A Quantitative Study in Cross Contamination

This article is based on data acquired during an evaluation of the quantifiable risk of cross contamination in an Oral Solid Dosage (OSD) facility. This article is intended to provide some quantitative data to an area in which perception and not reality is the norm. There is really no published data on cross contamination.

In 2005 it was clear that regulators around the world were considering adopting segregation and dedication for all "compounds of concern" such as genotoxic, mutagenic, carcinogenic, hormones, sensitizers and beta lactams. The impact to industry would be incalculable. The reason for this move was the perception that cross contamination was rampant.

Setting Limits

The perception is that there should be no cross contamination of one product by another, but how do you define "none". Some regulators have used zero as the limit, but it is impossible to demonstrate zero. Another method has been "below the level of current detection methods". In the two decades I have been in pharmaceutical containment I have seen the limit of detection for Naproxen Sodium fall from about 4 nanograms to 250 picograms. Looking at the data collected in this experiment if such a standard were applied then **all** pharmaceuticals should be produced in a segregated and dedicated way, including any handling of drug substance in the pharmacy or by caregivers. As will be seen later cross contamination of a single dosage is a greater risk than cross contamination of bulk API/ excipients prior to final blending/ mixing or other processes that ensure uniform distribution.

There are various ways limits can be set for pharmaceutical compounds. By far the most scientific is one based on toxicological data setting a health-based limit, such as an acceptable daily exposure (ADE). An ADE is a daily dose of a substance below which no adverse effects are expected by any route, even if exposure occurs for a lifetime. The same data is used to calculate occupational exposure limits (OELs). The major difference in the two terms is that the OEL is used to protect the operator/ worker whereas the ADE is used to protect the patient.

Design of Experiment

In this particular case the owner wanted to understand how effective their development scale OSD facility was for both operator protection and cross contamination. To determine if cross contamination was occurring air sampling, product contact and non-product contact surface swabs where taken as well as the surrogate/placebo sample test. These samples were used to see if they gave clues as to how cross contamination might occur, using data rather than perception. For occupational exposure, area and personnel sampling was used for iteration 1. The personnel sampling was omitted for iterations 2 and 3 as described below.

To robustly understand if cross contamination was occurring sequenced production of surrogate and placebo tablets was performed.

The Procedure

Basically the procedure was to run a surrogate material through the oral solid dosage process including end of run cleaning and then follow up with a placebo material run through the same processes, and with three iterations of the surrogate/placebo cycle. For each run area air samples and swabs were taken with placebo tablets pulled for testing at the start, middle, end of compression and after coating. 100 tablets were taken at the stated points, bagged separately and labeled. They were sent to a certified independent testing laboratory for analysis. The laboratory selected 3 tablets at random for sampling from each placebo batch and each sampling point (start, middle, end and coating for each of 3 iterations). Tablets with Naproxen Sodium as the active were made for each of the three surrogate batches. Table A shows the sequence of surrogate and placebo as well as the dose per tablet and total dosages manufactured in the batch.

Table A - Production Sequence

Production Sequence	Amount	
Surrogate 1 (S1)	300,000	300 mg Naproxen Sodium
Placebo 1 (P1)	300,000	300 mg placebo
Surrogate 2 (S2)	300,000	300 mg Naproxen Sodium
Placebo 2 (P2)	300,000	300 mg placebo
Surrogate 3 (S3)	300,000	300 mg Naproxen Sodium
Placebo 3 (P3)	300,000	300 mg placebo

Surrogate Run 1
As part of a surrogate test protocol, artificial events are not induced to represent worst case scenarios. Our experience shows that real world events regularly occur in surrogate runs because the operators are unfamiliar with the equipment. So surrogate run 1 demonstrates how real world conditions can occur without any artificial stimulus. The full industrial hygiene (IH) protocol sampling was to occur for each surrogate iteration. Due to the amount of time taken and the incidents described below, it was decided to dispense with IH sampling for surrogate runs 2 and 3.

What occurred
The system was new and had undergone IQ, OQ and PQ. The staff was not very familiar with the equipment and its operation which during a surrogate run is preferable to mimic real world conditions.

- Material was weighed in an isolator and passed into a bin connected by a split butterfly valve. In designing the system no provision had been made for misalignment or support of the bin when docked. As a result the bin was placed by the bin handler as accurately as possible.
- Once docked the bin handler was removed to allow the operator access to the isolator.

