CAS 80-05-7). While not looking much like a steroid, at high concentrations, it has been reported to demonstrate some estrogenic activity. BPA is not, however, a potent estrogenic compound and should not be viewed in the same category as estrogen itself, in terms of hazard.

While the structure of the molecule alone cannot tell one whether or not hormonal activity will be realized, it does make sense to question this a bit more closely when the structure is steroidal in nature. It is also important to recognize that sometimes compounds which look nothing like steroids can demonstrate hormonal activity. For example, in the agrochemical industry, it is not uncommon to discover compounds that look nothing like steroids demonstrating hormonal activity. Many research compounds in this industry vertical fail to make it to development due to their endocrine activity.

When we think about hormonal activity - estrogen, testosterone, progesterone and its derivatives are what come to mind. These are all highly potent hormonal compounds that have major effects on the endocrine system. Often, hormonal compounds of this type have very low occupational exposure limits (OEL)s and permitted daily exposure (PDE) values and require extensive containment and care in handling. Compounds that have this level of estrogenic, androgenic or thyroid activity are of high concern in both a laboratory and manufacturing environment. Additionally, these types of compounds have a long storied history of adverse health effects in pharmaceutical manufacturing employees.

Large number of tests required to determine hormonal activity

With this in mind, the original question should be modified from “What tests are necessary to determine hormonal activity?” to “How likely is it that this compound will demonstrate highly potent hormonal activity?” Addressing the original question would require large numbers of tests that would extend well beyond what would normally be performed at an early stage of development for a compound.

Switch the focus to risk assessment and management

What we can address is the relevant risk-oriented question: How likely is it that this compound will demonstrate highly potent hormonal activity? Low occupational exposure limits and low permitted daily exposure values are what we need to be concerned with in a laboratory or manufacturing environment. Consider BPA as an example, while it can demonstrate a low level of estrogenic activity, it does not require Category 4 handling practices or containment. Soy is another great example that demonstrates what is called phytoestrogenic activity. Soy contains

isoflavones that can bind to estrogen receptors in the body, but at a very weak level. Soy is neither highly potent, nor is it hazardous.

Addressing the question, "How likely is it that a compound will demonstrate highly potent hormonal activity? "

To address the question of whether an active pharmaceutical ingredient (API) demonstrates highly potent hormonal activity from the perspective of laboratory or manufacturing safety, a number of *in vitro* assays are available. These assays are as follows:

- **Binding or functional activity assays**. First, does the binding and/or functional activity at estrogenic, androgenic or thyroid receptors demonstrate significant potency? There are typical cutoffs for determining this; for example, the percentage of activity in a 10 micromolar screen. These assays are called binding or functional activity assays. There are vendors that provide these capabilities at a reasonable cost.
- **Transcriptional activation assays**. A second type of assay is called a transcriptional activation assay. Here, compounds are tested to see if they stimulate the expression (from DNA) of pertinent hormonally-relevant receptor subtypes. While a compound can have activity at, for example, the estrogen receptor, without activating its transcription, this type of assay has proven useful and is a complement, but not a replacement, for the binding/functional assays mentioned above. Low levels of transcriptional activation at a battery of hormonally-related receptors is considered to be supportive evidence of a lack of hormonal activity. Again, there are vendors that provide these capabilities to perform such assays.

Hormonal activity impacting developmental and reproductive systems

As a matter of course, APIs will undergo developmental and reproduction studies during their clinical development. Often, significant hormonal activities express themselves by affecting fetal development or reproduction. The results of these *in vivo* studies are useful to have available when the potential for exposure is greater than what may occur on the laboratory scale. Metabolism, distribution, etc. are all in play in these *in vivo* studies. This means that should a metabolite, for example, demonstrate hormonal activity, it will be expressed in these studies, while it may not be in the *in vitro* studies explained above. For many active pharmaceutical ingredients (API)s, these studies would be performed as a matter of course and it would be useful to have the results to support the safety assessment of a significant campaign where exposures may be greater than in a laboratory or early stage development environment.

When the results of developmental/reproductive studies are unknown, you will see the application of appropriate adjustment factors (AF's) in the OEL and ADE equations. These AF's are intended to protect workers in the event that compounds are ultimately found to have effects in these areas. Developmental/reproductive effects may be due to hormonal influences, so these AF's are designed to protect for these possibilities. When compounds are actually known to demonstrate effects with regard to development or reproduction, AF's may be even larger.

In summary, the focus should not be on whether a compound demonstrates hormonal activity or not, but what are the risk-management processes that employed to ensure the prevention of occupational exposures and cross-

contamination. This can be achieved by understanding what are the numerical OEL and PDE values, and what controls and procedures are in place to minimize the overall risk.

Reference: What determines if a compound has hormonal activity and are all hormonal compounds highly potent? (affygility.com)