- The active was added to the bin from the isolator. At this point the bin and contents were hung off the base pan of the isolator at a weight of about 150 kg after dispensing into the bin.
- The bin handler was placed and an attempt to disconnect the split butterfly valve (SBV) was made. Eventually a rubber hammer was used. When the valve finally parted, the isolator base sprang up by 1 ½" or so shaking both parts of the SBV to open and allowing product to escape. The energy produced caused visible powder clouds.

As part of the fluid bed processing function a compressed air pulse is used to clear the sock filter. This pulse is injected on the exhaust side of the filter sock and is meant to dislodge product into the product bowl. As configured a gasket had not been cut to profile on a relief vent. As a result the pressure pulse had no where to go (the exhaust valve is closed during purge) and the relief valve actuated allowing the over pressure to be relieved. As designed the fluid bed processor relieved into the technical space, designed to withstand relief and control its efflux to atmosphere via the exhaust HEPA filters. The technical space has its own HEPA in/ out filtration and has material air locks (MALs) and personnel air locks (PALs) to contain the space from the external cGMP corridor and the environment.

In addition a pulse purge on the vacuum transfer caused visible and measurable emission. This occurred because the quick connects on the vacuum transfer were not identified due to incorrect installation. As a result the pressure pulse was not vented and found every weak spot in the system (notably no gasket was present on the spray granulation plate of the fluid bed processor) and a visible powder plume resulted.

A technician rectified the items above before surrogate run 2 and the problem did not recur. However to be monitoring the equivalent of an explosion venting of a fluid bed processor was a unique experience and provides some very valuable data.

The design for off loading the fluid bed processor was:

- vacuum discharge to bin
- bin docks to mill
- mill discharges to bag
- bag is placed in the isolator
- isolator discharges to blending bin

Because significant exposure occurred in surrogate run 1, it was decided to discontinue IH data collection. However area sampling in all the rooms in which the operations occurred, the in suite corridor, the GMP corridor and the technical space continued to be monitored for each iteration. This was done so that airborne concentration based on emission could be compared with the placebo tablets to see if there was any correlation between air concentration and cross contamination.

The Data

The main purpose of the experiment was to show how much of the surrogate was present in the three placebo runs, regardless of the route of exposure. A test like this is holistic as it includes all routes of exposure.

Table B – Results of PlaceboTest

S = start, M= Middle, E= End, C= after coating P= Placebo Run

Placebo Tablets mcg/tablet			
	1P	**2P**	**3P**
S1	0.019	0.025	0.170
S2	0.025	0.029	0.160
S3	0.020	0.023	0.210
M1	0.019	0.024	0.200
M2	0.021	0.024	0.170
M3	0.018	0.021	0.340
E1	0.019	0.026	0.190
E2	0.025	0.025	0.180
E3	0.018	0.031	1.300
C1	0.034	0.020	0.170
C2	0.019	0.031	0.160
C3	0.021	0.023	0.200

The table shows the concentration in micrograms of Naproxen Sodium in the placebo tablets for each of the three runs.

Figure 1 - Results of Placebo Testing

From 100 tablets collected at the each of the stages (beginning, middle and end of the compression stage and after coating), 3 samples were randomly collected from each sampling stage for analysis at an internationally recognized laboratory with a well developed method for detecting Naproxen Sodium. In the graph, Placebo run 3 (P3) shows results that are significantly out of line with Placebo run 1 (P1) and Placebo run 2 (P2). To keep things in perspective even at the results of Placebo run 3 it would pass the FDA Genotoxic limit of 1.5 mcg/day, although it is very close to the limit.

Figure 1 – Placebo Results

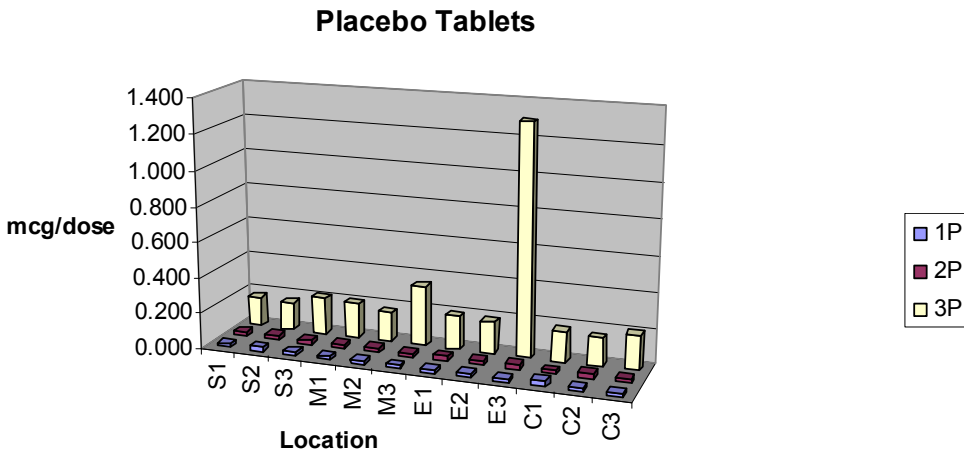
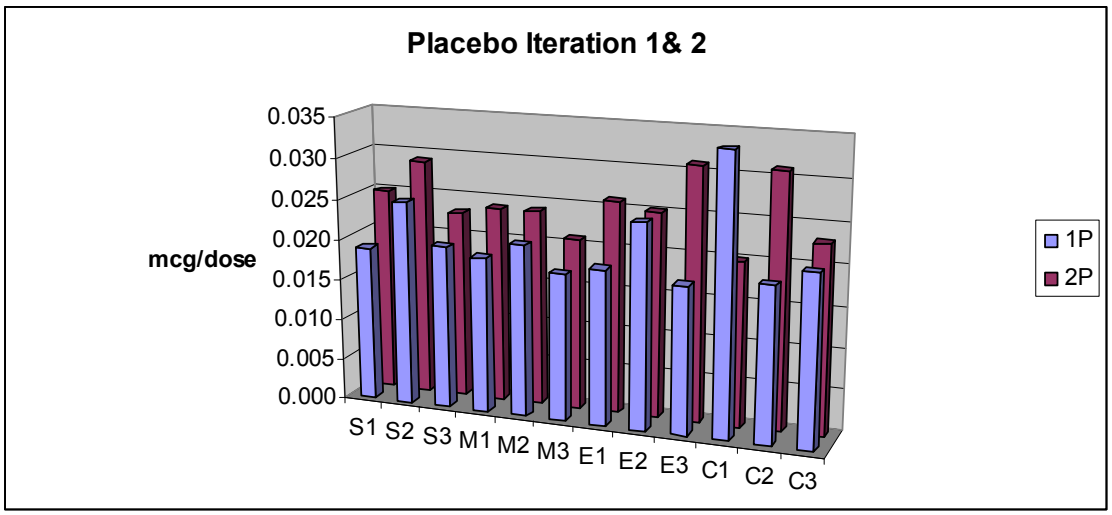


Figure 2 – Placebo Results for Iterations 1 and 2

The graph below shows Placebo runs 1 and 2 which show a consistent set of results.



Placebo runs 1 and 2 are consistent in the range (0.18 – 0.34 mcg/tablet). Placebo run 3 was significantly worse and had an outlier.

What caused Placebo run 3 to return inconsistent results? It could have been contamination by being in the same shipper as the surrogate tablets. But it is highly unlikely that it would lead to such consistent results, other than the outlier. Additionally Placebo run 2 was in the same box as Placebo run 3 and was consistent with Placebo run 1 which was sent separately. All samples were in zip lock bags. The laboratory sampled another set of tablets which verified the results consistent with the other samples from Placebo run 3 without an outlier. The most likely explanation is that this is real data and that Placebo 3 was contaminated at a higher level than Placebo runs 1 and 2 at some point and that the outlier could represent a tablet that was additionally contaminated when in single dosage form.

A product is more vulnerable to cross contamination when it is in a single dosage form because the amount of the contaminating compound needs to be below the ADE to keep the risk of cross contamination low because there is no expectation of uniform dispersion once in the dosage form. However before this stage the limit would be the number of daily doses present in the batch times the ADE (300,000 daily dose x 1.5 mcg/day = 0.45 grams).

Therefore final blend transfer, compression, coating and packaging are the most vulnerable operations for cross contamination and processes prior to blend uniformity are less vulnerable by significant orders of magnitude. This may seem counter intuitive, it is not.

What caused this increase in carry-over?

1. Was it sedimentation from the concentrations created in the process rooms and technical space and tabulated below?
2. Was it mechanical transfer?
3. Was it retention on critical product contact surfaces?

Airborne Concentration

Note the data below is based on long duration samples of 5 – 9 hours.

Table C – Airborne Concentration Results

Airborne Concentration mcg/m3/Duration								
	1S A Form	**1S Gran Mill/B**	**1S Comp**	**1P**	**2S**	**2P**	**3S**	**3P**
A Granulation	0.1600	0.6900		0.0060	0.6200	0.0180	0.1500	0.0041
B Granulation	0.0920	0.7500		0.0032	0.3300	0.0150	0.1500	0.0013
Compression			0.0023	0.0002	0.0072	0.0016	0.0086	0.0005
Coating			0.0025	0.0003	0.0007	0.0007	0.0051	0.0002
Corridor in Suite	0.0005	0.0400	<0.0002	0.0002	0.0015	0.0005	0.0043	< 0.0002
Corridor outside	<0.0002	0.0035	0.0005	0.0005	0.0033	0.0006	0.0004	0.0004
Tech space	28.0000	230.0000	0.0800	14.3000	100.0000	5.0000	41.0000	5.6000

1S A Form = Formulation in the first surrogate run where the split butterfly valve exposure event occurred
1S Gran/ Mill B = Granulation, first surrogate run where the fluid bed processor venting occurred
1S Comp = Compression/ coating first surrogate run
1P = first placebo run, backgrounds, no personnel samples
2S = second surrogate run no personnel samples
2P = second placebo run no personnel samples
3S = third surrogate run no personnel samples
3P = third placebo run no personnel samples

Location of samplers
Granulation – two background samples at different corners of the room
Compression – background during operation of the press
Coating – background during operation of the coater
Corridor in Suite – single door to process room, pressure cascade to the process room
Corridor Outside – cGMP corridor outside the suite protected by airlocks with two chambers
Tech Space – area sample in the technical space during the surrogate and placebo runs

Figure 3 – Layout of Facility

The data are very low in the process rooms despite two visible dust cloud events. The technical space is a different story but there is no route for this material to return to the placebo and by iteration 3 the results even in the technical space are far lower than iteration 1. The conclusion is that airborne concentration does not affect the carryover in this case.

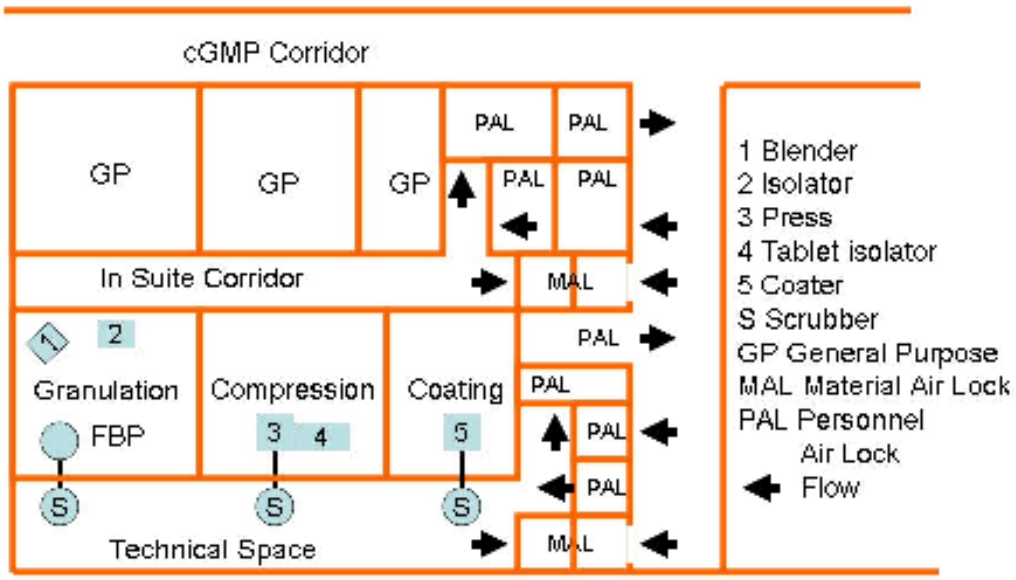
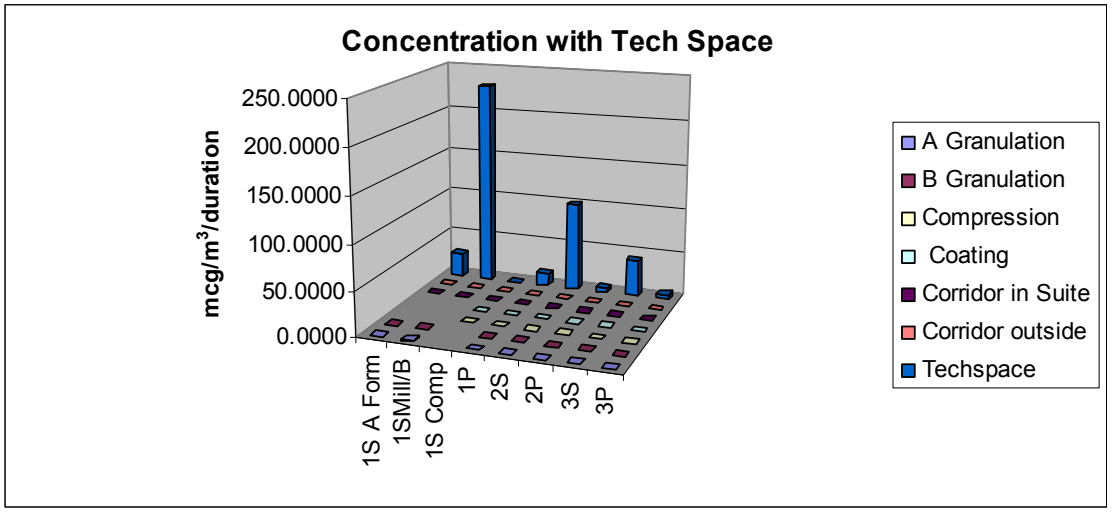


Figure 4 – Air Concentrations all spaces sampled

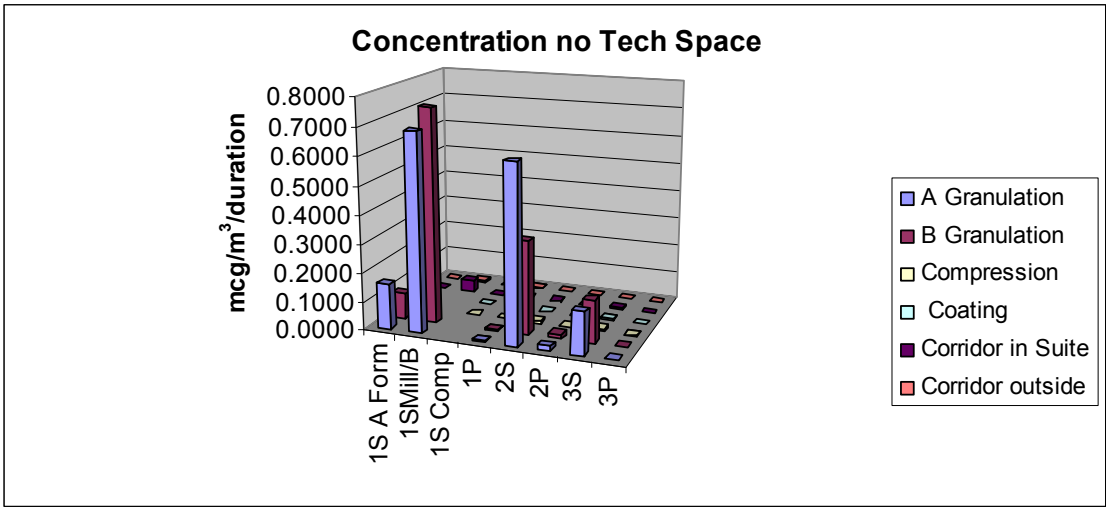
This graph includes the technical space results which graphically over power the much lower non-technical space results shown below. The technical space airborne concentrations are of interest because the fluid bed processor, press and coater all used scrubbers to collect the dust. Scrubbers are very poor dust collectors (as can be seen by the results) in addition the fluid bed processor vented to the technical space in iteration 1.



The technical space is negative in pressure to the other areas and is protected by MAL and PAL and is vented to the outside via HEPA Filters. It is constructed like the manufacturing rooms. When the result of the other areas are compared to the results in the technical space the data becomes insignificant. The technical space figures are very high for the Fluid Bed Processor (FBP) venting, but drop to lower levels during compression, so the room air handling dealt with clearing out the concentration. The figures dropped iteration to iteration. The concentrations in the technical space make it clear that the wet scrubbing is not efficient at removing particulate. The press and coater scrubbers are much more effective, or have considerably less load than the FBP scrubber.

Figure 5 – Air Concentrations except for Technical Space

This graph represents the concentration without the technical space figures. Granulation continued to be significantly higher than other results even though the results got better. This may be an improvement in technique, but is more likely caused by the pulse purge "finding" weak spots in the fluid bed processor connections.

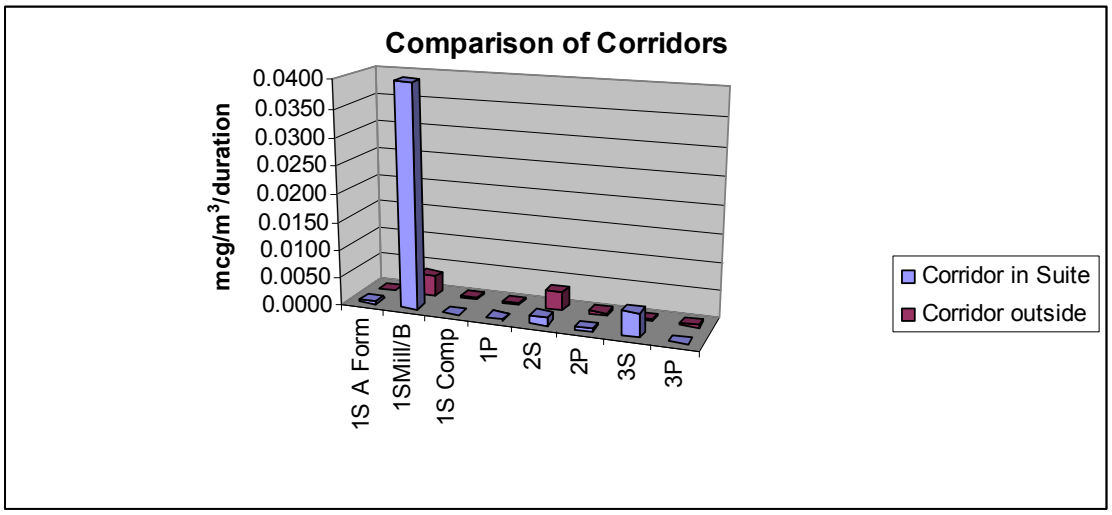


The non technical space concentrations are interesting because:

1. The in suite corridor with single door to the process rooms performed very well.
2. The air handling system effectively cleaned up the airborne concentrations between the iterations
3. The concentrations in the granulation room fell with each iteration and were very low during placebo operations showing excellent clean up by the air handlers.
4. The in suite corridor and external corridor were inconsistent; the external corridor had unexpectedly high concentrations when compared with the in suite corridor.

The granulation process caused room concentrations but as the iterations proceeded and the staff got more familiar with the process the concentration reduced. The issues with the granulation process lead to a higher reading in the common corridor, but these results are much lower than would be expected for an open process. The figures above would be acceptable for a compound with an OEL of 1 mcg/m^3/8 hours.

Figure 6 – Air Concentrations in the Corridors



Other than the issues with the surrogate 1 run (1S) the in-suite corridor performed extremely well, in fact at times better than the corridor outside the suite. This defies logic, but a possible explanation is the concentration in the technical space migrated through the building structure to the corridor.

The final analysis is the swabs; the area of interest is the product contact surface values (PC). However, the tablet samples were collected in the tablet isolator and it is highly probable that contamination occurred here for the outlier (see swab results). If this is the case, more work needs to be done to understand what caused the contamination. One hypothesis is that material residual in the isolator and on the gloves was mechanically transferred to a tablet during collection. With only one data point there is too little evidence that this was the case, but it is the most likely option since the press product contact swabs provided excellent results. More work is required, but it is possible that three classifications of contact surfaces are required. For example:

Product Contact (PC)	In product contact requiring cleaning to the best possible results comfortably below the hazard-based limit
Product Near Contact (PNC)	Surfaces such as an isolator wall, floor and especially gloves. May require cleaning to the standard of product contact surfaces
Non-product Contact (NPC)	Surfaces such as floors, walls and ceilings in processing rooms, etc. A visually clean limit should be sufficient for these surfaces

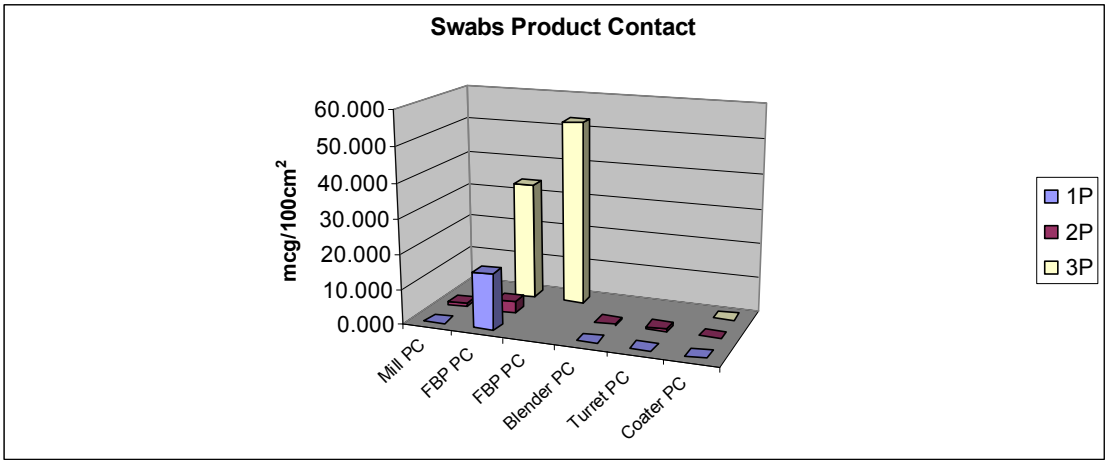
This would be in line with the statement that the highest risk of cross contamination occurs once the product is in dosage form.

The figures are unremarkable except for the fluid bed processor and the material and personnel airlocks.

Table D – Swab Results

Swabs	mcg/100 cm ^{2}		
Location	**1P**	**2P**	**3P**
Mill PC	0.150	1.000	
Isolator Floor	1.600	3.300	1.400
FBP Product Contact	16.000	3.300	34.000
FBP Product Contact			53.000
Blender Product Contact	0.140	0.330	
Granulation Floor	14.000	1.300	5.100
Tablet Isolator Non Product Contact	0.130	32.000	3.600
Turret Product Contact	0.028	0.750	
Coater Product Contact	<0.010	0.011	0.130
MAL 1 Floor	0.150	3.100	1.100
MAL 2 Floor	0.340	3.300	2.800
PAL 1 Floor	0.340	19.000	
PAL 1 Bench	0.120	0.840	2.600
PAL 1 Floor	2.700		
PAL 2 Floor	0.036	0.320	0.130

Figure 7 – Swab Results



The issue of concern is the fluid bed processor, because it has by far the largest surface area. When calculating cleaning limits the shared surface area is taken into account where the larger the shared surface area as typically found on V blenders and fluid bed processors, the lower the concentration has to be to meet the criteria.

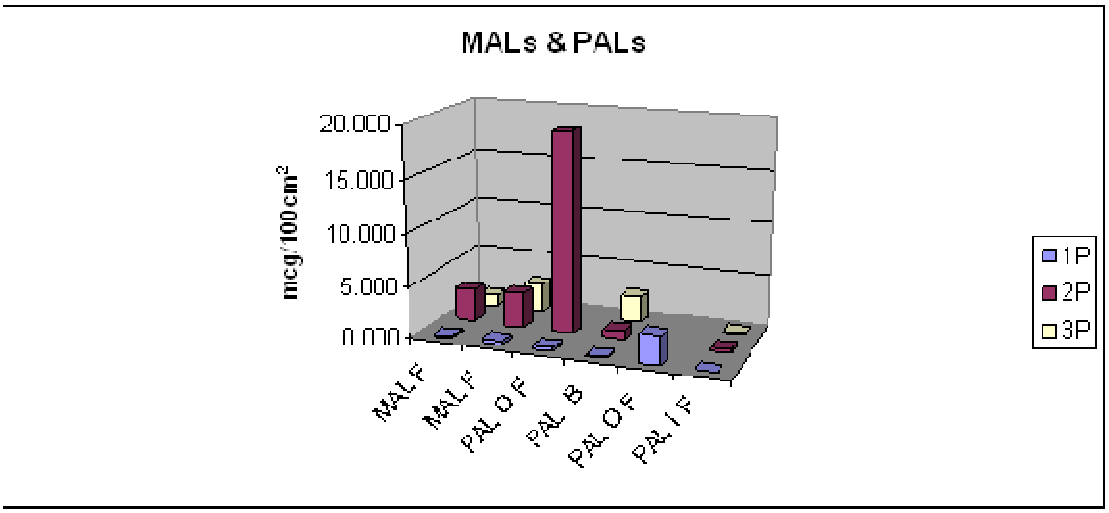
What it does show is a significant increase in concentration in the placebo run 3 coinciding with the increase in the placebo run 3 tablets. All the data was taken post cleaning from the previous surrogate batch. The placebo run 1 should be the worst, using current logic because the airborne concentrations are higher. Placebo run 2 was better,

while placebo run 3 was much worse. During the swab recovery the CIH taking the samples visually identified a contaminated area and took an additional swab. It is clear that the concentration in iteration 3 is far higher than the other runs and undoubtedly is the cause of the increase in concentration in placebo run 3 but due to blending after fluid bed processing is unlikely to cause the outlier.

The reasons for this failure and their detection under normal cGMP operation are the key lessons to be learned. In the end it is all about cleaning. In addition it does show that airborne sedimentation and mechanical transfer in most cases are a distraction rather than a cause.

Note the tablet isolator figures. Tablets were recovered at this point except for coating. Note the results for the coater are getting progressively worse. It is highly probable that the outlier E3 (Table B) in the third placebo run was contaminated by a single (very small particle) as a result of collection in the tablet press isolator which was contaminated significantly in placebo runs 2 and 3 due to failure to clean effectively and failure to inspect. The isolator had no lighting so identifying visually clean was difficult.

Figure 8 – Swab Results for each iteration – Airlocks



The events in the material and personnel airlocks did not follow the pattern of events in the process rooms and this is indicative of the random nature of results in airlocks. The airlocks tested were double chamber with separate in and out chambers. As a basic rule, expensive and complex airlocks can be defeated by operator technique.

The material airlock data showed that the second iteration had issues. As for the personnel airlocks, the same effect was seen in placebo run 2 while the bench remained relatively clear of contamination.

Again there is no evidence of carryover from non-product contact swabs except for the tablet press isolator which was used to capture the tablets.

Conclusions and Lessons Learned

The test runs as performed represent a true worst case scenario. Are there improvements and controls that can reduce the values seen?

1. Set acceptance limits for cleaning, swab and visual inspection and then monitor performance. Use the hazard-based calculation based on the ADE.
2. The surrogate chosen, the process equipment selected were all worst case. The important factor to consider is the shared surface area to volume processed ratio. The larger the shared surface area ratio the lower the rinse or swab limit will be. The fluid bed processor is significantly the largest shared surface area in this case.
3. Investigation for contamination pathways for fluid bed processor. The supply and exhaust ducts are undoubtedly contaminated but are not cleanable. Out of sight is not out of mind.
4. Vent fluid bed processor to roof. Discharging to the technical space for explosion relief is not recommended. It is far better to use a 12 bar rated construction with suitable valves.
5. Replace the scrubber with a dust collector for the fluid bed processor and keep the technical space clean.
6. Evaluation on a case by case basis is essential to ensure that anomalies are investigated.
7. Improve MAL and PAL operation, procedures and wipe down after use. Complex MALs and PALs are not necessarily better or necessary.
8. Split Butterfly Valves should never act as the support for equipment
9. Compensators for docking inaccuracies are essential
10. Bins should be on a docking station which allows accurate docking to take place and supports the bin rather than manual alignment.
11. Expect the unexpected.
12. There was no correlation between airborne concentration and cross contamination.

The results show cross contamination occurring at measurable levels. Because it can be measured does not mean it is unacceptable; cross contamination in the worst case was 1.3 mcg/dose. That is 1.3 millionths of a gram. Say the ADE is 10 micrograms, was the risk to the patient unacceptable? The real issue with the results shown is that they were not consistent.